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

007158

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Benefin, Toxicology Chapter of the Registration Standard

To: Chuck Kent, Chief
Registration Branch
Special Review and Reregistration Division

From: John H.S. Chen, D.V.M.
Microbiologist
Review Section I
Toxicology Branch-HFAS
Health Effects Division (TS-769)

Thru: Robert P. Zendzian, Ph.D.
Toxicology Registration Standard Coordinator
Health Effects Division (TS-769)

Albin Kocaialski, Ph.D., Head
Registration Standards and Special Review Section

William Burnam, Acting Director
Health Effects Division (TS-769)

Attached is the Toxicology Chapter of the Registration standard for Benefin. The following portions of this chapter are available. You may obtain a copy from this reviewer.

A. Toxicology Summary
B. Toxicology Profile
C. Data Gaps
D. ADI Reassessment
E. Toxicological Issues
F. Toxicology Summary Tables
H. One Liners

cc: Kocaialski
Zendzian
Coberly

007158

Toxicology Chapter

of the

Benefin

Registration Standard

Prepared by

John H.S. Chen, D.V.M.
Microbiologist
Review Section I
Toxicology Branch II
Health Effects Division

007158

TABLE OF CONTENTS

007158

A. Toxicology Summary

Benefin is a preemergent herbicide (N-butyl-N-ethyl-Alpha, Alpha, Alpha-trifluoro-2,6-dinitro-P-toluidine). Benefin is registered for uses on terrestrial non-food crops (i.e., tobacco, ornamental plants, forest trees. and turfgrasses) and may be applied to terrestrial food such as alfafa, clover, lettuce, and peanuts.

Benefin possesses a low order of acute oral, and inhalation toxicity to mammals (category IV and III, respectively). However, no data are available on the ability of benefin to produce acute dermal, primary eye irritation, and primary dermal irritation. A delayed neurotoxicity study is not required for benefin.

Available subchronic oral dosing studies with benefin are not adequate for assessment of subchronic toxicity. No acceptable subchronic dermal study (21-day) is available. This study is required for benefin. A subchronic inhalation study (90-day) will be required if residues of benefin occur in dried tobacco.

Available chronic feeding studies with benefin in rats or dogs, and oncogenicity studies in rats and mice are not adequate for assessment of chronic toxicity and oncogenicity in the rodent and the non-rodent. Chronic toxicity studies in rats and dogs and oncogenic studies in rats and mice are required for benefin.

Available teratology study with benefin in the rat is adequate for assessment of teratogenicity. In this study, the NOEL for maternal toxicity was established at 225 mg/kg/day. The developmental toxicity was established at 1000 mg/kg/day (HDT). The teratology study with benefin in the rabbit was not conducted in accordance with the method recommended by the Pesticide Assessment Guidelines Hazard Evaluation Series 83-2, therefore, a teratology study in the rabbit is required to satisfy this requirement.

Available reproduction study in the rat is not adequate for assessment of reproductive toxicity. A reproductive study in the rat is required for benefin.

Sufficient data are available to satisfy the data requirement for mutagenicity study in the categories of gene mutation and other genetic effects (DNA). In a Salmonella/Mammalian Microsome Mutagenicity Test, benefin did not demonstrate mutagenic activity against the Salmonella typhimurium strains TA1535, TA1537, TA1538, TA100, and TA98 in the presence and absence of metabolic activation at the concentrations from 25 to 750 ug/plate. Although the gene mutation study with benefin in the cultured L5178Y mous lymphoma cells is deficient at the present time, the study can be upgraded to acceptable upon receipt of additonal data under non-activation condition. In the rat hepatocyte unscheduled DNA synthesis assay, benefin did not cause DNA damage and inducible repair in this study. However, the in-vivo sister chromatid exchange assay with benefin in Chinese hamsters is unacceptable. A chromosomal aberration study is required for benefin.

No metabolism study is available for benefin.

Temporary tolerance have not yet been established for residues of benefin in or on the terrestrial food crops.

B. Toxicology Profile

81 Series Acute Toxicity

81-1 Acute Oral

Sufficient data are available to show that technical benefin has a low acute oral toxicity to rats (MRID 00024255) The acute oral LD_{50} for rats was greater than 10 g/kg (combined sexes). Toxicity Category IV.

81-2 Acute Dermal

No acceptable acute dermal toxicity is available for technical benefin. A study is required.

81-3 Acute Inhalation

Sufficient data are available to show that a mist of dimethyl formamide containing 5% benefin has no adverse effect in rats following a single uninterrupted exposure by inhalation route (MRID 00024275). The acute inhalation LC_{50} was greater than 1.33 mg/L/1 hr. Toxicity Category III.

81-5 Primary Eye Irritation

No primary eye irritation study is available for benefin. A study is required.

81-6 Dermal Sensitization

SEE DER

Sufficient data are available to show that the technical benefin is not a sensitizing agent in guinea pigs when 5% suspension were administered dermally (MRID 00144283).

81-7 Acute Delayed Neurotoxicity

No acute delayed neurotoxicity study is available for benefin. However, this test is required only for organophosphate compounds which inhibit cholinesterase. Benefin is not an organophosphate, therefore, a study is not required.

82 Series Subchronic Toxicity

82-1 Subchronic Oral

No acceptable 90-day feeding study either in the rat or in the dog is available for benefin. This data requirement is waived based on the requirement for chronic studies in two species.

82-2 Suchronic Dermal (21-day)

No acceptable subchronic study is available for benefin. A study is required.

82-3 Subchronic Dermal (90-day)

No 90-day dermal study is available for benefin. However, the study is not required because the existing acceptable end-uses (present use pattern) should not result in repeated skin contact for extended period.

82-4 Subchronic Inhalation

No subchronic inhalation study is available for benefin. A subchronic inhalation (90-day) study will be required if residues occur in dried tobacco.

82-5 Subchronic Neurotoxicity

No data are available on the subchronic neurotoxicity of benefin. Since an acute neurotxicity study is not required and there is no evidence of neurotoxicity in mammalian species, this study is not required.

007158

83 Series Chronic Toxicity

83-1 Chronic Toxicity

Available data are insufficient to satisfy the data requirements for chronic oral toxicity of benefin (MRID 00037675; 00037678). Chronic toxicity studies are required in two species (rodent and nonrodent).

83-2 Oncogenicity

Available data are insufficient to satisfy the data requirements for oncogenicity of benefin (MRID 0037675; 40301901; 41537401). Oncogenicity stduies in the rat and the mouse are required.

83-3 Teratogenicity

Available data are sufficient to satisfy the data requirements for teratogenicity of benefin in the rat only.

In the rat teratology study (MRID 00147535; 40128001; 40410000; 40410001), pregnant rats were fed 0, 50, 225, 475, and 1000 mg/kg/day of benefin on gestation days 6 through 15. There was no evidence of developmental toxicity observed in any of the dosed groups. However, maternal body weight was decreased at 475 mg/kg/day dose group. The developmental toxicity NOEL was established at 1000 mg/kg/day (HDT). The maternal toxicity NOEL was determined to be 225 mg/kg/day.

No acceptable rabbit teratology study is available for benefin. A rabbit teratology study is required.

83-4 Reproduction

Available data are insufficient to satisfy the data requirements for reproductive toxicity of benefin (MRID 00037676). A study is required.

84 Series Mutagenicity Testing

Sufficient data are available to satisfy the data requirements for mutagenicity of benefin in the categories of gene mutation and the other genetic effects (DNA).

In the Salmonella/Mammalian-Microsome Mutagenicity Test (MRID 00160863), benefin did not demonstrate mutagenic activity against the Salmonella typhimurium strains TA1535, TA1537, TA1538, TA100, and TA98 in the presence and absence of metabolic activation at the concentrations tested (i.e., activated condition: 25, 50, 100, 200, & 300 ug/plate; non-activated condition: 125, 250, 500, & 750 ug/plate).

In the gene mutation assay in cultured L5178 mouse lymphoma cells, benefin was nonmutagenic in this assay in the presence of metabolic activation at the concentrations tested (i.e., 0.5 through 100 ug/ml). The results obtained under the non-activated condition are incomplete at the present time. However, the study under the non-activated condition can be upgraded upon receipt of additional data (MRID 160866). A new study is not required.

In the rat hepatocyte unscheduled DNA synthesis, benefin was tested from 50 to 1000 ug/ml. Benefin did not cause DNA damage and inducible repair in this assay at the concentrations tested (MRID 00160865).

Available chromosomal aberration study with benefin (MRID 00160864) is unacceptable. A chromosomal aberration study is required.

85 Series Special Studies

85-1 Metabolism

No metabolism study is available for benefin. A study is required.

85-2 Domestic Animal Safety

Studies not required at this time.

85-3 Dermal Absorption

Studies not required at this time.

C. Toxicology Data Gaps and Data Requirements

Technical benefin is registered for uses on non-food crops and may be applied to terrestrial food such as alfafa, clover, lettuce, and peanuts. The following studies are required for these registered uses (i.e. 158.135 Toxicology Data Requirements):

1. Data Requirements:

81-1 Acute Oral Toxicity
81-2 Acute Dermal Toxicity
81-3 Acute Inhalation Toxicity
81-4 Primary Eye Irritation
81-5 Primary Dermal Irritation
81-6 Dermal Sensitization

82-1 Subchronic Oral Toxicity in Two Species
82-2 Subchronic Dermal (21-day)
82-3 Subchronic Dermal (90-day)
82-4 Subchronic Inhalation

83-1 Chronic Toxicity in Two Species
83-2 Oncogenicity in Two Species
83-3 Teratogenicity in Two Species
83-4 Reproduction and Fertility Effects

84-2 Mutagenicity

85-1 Metabolism

2. Data Gaps

Based on this assessment of the toxicology data base, the following guideline toxicology studies have been identified as data gaps and are required:

81-2 Acute Dermal Toxicity
81-4 Primary Eye Irritation
81-5 Primary Dermal Irritation

82-1 Subchronic Toxicity in Two Species *
82-2 Subchronic dermal (21-day)
82-4 Subchronic Inhalation
83-1 Chronic Toxicity in Two Species
83-2 Oncogenicity in Two Species
83-3 Teratogenicity in rabbit
83-4 Reproduction in Rat

84-2 Chromosomal Aberration

85-1 Metabolism

* This data requirement is waived based on the requirement for chronic studies in two

007158

D. ADI Reassessment

Temporary tolerances have not yet been established for residues of benefin in or on the terrestrial food crops such as alfafa, clover, lettuce, and penauts.

The Provisional Acceptable Daily Intake (PADI) for benefin is based on a rat teratology study (MRID 00147535; 40128001; 40410001) with a no-observed-effect level of 225 mg/kg/day for maternal toxicity. Utilizing a safety factor of 3000 , the PADI was calculated to be 0.075 mg/kg/day. Since there are no other toxicology data available to support the PADI (PADI of 0.075 mg/kg/day), the proposed PADI for benefin is submitted to Health Effects Division ADI Committee for verification.

E. Toxicological Issues

There were no toxicological issues at this time. The toxicological data gaps must be filled for a complete evaluation.

TABLE A
GENERIC DATA REQUIREMENTS FOR BENEFIN

:a Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/
.135 Toxicology					
te Testing:					
1 - Acute Oral Toxicity - Rat	TGAI	AB	Yes	00024255	No
2 - Acute Dermal Toxicity - Rabbit	TGAI	AB	No		Yes
3 - Acute Inhalation Toxicity - Rat	TGAI	AB	Yes	00024275	No
4 - Primary Eye Irritation - Rabbit	TGAI	AB	No		Yes
- Primary Dermal Irritation - Rabbit	TGAI	AB	No		Yes
- Dermal Sensitization - Guinea pig	TGAI	AB	Yes	00144283	No
- Acute Delayed Neurotoxicity - Hen	TGAI	AB	No		No 4/

007158

TABLE A
GENERIC DATA REQUIREMENTS FOR BENEFIN

a Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/
.135 Toxicology (con'd)					
Chronic Testing:					
1 - 90-day Feeding - Rodent	TGAI	AB	No	00024651	No 5/
Nonrodent	TGAI	AB	No	00024652	No 5/
! - 21-day Dermal - Rabbit	TGAI	AB	No	00160868	Yes
- 90-day Dermal - Rabbit	TGAI	AB	No		No 6/
- 90-day Inhalation - Rat	TGAI	AB	No		Yes 7/
- 90-day Neurotoxicity - Hen	TGAI	AB	No		No 4/

TABLE A
GENERIC DATA REQUIREMENTS FOR BENEFIN

Data Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/
158.135 Toxicology (cont'd)					
Chronic Testing:					
83-1 - Chronic Toxicity - Rat	TGAI	AB	No		Yes
Nonrodent (dog)	TGAI	AB	No		Yes
83-2 - Oncogenicity - Rat	TGAI	AB	No		Yes
Mouse	TGAI	AB	No		Yes
83-2 - Teratogenicity - Rat	TGAI	AB	Yes	00147535 40128001 40410001	No
Rabbit	TGAI	AB	No		Yes
83-4 - Reproduction - Rat	TGAI	AB	No		Yes

TABLE A
GENERIC DATA REQUIREMENTS FOR BENEFIN

Data Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/
158.135 Toxicology (cont'd)					
Mutagenicity Testing:					
84-2 - Gene Mutation Bacteria	TGAI	AB	Yes	00160863	No
Mammalian Cells (L5178Y)	TGAI	AB	No	00160866	No 9/
84-2 - Chromosomal Aberration	TGAI	AB	No		Yes
84-2 - Other Genetic Effects DNA	TGAI	AB	Yes	00160865	No
Special Testing:					
85-1 - General Metabolism	PAIRA	A	No		Yes
85-2 - Domestic Animal Safety	Choice	HI	No		No 8/
85-3 - Dermal Absorption	Choice	HI	No		No 8/

1/ Composition: Material to be tested is Technical Grade Active Ingredients; PAIRA = Purified Active Ingredient Radiolabelled;
2/ The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Nonfood; C = Aquatic, Food Crop; D = Aquatic, Nonfood; E = Greenhouse, Food Crop; F = Greenhouse, Nonfood; G = Forestery; H = Domestic Outdoor; I = Indoor; IP = Industrial Preservative.
3/ Unless otherwise specified data must be submitted no later than six months after publication of the standard; 82-1 - 12 months; 82-2 - 7 months; 82-4 - 12 months; 83-2 - 42 months; 83-3 - 12 months; 84-2 - 10 months; 85-1 - 14 months.
8/ Because benefin is not an orhanophosphate, a neurotoxicity study is not required on benefin.
9/ This data requirement is waived because chronic studies are required in two species.
8/ This study is not required because the existing acceptable end-uses should not result in repeated human skin contact for extended period.
8/ A subchronic inhalation study will be required if residues occur in dried tobacco.
8/ These studies are not required under the present use conditions.
8/ The study can be upgraded to acceptable upon receipt of incomplete data.

007158

G. Bibliography

00024255 Worth, H.M. et al. (1965) Toxicological studies with N-Butyl-N-Ethyl-Alpha, Alpha, Alpha-Trifluoro-2, 6-Dinitro-P-Toluidine, Benefin. Received 7/16/65 under 1471-55.

00024275 Worth, H.M. (1964) Toxicity Studies on Mice, Dogs, Rats, and Rabbits. Received 10/1/65 under 1471-55.

00024651 Worth, H.M. et al. (1966) Subacute toxicity of benefin to rats. Received 3/15/66 under unknown admin. no.

00024652 Worth, H.M. et al. (1966) Subacute toxicity of benefin to dogs. Received 3/15/66 under unknown admin. no.

00037675 Gibson, W.R. et al. (1973) A study of the effect on rats from ingestion of benefin for two years. Received 8/5/76 under 1471-92.

00037676 Adams, E.R. (1973) A multigeneration rat reproduction study with benefin. Received 8/5/76 under 1471-92.

00037677 Markam, J.K. et al. (1973) A teratology study on benefin in the rabbit. Received 8/5/76 under 1471-92.

00037678 Gibson, W.R. (1973) A two year toxicity study of benefin administered orally to beagles. Received 8/5/76 under 1471-92.

00144283 Mattingly, C. (1984) A guinea pig snesitization study of benefin, compound 54521. Received under unknown admin. no.

00160863 Rexroat, M. (1985) The effect of benefin (EL-100, compound 54521) on the induction of reverse mutations in Salmonella typhimurium.

00160864 Neal, S. (1985) The effect of benefin (EL-110, compound 54521) on the in vivo induction of sister chromatid exchange in bone marrow of Chinese hamsters.

00160865 Hill, L. (1985) The Effect of benefin (EL-110, compound 54521) on the induction of DNA repair synthesis in primary cultures of adult rat hepatocytes.

00160866 Bewsey, B. (1985) The effect of benefin on the induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells.

089159

00160868 Brown, G. (1986) Subchronic (21-day) dermal toxicity study in New Zealand white rabbits with technical benefin.

40128001 Elanco Products Co. (1987) Submission of corrected rat teratology data in support of application for registration of benefin.

40301900 Koenig, G. et al. (1987) Gross pathology findings in a two year oncogenic mouse study with benefin. Interim report.

40410000 Elanco Products Co. (1987) Submission of supplemental data for benefin teratology study.

40410001 Byrd, R. (1987) Response to the Envrionmental Protection Agency review of a rat teratology study with benefin.

40537401 Koenig, G. et al. (1987) Interim report of histopathologic findings in a two-year oncogenic mouse study with benefin (EL-110, compound 54521).

40569101 Koenig, G. (1987) Benefin data call-in chronic toxicology data from a two-year oncogenic mouse study with benefin to support food crop use.

Tox Chem No. 130

File Last Updated _____ Current Date 11/15/88

Benefin

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Category	Core Grade Doc. No.
Acute Oral LD_{50} - rat; Lilly Tox. Lab.; 7/1965	Benefin 54521	MRID 00024255	LD_{50} > 10 g/kg	IV	Minimum 003481
Acute Oral LD_{50} - mouse; Lilly Tox. Lab.; 7/1965	Benefin 54521	MRID 00024255	LD_{50} > 5 g/kg	IV	Minimum 003481
Acute Inhalation LC_{50}-rat; Lilly Tox. Lab.; 7/24/64	Benefin 54521	MRID 00024275	LC_{50} > 1.33 mg/L	III	Minimum 003481
Dermal Sensitization - guinea pig; Lilly Res. Lab.; GO1184; 3/14/84	Benefin 54521 98.2% Purity	MRID 00144283	Non-sensitizer (female only) Level tested: 5% in ethanol		Guideline 006287
90-day Feeding - rat; Eli Lilly Labs.; RO524; 3/15/66	Benefin 54521	MRID 00024651	NOEL = 5000 ppm LEL = 10000 ppm (retarded growth and inclusion bodies in hepatic cells) Levels tested: 1250, 2500, 5000, 10000, & 20000 ppm		Supplementary 003482
90-day Feeding - dog; Eli Lilly Labs.; D-96-64; 3/15/66	Benefin 54521	MRID 00024652	NOEL = 500 ppm LEL = 2000 ppm (weight loss, decreased food consumption) Levels tested: 500, 2000, & 8000 ppm		Supplementary 003482
21-day Dermal Toxicity - rabbit; Lilly Res. Labs.; RO2185; 1/15/86	Benefin 54521 (Lot No. 231EF4; 97.3% Purity)	MRID 00160868	NOEL = 100 mg/kg Dose levels tested: 100, 325, & 1000 mg/kg (incomplete biochemstry determination)		Supplementary
2-year Feeding -dog; Lilly Res. Labs.; 92-65; 8/5/76	Tech. Benefin 95.6% Purity	MRID 00037678	NOEL = not determined Dose levels tested: 5, 25, & 125 mg/kg (incomplete study)		Supplementary

Tox Chem No. [illegible] Benefin — File Last Updated _____ — Current Date 11/15/88

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Category	Core Grade Doc. No.
2-year Feeding - mouse; Eli Lilly Labs.; MO2785 & MO2985;	Benefin 54521	MRID 40301900 40537401 40569101	Interim report: Significant, increased incid. of liver nodules in the females of highest dose group was reported. Levels tested: 0, 6, 36, & 180 mg/kg in B6C3F mice.		Supplementary 006336
2-year Feeding and Oncogenicity - rat; Lilly Res. Labs.; R-0295; 8/5/76	Tech. Benefin (Lot No. X-11424, 95.6% Purity)	MRID 00037675	Systemic NOEL = not determined Oncogenic NOEL = not determined (incomplete study) Levels tested: 0, 0.1, & 0.5% benefin in the diet		Supplementary
Teratology - rabbit; Lilly Res. Labs.; B-O-7-68; 4/8/68	Tech. Benefin (Lot No. 858929, 96.8% Purity)	MRID 00037677	Maternal NOEL = not determined Developmental NOEL = not determined Levels tested: 0, 50, & 100 mg/kg		Supplementary
Teratology - rat; Hazleton Labs.; 6180-101; 6/18/85	Benefin 54521 (Lot No. 231FF4, 97.3% Purity)	MRID 00147535	Maternal NOEL = 225 mg/kg Maternal LEL = 475 mg/kg (decre. body wt.) Developmental NOEL = additonal data required Levels tested: 0, 50, 225, 475, & 1000 mg/kg		Supplementary 005443
Teratology - rat; Hazleton Labs.; 6180--101; 113/87	Benefin 54521 (Lot No. 231FF4)	MRID 40410001 40410000 40128001	Developmental NOEL = 1000 mg/kg Levels tested: 0, 50, 225, 475, & 1000 mg/kg		Guideline 006693
3-Generation Reproduction - rat; Lilly Res. Labs.; R-0305	Tech. Benefin (Lot No. X-11424, 95.6% Purity)	MRID 00037676	Reproductive Toxicity NOEL = not determined Parental Toxicity NOEL = not determined Levels tested: 0.1 & 0.5% of benefin		Supplementary

00158

18

Tox Chem No. 130 Benefin | File Last Updated _____ | Current Date 11/15/88

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Category	Core Grade Doc. No.
Mutagenicity - Ames test; Lilly Res. Labs.; 850624AMS2598 & 850708 AMS2598	Tech. Benefin Batch No.231EF4 97.3% Purity	MRID 160863	Nonmutagenic to TA1535, TA1537, TA1538, TA100 & TA98 strains of S. typhimurium Levels tested: 25, 50, 100, 200, & 300 ug/plate with S9 mix; 125, 250, 500, & 750 ug/plate without S9 mix		Acceptable
Mutagenicity - UDS in rat hepatocytes; Lilly Res. Labs.; 850716UDS-2598 &850723UDS2598	Tech. Benefin Batch No. 231FF4 97.3% Purity	MRID 00160865	Benefin did not cause DNA damage and inducible renair in the rat hepatocytes Levels tested: 0.5, 1, 5, 10, 50, 100, 500, & 1000 ug/ml		Acceptable
Mutagenicity - Gene mutation in L5178Y Cells; Lilly Res. Labs.; 8501612MLA2598 & 850724-MLA2598; 10/29/85	Tech. Benefin 54521 (Batch No. 231EF4, 97.3% Purity)	MRID 00160866	Nonmutagenic in L5178Y cells either in the presence or absence of S9 mix (Deficiency: inadequate high dose) Levels tested: 5, 10, 15, 20, 25, 30, 35, & 40 ug/ml without S9 mix; 0.5, 1, 10, 20, 40, 60, 80, & 100 ug/ml with S9 mix		Unacceptable
Mutagenicity - SCE in bone marrow of Chinese hamster; Eli Lilly Res. Labs.; 850722SCE2598; 11/7/85	Tech. Benefin 54521 (Batch No. 231EF4, 97.3% Purity)	MRID 00160864	Benefin did not induce sister chromatid exchange in bone marrow of Chinese hamsters Levels tested: 200, 300, 400, & 500 mg/kg (incomplete study)		Unacceptable

81-1 - Rat - Acute Oral Toxicity

Reviewed by: John H.S. Chen JHS Chen 11/2/87
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui
Section I, Toxicology Branch II (TS-769C) Quang Q Bui

007158

DATA EVALUATION REPORT

Study Type: Acute Rat Oral Toxicity

Tox. Chem. No.: 130

MRID No.: 24255

EPA File Symbol:

Test Material: Technical Benefin (EL-100, 54521)

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

Study Number(s):

Sponsor: Division of Eli Lilly and Company, Indianapolis, Ind.

Testing Facility: Lilly Toxicology Laboratory, Greenfield, Ind.

Title of Report: Toxicological Studies with N-butyl-N-ethyl- Alpha, Alpha, Alpha-trifluoro-2, 6-dinitro-p-toludine, Benefin

Author(s): H.M. Worth and R.C. Anderson

Report Issued: July, 1965

Conclusions:

$LD_{50} > 10$ g/kg (male and female)

Level tested: 10 g/kg

Classification of Data: Core Minimum

The following is a copy of the original review of this study as prepared by Edwin Budd dated 7/19/76

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SUBJECT: Consideration of Additional Toxicity Data Supplied by Ed Derrenbacher on 8590-UTR, 961-GGN and 1491-GEL.

DATE: 7/16/76

FROM: Ed Budd / Toxicology Branch

007158

TO: Robert Taylor / PM #25

Additional toxicity data (accession number 094594, 7F0514, 2/21/67) was submitted to me for evaluation (by Ed Derrenbacher) regarding the new registration of EPA Reg. No's 8590-UTR, 961-GGN and 1491-GEL all of which contain 0.84% to 1.00% Balan® (benefin) as the sole active ingredient in a fertilizer mixture. The test material used for all the toxicity tests in the additional data was a "mixture in the 1:2 ratio" of Balan® and Vernam™. This test material is not acceptable. Each applicant must submit acute toxicity data on the particular and specific formulation he intends to register.

In addition, note those changes that have been made in the review of July 7, 1976 for Greenlawn Plus 21-5-7 (EPA No. 8590-UTR) and Lebanon County Club 12-4-8 Fertilizer (EPA No. 961-GGN).

Ed Budd

William Dissen 7/19/76

EPA Form 1320-6 (Rev. 6-72)

[redacted]

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY Ebdoep 7/7/76

SUBJECT: Application for new registration
Greenlawn Plus 21-5-7 007148 DATE: July 7, 1976
E.P.A. No. 8590-UTR

FROM: Ed Budd / Toxicology Branch

TO: Robert Taylor / PM #25

Conclusion: Applicant must submit acute toxicity data on the formulated product & other data

Discussion: The applicant has applied for new registration of a herbacide formulation containing: 1.00 % Balan™ (benefin) as the active ingredient. His method of support is 1B and the following accession number has been referenced for toxicology data on the technical product: 090606. The following is an evaluation of the pertinent acute toxicology data contained in this jacket. (all tests on technical benefin).

Acute oral LD_{50} (adult rats) – data acceptable
LD_{50} > 10,000 mg/kg

Acute oral LD_{50} (mice) — data acceptable
LD_{50} > 5,000 mg/kg

Acute inhalation LC_{50} (rats) – data acceptable.
LC_{50} > 1.33 mg/liter

Acute dermal LD_{50} (rabbits) — data acceptable
(LD_{50} > 100 mg/kg)

Primary eye irritation (rabbits) – data not acceptable

Primary skin irritation (rabbits) – data not acceptable

~~Although the eye and skin irritation tests for technical benefin are not acceptable, it is felt that if the applicant submits satisfactory data on the formulated product (see other side of this sheet), the potential hazard to human safety will be adequately defined for the subject product~~

EPA Form 1320-6 (Rev. 6-72)

OIFR [redacted]

In addition to the toxicity data on the technical product which has been referenced, the applicant must submit acute toxicity data on the formulated product. The following tests will be required:

Acute oral LD_{50} (rats)
Acute dermal LD_{50} (rabbits)
Primary eye irritation (rabbits)
Primary skin irritation (rabbits).

Note – Ask applicant to explain how he calculated the percent of active ingredient (benfin) in the formulated product (given as 1.00 % on the label).

Additional toxicity data that will be required for registration of the subject product are:

acute dermal LD_{50} — technical
primary eye irritation — technical
primary skin irritation — technical
teratology study — technical

81-3 - Rat - Acute Inhalation Toxicity 007158

Reviewed by: John H.S. Chen 11/2/88
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: Acute Rat Inhalation Toxicity Tox. Chem. No.: 130

MRID No.: 24275 EPA File Symbol:

Test Material: Technical Benefin (EL-110, 54521)

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

Study Number(s):

Sponsor: Division of Eli Lilly and Company, Indianapolis, Ind.

Testing Facility: Lilly Toxicology Laboratory, Greenfield, Ind.

Title of Report: Toxicity Studies on Mice, Dogs, Rats and Rabbits

Author(s): H.M. Worth

Report Issued: July 24, 1964

Conclusions:

LC_{50} > 1.33 mg/L

Level tested: 1.33 mg/L

Classification of Data: Core Minimum

The following is a copy of the original review of this study as prepared by Edwin Budd dated 7/19/76

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SUBJECT: Consideration of Additional Toxicity Data Supplied by Ed Perrenbacher on 8590-UTR, 961-GGN and 2491-GEL.

DATE: 7/16/76

007158

FROM: Ed Budd / Toxicology Branch

TO: Robert Taylor / PM # 25

Additional toxicity data (accession number 094594, 7F0514, 2/21/67) was submitted to me for evaluation (by Ed Perrenbacher) regarding the new registration of EPA Reg. No's 8590-UTR, 961-GGN and 2491-GEL all of which contain 0.84 % to 1.00 % Balan® (benefin) as the sole active ingredient in a fertilizer mixture. The test material used for all the toxicity tests in the additional data was a "mixture in the 1:2 ratio" of Balan® and Vernam™. This test material is not acceptable. Each applicant must submit acute toxicity data on the particular and specific formulation he intends to register.

In addition, note those changes that have been made in the review of July 7, 1976 for Greenlawn Plus 21-5-7 (EPA No. 8590-UTR) and Lebanon County Club 12-4-8 Fertilizer (EPA No. 961-GGN).

Ed Budd

William Dixon 7/19/76

EPA Form 1320-6 (Rev. 6-72)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Re for OEP 7/71

SUBJECT: Application for new registration 007158
Lebanon Country Club 12-4-8 Fertilizer DATE: July 7, 1976
E.P.A. No. 961-GGN

FROM: Ed Budd / Toxicology Branch

TO: Robert Taylor / P.M. #25

Conclusion: Applicant must submit acute toxicity data on the formulated product and other data

Discussion: The applicant has applied for new registration of a herbicide formulation containing 0.84% Balan™ (benefin) as the active ingredient. His method of support is 2B and the following accession numbers have been referenced for toxicology data on the technical product: 090606, 090607, 100426, 100663, 114700 and 121005. The following is an evaluation of the pertinent acute toxicology data contained in these packets. (All tests on technical benefin).

Acute Oral LD_{50} (adult rats) – data acceptable
LD_{50} > 10,000 mg/kg

Acute Oral LD_{50} (mice) — data acceptable
LD_{50} > 5,000 mg/kg

Acute inhalation LC_{50} (rats) — data acceptable
LC_{50} > 1.33 mg/liter

Acute Dermal LD_{50} (rabbits) – data acceptable
LD_{50} > 200 mg/kg

Primary Eye Irritation (rabbits) – data not acceptable

Primary Skin Irritation (rabbits) – data not acceptable

~~Although the eye and skin irritation tests for technical benefin are not acceptable, it is felt that if the applicant submits satisfactory data on the formulated product (see other side of this sheet), the potential~~

EPA Form 1320-6 (Rev. 6-72)

OVER

~~Hazard to human safety will be adequately defined for the subject product.~~

In addition to the toxicity data on the technical product which has been referenced, the applicant must submit acute toxicity data on the formulated product. The following tests will be required:

acute oral LD_{50} (rats)
acute dermal LD_{50} (rabbits)
primary eye irritation (rabbits)
primary skin irritation (rabbits).

Note — Ask applicant to explain how he calculated the percent of active ingredient (benefin) in the formulated product (given as 0.84 % on the label).

Additional toxicity data that will be required for registration of the subject product are:

acute dermal LD_{50} ——— technical
primary eye irritation — technical
primary skin irritation — technical
teratology study ——— technical

82-1 - Rat - Subchronic Toxicity

007158

Reviewed by: John H.S. Chen John H.S. Chen 4/2/88
Section I, Toxicology Branch II (TS-769C);
Secondary reviewer: Quang Q. Bui Quang Q. Bui
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: 90-day Rat Feeding

Tox. Chem. No.: 130

MRID No.: 24651

EPA File Symbol:

Test Material: Technical Benefin (Compound 54521)

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

Study Number(s): R-0524

Sponsor: Division of Eli Lilly and Company, Indianapolis, Ind.

Testing Facility: Lilly Toxicology Laboratories, Greenfield, Ind.

Title of Report: Subacute Toxicity of Benefin to Rats

Author(s): H.M. Worth, E.C. Pierce, P.N. Harris, W.J. Griffing, and R.G. Anderson

Report Issued: March 15, 1966

Conclusions:

NOEL = 5000 ppm
LEL = 10000 ppm (retarded growth and inclusion bodies in hepatic cells)

Levels tested: 1250, 2500, 5000, 10000, and 20000 ppm

Deficiencies: no data for clinical observations, body weight, food consumption, ophthalmological examination, clinical biochemistry determination and a complete necropsy and histopathologic examinations in this study.

Classification of Data: Supplementary without the possibility of upgrading

The following is a copy of reviewed study (Document No. 003482) as prepared by P.D. Baron dated 3/20/68.

January, 1966:

Subacute Toxicity - Rats:

Harlan strain weanlings, 10 males and 10 females per group, were fed a benefin-containing mash diet on the following levels for 92 days: 0; 1,250; 2,500; 5,000; 10,000; and 20,000 ppm.

Records kept weekly on body weight and food consumption. Complete blood counts and miscroscopic pathology done at conclusion.

Results:

1) Little mortality - no correlation with benefin.

2) No significant differences in food consumption.

3) Feed efficiency (gms weight gained/100 gms feed consumed) decreased on 2 highest levels in females and showed a gradual decrease in males with increasing dose.

4) In both sexes there were trends for hematocrit, hemoglobin, and RBCs to be lower as dose increased - statistically significant in females.

5) Mean organ/body weight ratios:
In males - significantly larger ratios for kidney and liver on 2500 ppm and above levels; and for thyroid and adrenal on two highest levels.
In females - liver ratios were significantly higher on 2500 ppm and above levels; kidney and thyroid values higher on 2 highest levels; body weights significantly lower on 2 highest levels.

6) Necropsy:
Fatty metamorphosis of the liver was found on all rats - somewhat more in treated than controls. Hyaline globules in renal convoluted tubules - in all rats but the control females - were found conspicuously more in treated than controls; especially in males. Fatty degeneration of kidneys was generally found - especially high in both female and male controls! A solitary kidney abscess was found on one 20,000 ppm female, and cystitis on one 20,000 ppm male. There was no interference with spermatogenesis. A strictly chemical effect was the "inclusions" found in liver cells of one 10,000 ppm male, and 4 males and 1 female on 20,000 ppm. This was found to be carbohydrate in nature.

007158

General Summary

Benefin Subacute Toxicity

Rat Study - R0524

Duration: Three months

Number started: Ten males, ten females, on each level

Levels ppm:	1250	2500	5000	10,000	20,000	Controls
Mortality:	M-0	0	0	0	1	4
	F-1	2	2	0	0	1

Results: There was retarded growth in the 10,000 and 20,000 ppm groups. An apparent dose-related trend toward a depression of the red cell components was observed. No findings from gross or microscopic pathology examination were attributed to the Benefin treatment excepting inclusion bodies in the liver cell cytoplasm of one male at 10,000 ppm and four males and one female fed 20,000 ppm.

Comment: The red cell component depression was within the range of normal values for this laboratory and at the 1250 ppm level was not of consequence.

Safe level, ppm: 1250

30

007158

82-1 - Dog - Subchronic Toxicity

Reviewed by: John H.S. Chen John H.S. Chen 11/2/88
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui Quang Q. Bui
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: 90-day Dog Feeding

Tox. Chem. No.: 130

MRID No.: 24652

EPA File Symbol:

Test Material: Technical Benefin (Compound 54521)

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

Study Number(s): D96-64

Sponsor: Division of Eli Lilly and Company, Indianapolis, Ind.

Testing Facility: Lilly Toxicology Laboratories, Greenfield, Ind.

Title of Report: Subacute Toxicity of Benefin to Dogs

Author(s): H.M. Woth, E.G. Pierce, P.N. Harris, and R.G. Anderson

Report Issued: March 15, 1966

Conclusions:

NOEL = 500 ppm
LEL = 2000 ppm (weight loss, decreased food consumption)

Levels tested: 500, 2000, and 8000 ppm

Deficiencies: inadequate numbers of animals of each sex/dose used, no statistical analysis of data, no data for clinical biochemistry determinations and histopathologic findings in this study

Classification of Data: Supplementary without the possibility of upgrading

The following is a copy of reviewed study (Document No. 003482) as prepared by P.D. Baron dated 3/20/68

Subacute toxicity - Dogs:

Note: Age, and previous history of animals, not given.

Mongrel dogs, 3 males and 3 females per group, were dosed by capsule daily at the following estimated levels for 3 months: 0; 500; 2000; 8000 ppm. Blood and urine studies were done before testing and throughout test duration. Body weight recorded weekly.

Results:

1) Occasional vomiting of one dog on 500 ppm level.

2) Weight loss in 3 dogs and vomiting in 2 - on 2000 ppm level.

3) Weight loss noted on all animals in 8000 ppm level.

4) Particles of unchanged benefin noted in feces of all treated animals.

5) Marked reduction in food consumption of all animals on 2000 and 8000 ppm levels. (Extent of cachexia in 4 of these at 8000 ppm resulted in early sacrificing of animals.)

6) Mean organ-body weight ratios showed a marked increase at 8000 ppm level for liver, kidney, heart, and adrenal glands.

7) At 2000 and 8000 ppm levels, depression in blood chemistry levels occurred.

8) At 8000 ppm level, also found enzyme changes.

9) At 8000 ppm, inhibition of spermatogenesis occurred in one dog, and emaciation in 3 others.

10) Benefin found in all fat samples of treated dogs.

007158

GENERAL SUMMARY

Section C - Benefin Subacute Toxicity
Dog Study D96-64

Duration:	Three months
Number started:	Three males, three females on each level.
Levels ppm:	500, 2000, 8000, and control
Route and frequency:	PO daily
Result:	All survived and were normal in all respects at 500 ppm. At 2000 ppm all animals survived, half lost weight. In several there was a depression in red cell components. At 8000 ppm, all lost weight and showed depression in red cell components. Four became cachectic and were killed for necropsy after doses 49, 50, 50, and 85 respectively. Effects found in hematology and pathology were attributed to inanition.
No effect level:	500 ppm

82-2 - Rabbit - 21-Day Dermal Toxicity 007158

Reviewed by: John H.S. Chen 4/30/85
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: 21-Day Dermal Toxicity Tox. Chem. No.: 130

MRID No.: 160868 EPA File Symbol:

Test Material: Technical Benfin (EL-110, Compound 54521; Lot No. 231EF4; 97.3% Purity)

Synonyms/CAS No.:

Study Number(s): 302185

Sponsor: Division of Eli Lilly and Company, Greenfield, Indiana

Testing Facility: Lilly Research Laboratories, Greenfield, Indiana

Title of Report: Subchronic (21-day) Dermal Toxicity Study in New Zealand White Rabbits with Technical Benefin (EL-110, 54521)

Author(s): G.R. Koenig, W.H. Jordan, and G.E. Brown

Report Issued: January 15, 1986

Conclusions:

Benefin technical caused dose-related persistent dermal irritation, and secondary hematologic effects in the subchronic (21-day) rabbit dermal toxicity study at the dose levels tested.

Dose levels tested: 100, 325, and 1000 mg/kg

NOEL for Repeated Dose Dermal Toxicity = <100 mg/kg

Classification of Data: Supplementary

(Deficiencies: incomplete biochemistry determinations and no clear demonstration of A NOEL in this study)

Title of Study: Subchronic (21-day) Dermal Toxicity Study in New Zealand White Rabbits with Technical Benefin (EL-110, 54521)
Study No. B02185, January 15, 1986

I. Materials and Treatment Schedule:

1. Test Animals

New Zealand white rabbits of both sexes (12 to 16 weeks of age; 2.87 ± 0.31 kg for males and 2.79 ± 0.28 kg for females) were used in this study. The animals were acclimated to laboratory condition for at least two weeks prior to dosing, and were housed individually in stainless steel cages with wire mesh floors under an environmentally controlled room. Animals were provided with a constant supply of Purina certified high fiber rabbit chow No. 5325 and Greenfield city water was continuously available.

2. Test Material

Technical benefin (EL-110, Compound 54521; Lot No. 231EF4; 97.3% Purity)

3. Treatment Schedule

Prior to dosing, each rabbit was prepared by clipping the skin of the back free of hair. Following application of the test material to a gauze pad, the torso of each rabbit was wrapped with an elastic bandage. Dressings were removed after six hours and the application sites were rinsed with tap water and dried. The control animals received the same application procedure but without test material. The test material was administered topically to the skin of rabbits for 21 days. Following were the animal groups according to the treatment schedule:

Group	No. of Rabbits Male	Female	Daily Dose (mg/kg)	No. of Dose Days
Control	5	5	0	21
Benefin-Low	5	5	100	21
" -Mid	5	5	325	21
" -High	5	5	1000	21

II. Reported Methods and Results:

1. Observations - The physical conditions of animals were inspected daily for signs of toxicity and mortality.

   Results: All rabbits survived until study termination except one male in the low-dose (100 mg/kg) group, which died after 7 days on test with pneumonia. Results of physical and ophthalmic examinations conducted pretest and at study termination showed no treatment-related findings in this study.

2. Body weights were recorded at test initiation, then weekly thereafter for the 21-day study.

   Results: No differences were observed between the control and the dosed animals for mean body weight or mean body weight gain during the course of this study.

3. Food Consumption was recorded initially then weekly thereafter for the 21-day study.

   Results: There was a statistically significant decrease in food consumption in the high-dose (1000 mg/kg) males only (Table 2.1 attached).

4. Dermal Irritation was graded daily using an eight-point scale for erythema and edema according to the method of Draize (J. Pharmacol. and Exp. Therap. 82: 377-390, 1944).

   Results: There were dose-related persistent dermal changes (erythema and edema) observed in the dose groups. Dermal irritation was similar for all benefin treatment group but the area of involment was dose dependent. Such dermal irritation was characterized by slight to severe erythema and edema, which persisted from study initiation through termination. In rabbits of all dose groups, moderate to severe irritation was also accompanied by cracked and bleeding skin (Figure 1 attached).

5. Blood was collected prior to test initiation and near termination of the study.

   (a) Hematology - Parameters (X) were examined

   (X) Erythrocyte Count (RBC)
   (X) Hemoglobin (HGB)
   (X) Packed Cell Volume (PCV)
   (X) Leukocyte Count (WBC)
   (X) Thrombocyte Count
   (X) Differential Leukocyte Count
   (X) Mean Corpuscular Volume (MCV)
   (X) Mean Corpuscular Hemoglobin (MCH)
   (X) Mean Corpuscular Hemoglobin Concentration (MCHC)
   (X) Erythrocyte Morphology

(a) Hematology - continued

Results: A statistically significant increase in the mean thrombocyte count was observed in the high-dose (1000 mg/kg) males and females. There was also a statistically significant increase in the mean basophilic count found in the high-dose males. No treatment-related effects on other hematology parameters were observed in this study (Table 4 attached).

(b) Clinical Chemistry - Parameters (X) were examined

(X) Alkaline Phosphatase (ALP)
(X) Alanine Transaminase (ALT)
(X) Glucose
(X) Blood Urea Nitrogen (BUN)
(X) Creatinine
(X) Total Bilirubin

Results: There was a statistically significant decrease in mean ALP value for male and female rabbits in the high-dose group (1000 mg/kg). No indication of treatment-related effects on other clinical chemistry parameters was observed in this study (Table 5 attached).

6. Sacrifice and Pathology:

At the termination of study, all of the surviving animals were sacrificed. These animals were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

(XX) Kidney
(XX) Liver
(XX) Heart
(X) Aorta
(X) Trached
(X) Lung
(X) Spleen
(X) Thymus
(X) Lymph Node
(X) Salivary Gland
(X) Pancreas
(X) Esophagus
(X) Stomach
(X) Duodenum
(X) Jejunum
(X) Ileum
(X) Colon
(X) Ovary
(X) Uterus
(XX) Adrenal
(XX) Thyroid
(X) Testis
(X) Prostate
(X) Skin
(X) Mammary Gland
(X) Skeletal Muscle
(X) Urinary Bladder
(X) Bone
(X) Bone Marrow
(X) Eye
(X) Cerebrum
(X) Cerebellum
(X) Brain Stem
(X) Pituitary
(XX) Ovary
(XX) Testes

(a) Organ Weight:

Results: The statistically significant decrease in absolute kidney weight was observed in males of the high dose group (1000 mg/kg). This decrease in kidney weight was not seen when kidney weight

(a) Organ Weight: continued

relative to body weight was compared. There was also a statistically significant increase in absolute thyroid weights observed in middle and high dose females (325 mg/kg and 1000 mg/kg). However, based on the histologic evaluation of the thyroid, no morphologic differences were detected between the thyroids of control and treated rabbits. There were no indications of treatment-related effects on other organ weights.

(b) Histopathology

The only lesions related to treatment were those observed in the skin at the application site as follows:

Skin	Dose (mg/kg)							
	0		100		325		1000	
	M	F	M	F	M	F	M	F
No. Examined:	5	5	4	5	5	5	5	5
Slight chronic active dermatitis	-	-	-	-	-	-	1	-
Minimal active dermatitis	-	-	-	1	-	-	1	1
Slight acute dermatitis	-	-	3	1	2	3	0	2
Moderate acute dermatitis	-	-	1	3	3	2	3	2

There were no other compound-related alterations found in any organ or tissue examined from the treatment groups in this study.

7. Statistical Evaluation:

The statistical method described by Dunnett (Biometrics 20: 482-491, 1964) was used in the analysis of difference (at each time point) between control and treated group means for which data are generally distributed normally (body weight, weight gain, hematology, clinical chemistry and organ weight data). The homogeneity of variances was tested by the method of Bartelett (Principle and Procedures of Statistics, pp. 347-349, 1960, McGraw-Hill, New York, N.Y.). All references to statistical significance in this report represent a "P" value <0.05.

III. Reviewer's Conclusions:

1. There were no toxic effects on clinical observation, mortality, or adverse effects on body weight, food consumption, ophthalmic examination and organ weight in the rabbits treated with technical benefin when administered topically to animals at doses up to 1000 mg/kg during this study.

2. Treatment-related adverse effects on hematology and clinical chemistry values were observed in the rabbits treated with technical benefin at 1000 mg/kg during the 21-day dermal toxicity study.

3. There were dose-related, persistent, dermal irritations (erythema and edema) observed in all the dose groups (i.e., 100, 325 and 1000 mg/kg). The evidence of dermal irritations in treated rabbits was further supported by the significantly increased incidences of minimal and acute dermatitis in the skin at the application site observed in the animals of same treated groups (histopathologic examinations).

4. However, the evaluation of suchronic (21-day) dermal toxicity study in rabbits with technical benefin cannot be accomplished due to the following reporting deficiencies:

   i. The clinical biochemistry determinations for calcium, phosphorous chloride, sodium and potassium in the blood samples of all animal groups were not not given in this report.

   ii. There is no clear demonstration of A NOEL in this study (NOEL =<100 mg/kg).

5. Since the submitted report is incomplete, the study is unacceptable in the present form.

Classification of Data: Supplementary

NOEL for Repeated Dose Dermal Toxicity = To be determined

BENEFIN TOX REVIEW 007158

Page_____ is not included in this copy.

Pages 39 through 48 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

83-1 - Mouse - Oncogenicity

Reviewed by: John H.S. Chen — John H.S. Chen 11/2/87
Section I, Toxicology Branch II (TS-769C);
Secondary reviewer: Quang Q. Bui
Section I, Toxicology Branch II (TS-769C) Quang Q. Bui

007158

DATA EVALUATION REPORT

Study Type: 2-year Oncogenic Mouse Study

Tox. Chem. No.: 130

MRID No.: 40301901 and 41537401

EPA File Symbol:

Test Material: Technical Benefin (EL-110, 54521)

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

Study Number(s): M02785, M02885, and M02985

Sponsor: Division of Eli Lilly and Company, Greenfield, Indiana

Testing Facility: Lilly Research Laboratories

Title of Report: Two-Year Oncogenic Mouse Study with Benefin

Author(s): G.R. Koenig and W.H. Jordan

Report Issued: August 7, 1987

Conclusions:

Interim Report: An increased incidence of gross liver nodules was reported for high dose females (dietary concentration of 0.15%)

Levels tested: 0, 0.005, 0.03, and 0.15% of benefin in the diet

Classification of Data: Supplementary

The following is a copy of this original review of this study as prepared by John H.S. Chen dated 9/21/87.

007158

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 2985. 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.

50

007158

83-1 - Dog - Chronic Toxicity

Reviewed by: John H.S. Chen John H.S. Chen 11/1/87
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui Quang Q. Bui 11/4/86
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: 2-Year Feeding study in Dogs Tox. Chem. No.: 130

MRID No.: 37678 EPA File Symbol:

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

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

Study Number(s): 92-65

Sponsor: Elanco Products Co., Indianapolis, Ind.

Testing Facility: Toxicology Division Lilly Research Laboratories

Title of Report: A Two Year Toxicity Study of Benefin Administered Orally to Beagles

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

Report Issued: August 5, 1976

Conclusions:

Systemic .OEL = To be determined

(because of incomplete study)

Levels tested: 5, 25, and 125 mg/kg

Classification of Data: Supplementary without the possibility of upgrading

007158

Title of Study: A Two Year Toxicity Study of Benefin Administered orally to Beagles. Study No. 92-65

I. Materials and Methods:

1. Test Material

Benefin (Lot No. X-11424; 95.6% Purity) in gelatin capsules

2. Animals

The study consisted of 32 healthy beagle dogs. Number of animals in each dose group was given below:

Dose Group mg/kg	No. of Animals male	female
0	5	3
5	4	4
25	4	4
125	4	4
Total	17	15

3. Statistical Analysis

The significance of difference of data between control and treated groups were not evaluated by the statistical analysis.

II. Reported Results:

1. Clinical Observations

All dogs were observed daily for mortality, moribundity, and clinical signs of physiologic and pharmacologic effects.

Results: Thirty dogs survived throughout the study. However, two dogs (No. 1984 and 3327) in the high dose group developed problems from mange and many of the treated animals had yellow mouths, skin, and sclera - the color of benefin. No other treatment-related changes were detected in test animals during the course of this study.

2. Body Weights

Individual body weights were recorded before the start of the treatment and then at the termination of study.

Results: No treatment-related effects on body weight gain was detected in animals treated with benefin.

II. Reported Results: continued

3. Food Consumption

Results: No result was reported for this study.

4. Ophthalmoscopy

Results: No result was reported for this study.

5. Hematology

Blood samples for hematological analysis were collected before the start of the treatment period and then periodically during the course of the study. The following parameters (X) were examined:

*(X) Hemoglobin (HGB)
*(X) Hematocrit (HCT)
*(X) Platelet count
(X) Sedimentation rate
*(X) Red and White cell counts
*(X) Differential counts
(X) Red blood cell morphology
*(X) Prothrombin time
(X) Whole blood clotting time

*Recommended by Subdivision F (October 1982) Guidelines

Results: consistent decrease in the mean values of RBC was observed in the 25 and 125 mg/kg dose groups throughout the study. The animals treated with 25 and 125 mg/kg had also an increase in platelet count values after the first three months of treatment. However, no statistical analysis of these data was given.

6. Clinical Chemistry

Blood samples for clinical chemistry determinations were collected before the start of the treatment period and then periodically during the course of the study. The following parameters (X) were examined:

Electrolytes	Enzymes	Other
*( ) Calcium	*(X) Serum glutamic-pyruvic transaminase	*(X) Glucose
*( ) Phosphorus	*( ) Serum glutamic-oxaloacetic transaminase	*(X) Blood urea nitrogen
*( ) Sodium	(X) Alkaline phosphatase	*( ) Total protein
*( ) Potassium		*( ) Albumin
*( ) Chloride		*( ) Creatinine
*( ) Magnesium		*( ) Cholesterol
		*( ) Bilirubin

*Recommended by Subdivision F (October 1982) Guidelines

6. Clinical Chemistry - continued

Results: There was an increase in the mean values of alkaline phosphatase in the 125 mg/kg dose group males and females when compared to that of the corresponding control males and females after three months of treatment in this study (Figure 16 attached). However, no changes in any of the other clinical chemistry determinations were observed. Complete results for the recommended parameters were not given in this study.

7. Urinalysis

Samples for urinalysis were carried out before the start of the treatment and then periodically during the course of the study. The following parameters (X) were examined:

*(X) Specific gravity
*(X) Glucose
*(X) Protein
*(X) PH
*(X) Occult blood
*( ) Ketone
*( ) Bilirubin

*Recommended by Subdivision F (October 1982) Guidelines

Results: No treatment-related effects were detected on the parameters tested in this study.

8. Sacrifice and Pathology:

All animals 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
*(X) Lung
*( ) Trachea
Digestive System
*(XX) Liver
*(X) Gall bladder
*(X) Pancreas
*(X) Stomach
*(X) Ileum
*(X) Jejunum
*(X) Colon
*( ) Rectum
*(X) Salivary gland

Urogenital
*(XX) Kidney
*(X) Uterus
*(XX) Ovaries
*(XX) Testes
*( ) Urinary bladder
Cardiovasc./Hemat.
*(XX) Heart
*(X) Lymph nodes
*( ) Aorta
*(X) Thymus
*(XX) Spleen
*(X) Bone marrow
Glandular
*(XX) Thyroid
*(XX) Adrenals
*( ) Parathyroid
*(X) Mammary gland

Neurologic
(X) Eyes
*( ) Pituitary
(X) Central nervous system
Other
*(X) Skeletal muscle
(X) Ear

*Recommended by Subdivision F (October 1982) Guidelines.

8. Sacrifice and Pathology - continued

(a) Organ Weights

Results: Slight increase in the mean values of relative liver weight was observed in the 125 mg/kg dose groups (males and females) when compared to that of the corresponding control groups (Table 2 attached). There were no other treatment-related effects on the organ weights examined.

(b) Gross Pathology

Results: Two dogs (No. 1984 and 3327) in the 125 mg/kg dose group were killed before the end of the experiment because of poor physical clinical condition. The cause of the deterioration of condition was not known. There were no macroscopical changes in the tissues of dogs which were considered to be treatment-related responses in this study.

(c) Microscopic Pathology - Selected histopathological changes

	Dietary Level mg/kg Male				Dietary Level mg/kg Female			
	0	5	25	125	0	5	25	125
No. Started	5	4	4	4	3	4	4	4
No. Evaluated	5	4	4	3	3	4	4	3
No. Killed before termination date	0	0	0	1	0	0	0	1
Organ/Findings:								
Thyroid								
Lymphocytic thyroiditis	0	0	1	0	0	1	0	0
Skin								
Mass cell tumor	0	0	0	0	0	0	0	1
Testes								
Focal diminution of spermatogenesis	0	0	0	1	-	-	-	-
Kidney								
Agenesis of right kidney	0	0	0	0	0	0	0	1

Results: There were no histopathological changes in the tissues of dogs which were considered to be treatment-related responses in this study.

007158

III. Study Author's Conclusion:

"... Dog No. 1984 had very poor teeth, and dog no. 3327 had a small mast cell tumor of the thoracic wall. The mast cell tumor is not an uncommon spontaneous neoplasm of dogs. The lesions in the remaining test animals were few and were not of the type which could be considered related to the test compound."

IV. Reviewer's Assessment of Results:

Test Material Analysis: Chemical analysis for the concentrations of benefin in the gelatin capsules was not included.

Chronic Study Results: The study design was incomplete and the conduct and reporting of specific areas were deficient as follows:

1. The results of body weights, food consumption, and opthalmology from the dogs treated with benefin in gelatine capsules were not given in this report.

2. The complete results of required parameters for the blood chemistry determination, the urine examination, and the examination in pathological changes were not presented. The following parameters were not examined: Blood Chemistry: calcium, phosphorous, sodium, potassium, chloride, magnesium, SGOT, protein, albumin, creatinine, cholesterol, bilirubin. Urine Examination: ketones, bilirubin. Histopathologic examination: trachea, rectum, urinary bladder, aorta, parathyroid, pituitary.

3. Statistical analysis of data was not performed.

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

BENEFIN TOX REVIEW 007158

Page_____ is not included in this copy.

Pages 57 through 58A are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007158

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

Reviewed by: John H.S. Chen John H.S. Chen 11/1/88
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui Quang Q. Bui 11/4/88
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: 2-Year Feeding and Oncogenic Study in Rats

Tox. Chem. No.: 130

MRID No.: 37675

EPA File Symbol:

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

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

Study Number(s): R-0295

Sponsor: Elanco Products Co., Indianapolis, Ind.

Testing Facility: Toxicology Division Lilly Research Laboratories

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

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

Report Issued: August 5, 1976

Conclusions:

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

Systemic NOEL - Not determined
(incomplete study)

Levels tested: 0.1, 0.5, and 1% of benefin in the diet

Classification of Data: Supplementary without the possibility of upgrading

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 necropsy.

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., 6%; 21 mo., 2%; 22 mo., 1%; 24 mo., 4%; Percentage of survival for females: 18 mo., 46%; 21 mo., 1%; 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 pretreatment 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) nerve
*(X) Stomach	(XX) Testes	
*(X) Colon	(XX) Uterus	Other
*(X) Jejunum	(XX) Ovaries	( ) Bone (sternum)
*(X) Ileum	Cardiovasc./Hemat.	*( ) Skeletal muscle
*(X) Colon	*(XX) Heart	(X) Skin
*(X) Salivary gland	*(X) Aorta	(X) Ear
*( ) 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 when 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:

(i) 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	3	3	3	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	3	3	0	2	4	4	3

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 by 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	26	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

5091-12-020

TABLE 1
Percentages of Survival of Rats Fed Benefin
in the Diet for 2 Years

Study R-0295

Time	Sex	Dietary Concentration 0	0.1%	0.5%	1.0%
Start	M	100	100	100	100
12 mo	M	100	92	75	96
18 mo	M	73	68	54	65
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 Error: Liver g	Kidney g	Heart g	Spleen g	Thyroid mg	Adrenal mg	Prostate g	Testes g
				Males					
0.0	618.3 ±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	0.581 0.032
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	0.686 0.052
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	0.767* 0.070
1.0	488.0[a] ±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	0.773 0.0
				Females					
								Uterus g	Ovaries mg
0.0	427.9 ±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	25.151 2.171
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	24.523 2.777
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	40.411* 5.203
1.0	No survivors								

[a] Since these values were from a lone survivor, statistical analyses were not deemed appropriate. 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.

5091-12-C31

83-3 - Rabbit - Teratogenicity

007158

Reviewed by: John H.S. Chen John H S Chen 10/28/68
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui Quang Q Bui
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: Rabbit Teratology

Tox. Chem. No.: 130

MRID No.: 37677

EPA File Symbol:

Test Material: Technical Benefin (Lot No. 858929; 96.8% Purity)

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

Study Number(s): B-0-7-68

Sponsor: Elanco Products Co., Indianapolis, Ind.

Testing Facility: Toxicology Division Lilly Research Laboratories

Title of Report: A Teratology Study on Benefin in the Rabbits

Author(s): J.K. Markham, G.R. Koenig, and N.V. Owen

Report Issued: April 8, 1968

Conclusions:

Maternal Toxicity NOEL = Not determined

Developmental Toxicity NOEL = Not determined

Dose Levels tested: 0, 50, and 100 mg/kg/day

Deficiency: incomplete study

Classification of Data: Supplementary
A new developmental study in rabbits is required

007158

Title of Study: A Teratology Study o Benefin in Rabbits
Study No. 3-0-7-68

I. Materials and Methods:

1. Test Material

Technical benefin (Lot No. 358929; 96.8% Purity) was prepared as a slurry of 20 mg of compound and 100 mg of feed per milliliter of distilled water and was given in dosage volume of 2.5 or 5.0 ml/kg. The control rabbits received 5.0 ml/kg dosage volumes of feed slurry.

2. Test Animals

The study consisted of 45 pregnant Dutch Belted rabbits which were artificially inseminated (gestation day 0) according to the method of Gibson et al. (Toxicol. and Appl. Pharmacol. 9, 398-407, 1966). Number of animals in each dose group was given below:

Dose Group (mg/kg/day)	No of Animals Pregnant Rabbits
0	15
50	15
100	15
Total	45

II. Reported Results:

1. Maternal Effects

A. Maternal Body Weight

Individual body weights were recorded on the day of insemination, the day of initial dosing and periodically thereafter (i.e., 0, 6, 12, 18, 21, and 27 gestation days).

Results: No compound-related effects on maternal body weight were detected in pregnant rabbits treated with benefin (Table 1-3 attached).

B. Food Consumption

Food consumption for individual animals was monitored daily during the study (i.e., 0 through 27 gestation days).

Results: Food consumption values were comparable for all groups (Table 2 attached).

C. Clinical Observations

Results: One rabbit (No. 2076) in the control group and one rabbit (No. 2143) in the 100 mg/kg dose group aborted on gestation day 27. Another rabbit (No. 2157) was killed on gestation day 28 due to the presence of mucopurulent material in the cervix of animal. The remainder of the rabbits (42) were survived throughout the study, sacrificed, and examined on day 28.

2. Developmental Effects:

A. Summary of reproductive data from rabbits treated with benefin

	Dosage (mg/kg/day)		
	0	50	100
No. females inseminated	15	15	15
No. aborted	1	0	1
No. killed in extremis	0	1	0
No. nonpregnant	4	3	1
No. litters with resorption	6	6	2
No. litters with live fetuses	10	9	13

Results: No adverse effects on reproductive data were observed from rabbits treated with benefin.

B. Summary of developmental data from rabbits treated with benefin

	Dosage (mg/kg/day)		
	0	50	100
No. pregnant females	11	12	14
Group Mean:			
No. corpora lutea	7.5	7.8	6.5
No. resorptions	0.7	1.6	0.2
No. life fetuses	5.7	5.9	4.8
No. implantations	6.5	5.7	5.1
Fetal weight (g)	27.4	27.2	27.3
% Male fetuses	51	51	41*

* Based on the following historical control data, similar ratio has previously observed in a large number of rabbit studies:

3-O-11-66: 58% Males
3-M-24-66: 36% "
3-M-30-66: 56% "
3-O-19-67: 43% "
3-C-2 -68: 61% "
3-D-47-68: 63% "

Results: No adverse effects on developmental data were observed from rabbits treated with benefin. Litter size (live fetuses) was reduced in both treated groups.

C. Summary of malformed fetuses

	Dosage (mg/kg/day)		
	0	50	100
No. litters examined	10	9	13
No. fetuses examined	57	43	63
No. fetuses with findings	21	15	18
% fetuses with findings	36.8	34.9	28.6

Results: No adverse effects were detected from the fetuses of rabbits treated with benefin.

D. Summary of fetal abnormalities (Table 7 attached)

	Dosage (mg/kg/day)		
	0	50	100
External Findings			
Multiple abnormalities	0	1	0
Exencephaly	0	0	1
White Spotted Condition	0	1	0
Skeletal Findings			
Incomplete development of frontal bones	0	0	1
13 Ribs			
Bilateral	9	10	9
Unilateral	8	2	9
Fused ribs	1	0	0
Bifurcated ribs	1	0	1
Non-articulated ribs	0	0	2
Misaligned sternal bars	2	1	0
Misaligned sternebrae	1	0	0
Incomplete development of sternebrae	0	1	0
Fused vertebrae	0	0	1

Results: Because of the low incidence and the lack of a dose response, the external abnormalities and the skeletal deviations found in the fetuses of the benefin-treatment groups were not considered to be related to treatment. The data should have been presented on a litter basis.

III. Study Author's Conclusion:

The study author concluded that the test material, benefin, produced no dose-related maternal toxicity at all levels tested and that there was no evidence of adverse developmental effects at any dose level.

IV. Reviewers' Assessment of Study Results:

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

1. As stated in the Guidelines (Subdivision F Guidelines), at least three treatment levels and a control group should be used, and the high dosage level should demonstrate maternal toxicity. Since the high dosage level selected for this study (100 mg/kg/day) produced no sign of maternal toxicity, it is questionable whether an appropriate high dose of the test material was chosen for this study. The number of treatment levels selected for the study is also inadequate.

2. Statistical treatment of test results was not performed in this study.

3. Lack of visceral examination and findings for all groups in this study.

4. Since the submitted information in this study are inconclusive, the study is judged supplementary in the present form.

5. Lack of supporting raw data.

6. Inadequate number of litters for evaluation.

Classification of Data: Supplementary

A new developmental toxicity study in rabbits is required.

007158

DeRoeck Ackerman 5-6-81

2

5091-12-206

TABLE 1.3

Mean Body Weights of Pregnant Rabbits (kg)
Rabbit Teratology Study B-0-7-68

Benefin mg/kg/day	Number of Animals	Gestation Day 0	6	12	18	21	27	Mean Percentage Change in Weight
0 (control)	11	2.42	2.45	2.43	2.41	2.37	2.37	- 2.0
50	12	2.44	2.43	2.38	2.29	2.31	2.30	- 5.5
100	14	2.37	2.42	2.36	2.31	2.31	2.31	- 2.1

*Based on percent change values of each rabbit.

74

(14)

TABLE 2

Mean Daily Food Consumption of Pregnant Rabbits (g)
Rabbit Teratology Study B-0-7-68

Benefin mg/kg/day	Number of Animals[a]	Gestation Days 0-5	6-18	19-27	Mean
0 (control)	11	91	45	59	60
50	10	88	51	52	59
100	14	91	55	59	68

[a]Values for rabbits that habitually spilled food in the cage were omitted from the calculation of the mean.

5091-14-207

007158

TABLE 7

Summary of Fetal Abnormalities

Rabbit Teratology Study B-0-7-68

Observation	Incidence of Abnormality			Cumulative Control Data
	Control	Benefin 50 mg/kg/day	Benefin 100 mg/kg/day[a]	
External				
Multiple Anomalies	0	1/43	0	1/1084
Exencephaly	0	0	1/69[b]	Related conditions have been observed.
White Spotted Condition	0	1/43	0	0
Skeletal				
Incomplete Development of Frontal Bones	0	0	1/63	11/1084
13 Ribs				
Bilateral	9/57 (15.8%)	10/43 (23.3%)	9/63 (14.3%)	181/1084 (16.7%)
Unilateral	8/57 (14.0%)	2/43 (4.7%)	9/63 (14.3%)	95/1084 (8.8%)
Fused Ribs	1/57	0	0	1/1084
Bifurcated Ribs	1/57	0	1/63	3/1084
Non-Articulated Ribs	0	0	2/63	0
Misaligned Sternal Bars	2/57	1/43	0	9/1084
Misaligned Sternebrae	1/57	0	0	3/1084
Incomplete Development of Sternebrae	0	1/43	0	15/1084
Fused Vertebrae	0	0	1/63	0

[a] 63 fetuses were recovered from the rabbits sacrificed on gestation day 28, and 6 additional fetuses were aborted by rabbit 2143 on day 27. All 69 were examined for external abnormalities, but the 6 aborted fetuses were not examined for skeletal defects.

[b] One of 6 fetuses aborted by rabbit 2143.

5091-12-217

(25)

76

007158

83-3 - Rat - Teratogenicity

Reviewed by: John H.S. Chen 4/1/88
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: Rat Teratology Tox. Chem. No.: 130

MRID No.: 147535, 40410000, 40410001, & 40128001 EPA File Symbol:

Test Material: Technical Benefin (Lot No. 251EF4; 97.3% Purity)

Synonyms/CAS No.:

Study Number(s): 6180-101

Sponsor: Eli Lilly and Company, Greenfield, Indiana

Testing Facility: Hazleton Laboratories, Madison, Wisconsin

Title of Report: Rat Teratology Study with Benefin

Author(s): Karen M. Mackenzie, Ph.D.

Report Issued: June 24, 1985

Conclusions:

Maternal Toxicity NOEL = 225 mg/kg
Maternal Toxicity LEL = 475 mg/kg (decreased body weight)

Developmental Toxicity NOEL = 1000 mg/kg (HDT)

Levels tested: 0, 50, 225, 475, & 1000 mg/kg

Classification of Data: Core Guideline

The following is a copy of this orginal review of this study as prepared by John H.S. Chen dated 9/8/86 and 5/5/88.

007158

REVIEWER

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

SEP - 8 1986

005443

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Benefin: 1471-71 (Record Number 164379): Review of Teratology Study with Benefin in Rats
Caswell Number 130, Accession No. 258788

FROM: John H.S. Chen, D.V.M.
Review Section #1
Toxicology Branch/HED (TS-769C)

TO: Robert Taylor, PM #25
Herbicide-Fungicide Branch
Registration Division (TS-767C)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

Petitioner:

Eli Lilly and Company
Greenfield, Indiana 46140

Action Requested:

Review and assessment of the teratology study with Benefin in rats, Hazleton Laboratories Ameriac, Inc. Study No. 6180-101, June 18, 1985.

Recommendation:

The registrant should be apprised of the deficiencies noted in this study, which are identified in the detailed review. The study may be upgraded on resolution of these deficiencies.

Study: Rat Teratology Study with Benefin Hazleton Laboratories Study No. 6180-101, June 18, 1985 (Authors: J.K. Markham and K.M. Macckenize). Accession No. 258788.

Procedure:

The method used to determine the potential maternal, embryotoxic and teratogenic effects of benefin 54521 (Lot No. 231EF4; 97.3% pure; Seven impurities were found, 0.1 - 1.1%) in pregnant female rats is outlined below:

1. Nine-week old female, nonpregnant Crl:CD (SD) BR rats were mated with 11-week old males. The females were checked daily for the presence of a vaginal plug or sperm in the vaginal smear.

2. Four groups of pregnant rats, 25 per group, were treated with Benefin 54521 dissolved in 10% acacia solution by oral gavage at 50, 225, 475, and 1000 mg/kg/day for 9 consecutive days (initiated on gestation day 6 and continuing up to and including day 15 of gestation). To ensure the prescribed dosage levels of the test compound, samples of the test mixtures from the initial preparation (daily prepared) were periodically analyzed.

3. All animals were observed daily for morbundity, death and obvious indications of a toxic effect. Individual maternal body weights were recorded on gestation days 0, 6, 11, 16 and at the time of sacrifice on day 20. Individual food consumptions was recorded for intervals between gestatin days 0 through 6, 6 through 11, 11 through 16, and 16 through 20. All dams were sacrificed on day 20 of gestation. The uterine weight was recorded and the ovaries were examined for gross abnormalities; the number of corpora lutea was recorded. After being examined externally, the uterus was opened along its entire length and the contents were examined.

4. All viable fetuses were examined externally for gross abnormalities and variations. Each fetus was examined for visceral abnormalities or stained for skeletal examination.

5. All statistical analyses were conducted for a minimum significant level of 5% comparing the treated groups to the control group ($P<0.05$). The following analyses were used:

(a) Body weight and food consumption were analyzed by using one-way analysis of covariance (ANCOVA). If this test was significant, Dunnett's test was used to determine significance between the control and treated group;

(b) One-way analysis of variance (ANOVA) with transformations (e.g., square root, log, reciprocal, are sin, and rank) was done on the following data for pregnant animals: body weight changes between day 0 and 20, gravid uterine weight, the number of corpora lutea and implants, implantation efficiency, and the number and percent of live and resorbed fetuses (Winer, B.J., Statistical principles in Experimental design, McGraw-Hill, New York, 2nd Edition 1971).

(c) The proportion of male and female fetuses with visceral and skeletal variations and anomalies and the proportion of litters with variant and anomalous fetuses was compared with the control groups by the cochran-Armitage test for trend and departure and a Fisher-Irwin (exact) test (Thakur A. et al., Fortran Program for Testing Trend and Homogeneity in Proportions, Comp. Prog. in Bio. Med., 19: 229-233, 1985).

Results:

1. Teratology Range-Finding Study

The preliminary study for dose selection of the test compound (Benefin 54521: Lot #219EF4 95.3% Purity) was conducted on pregnant rats using 5 dose levels of Benefin (50, 100, 225, 475, and 1000 mg/kg/day and one control group (5 rats/group). The general procedures used for the range-finding study were similar to that described in the teratology study.

Under the test conditions reported, survival for animals in this study was 100% in all groups. The mean maternal body weights on gestation days 11 and 16 and corrected weight changes from days 0 through 20 of gestation for the 475- and 1000 mg/kg/day groups were lower than those of the control in a dose-relaed manner. The mean food consumption values between gestation days 6 through 11 for the 475 mg/kg/day group and between gestation days 11 through 16 for the 475- and 1000 mg/kg/day groups were also lower than those of control in a dose-related manner. However, there were no statistically significant differencs in the mean number of corpora lutea or implants, implantation efficiency or the number of percent of live or resorbed fetuses. The reduced food consumption combined with the body weight loss in the treated dams at 1000 mg/kg/day demonstrated a maximum tolerated dose.

2. Clinical Examination

There were no treatment-related individual clinical observations noted during this study. However, two animals (Nos. C28304 and C28321) in the 1000 mg/kg/day group had alopecia.

3. Mortality

Survival for animals in this study was 100% in all groups.

4. Food Consumption - Mean Daily Food Intake (g)

Dose Levels mg/kg	Day of Gestation 0-6	6-11	11-16	16-20
0	24	26	27	29
50	24	26	27	29
225	24	24	27	30
475	24	22*	25*	29
1000	24	21*	25*	30

* Significantly different from the control P<0.05

Findings: The mean food consumption values between gestation days 6 through 11 and 11 through 16 for the 475- and 1000 mg/kg/day groups were significantly less than those of the control group.

5. Maternal Body Weights

Summary of Mean Body Weight and Weight Change Data

Treatment mg/kg	Body Weight on Days (g) 0	6	11	16	20	Weight Change Between Days (g) 0-6	6-11	11-16	16-20	0-20
0	243	272	299	337	397	30	27	38	60	154
50	242	272	296	333	392	30	25	37	59	151
225	241	267	289	327	388	26	22	38	60	147
475	244	273	286*	322*	383	29	13*	36	61	139
1000	240	269	283*	322*	386	29	14*	39	64	146

* Significantly different from the control at 0.05 level

Findings: The mean maternal body weight on gestation days 11 and 16 and the mean weight changes from days 6 through 11 for the 475 and 1000 mg/kg groups were significantly less (P<0.05) than those of the corresponding control group.

6. Urine Stains

Urine stains (from light yellow to orange) on the pan paper were observed following the initiation of dosing (Day 10 of gestation) for the 50 mg/kg group and the day following initiation of dosing (Day 7 of gestation) for the remaining treated groups. However, the occurance of this observation began to subside within 48 hours of the end of the dosing period (i.e., day 17 of gestation) and the urine apearance of all animals was normal at the time of cesarean section.

7. Postmortem Observations

Under the individual necropsy observations (number of animals with abnormal necropsy observatgions: control, 2; 50 mg/kg group, 5; 225 mg/kg group, 2; 475 mg/kg, 2; 1000 mg/kg group, 5), dilated renal pelvis was shown in all groups and enlarge hepatic lobes were also noted in all treated groups with similar frequency. There were no treatment-related observations for the pregnant rats at the time of cesarean section.

8. Cesarean Section Observation - Mean Values

Observations	Benefin (mg/kg)				
	0	50	225	475	1000
Number of animals on test	25	25	25	25	25
Number (percent) pregnant	24(96)	24(96)	24(96)	24(96)	25(100)
Corpora lutea	16	16	18	15	17
Implants	16	15	15	14	16
Implantation efficiency	96.8	97.1	88.7	92.5	91.6
Live fetuses	14.7	14.5	14.7	13.6	15.4
Percent live fetuses	100	100	100	100	100
Fetal viability	94.8	93.3	95.0	94.5	96.7
Sex Ration (M/M+F)	50.6	51.3	48.3	47.0	51.5
Male Fetal Weight (g)	3.6	3.5	3.5	3.5	3.5
Female Fetal Weight (g)	3.3	3.3	3.3	3.3	3.3
Resorptions*	0.8	1.0	0.8	0.8	0.5
Percent resorptions	5.2	6.7	5.5	5.5	3.3

* All were early resorptions except for one late resorption in Animal No. C26848 in the 50 mg/kg group.

Findings: There were no significant differences (P>0.05) in the mean number of corpora lutea, or implants, implantation efficiency, fetal weights, sex ratio, or the numbr of percent of live or resorbing fetuses. All fetuses were also survived during this study.

9. Number of Fetuses with External Abnormalities

One fetus (No. 4) from Animal No. C28236 in the 50-mg Benefin/kg group had atailia. Another fetus (No. 3) from Animal No. C28274 in the 225-mg Benefin/kg group had a thread-like tail and no anus. However, this fetal external abnormalities were isolated incidents and are not considered treatment related.

10. Number of Fetuses and Litters with Anomaly - Summary of Incidences (Males and Females)

	Fetuses					Litters				
Dose Levels (mg/kg)	0	50	225	475	1000	0	50	225	475	1000
Number Examined Skeletally	185	179	182	167	198	24	24	24	24	25
Skull										
Reduced ossification	4	12	6	12	9	4	6	5	5	9
Unossified hyoid	1	0	1	0	1	1	0	1	0	1
Vertebrate										
Absent vertebra/centra	1	1	2	1	0	1	1	2	1	0
Unossified vertebra/ centra	1	0	4	0	4	1	0	1	0	2
27 Presacral	0	0	0	0	1	0	0	0	0	1
25 Presacral	1	0	1	0	0	1	0	1	0	0
13 Presacral	0	1	0	0	0	0	1	0	0	0
Sternebra										
Bipartite	0	0	0	1	1	0	0	0	1	1
Ribs										
Wavy	1	0	0	0	0	1	0	0	0	0
Bent	0	3	0	0	0	0	1	0	0	0
Rudimentary	5	7	2	7	5	4	6	2	5	5
Extrafull unilateral	2	4	1	5	1	2	3	1	5	1
Reduced/interrupted ossification	1	0	0	0	0	1	0	0	0	0
7th Cervical	0	0	0	1	1	0	0	0	1	1
Absent	0	1	0	0	0	0	1	0	0	0
Pectoral Girdle										
Bent/malformed scapulae	0	0	0	1	0	0	0	0	1	0
Pelvic Girdle										
Malformed ileum	0	0	1	0	0	0	0	1	0	0
Total Number W/Skeletal Malformation	17	29	18	28	23	16	19	14	19	21
Number Examined Viscerally	167	168	117	157	187	24	24	24	24	25
Head										
Cleft palate	0	1	0	0	0	0	1	0	0	0
Aphakia	0	0	1	0	0	0	0	1	0	0
Circulatory										
Absent innominate	0	0	1	1	0	0	0	1	1	0
Accessory left subclavian	0	0	0	0	1	0	0	0	0	1
Gastrointestinal										
Dark brown-red diffuse areas or liver	0	0	5	1	4	0	0	5*	1	4
Urogenital										
Undescended testis	0	0	0	0	0	2	2	0	0	0
Total Number W/Soft Tissue Malformation	0	1	7	2	5	0	3	7	2	5

* Significantly different from the control at 0.05 level.

Findings: The number of litters with dark brown-red diffuse areas on the liver was significantly greater in the 225 mg/kg group when compared to the control group. The diffuse areas on the liver appeared to be hemorrhagic regions. There was no evidence of a dose-response relationship for this incidence of liver observations. No other significant differences were observed between the litters of treated groups and the litters of control group.

11. Number of Fetuses and Litters with Variation - Summary of Incidences (Males and Females)

	Fetuses					Litters				
Dose Levels (mg/kg)	0	50	225	475	1000	0	50	225	475	1000
Number Examined Skeletally	185	179	182	167	198	24	24	24	24	25
Vertebrae										
Centra abnormalities	10	6	5	11	18	7	5	4	7	12
Sternebra										
Unossified	10(M) 24(F)	17(M) 23(F)	21(M) 26(F)	*12(M) 25(F)	22(M) 34(F)	14	16	15	15	21
Total Number W/Skeletal Variation	44	46	52	48	74	21	21	19	22	33
Number Examined Viscerally	167	168	171	157	187	24	24	24	24	25
Urogenital										
Distended ureter	13	21	10	7	2	9	13	9	4	2
Dilated renal pelvis	12	24	21	9	9	10	16	14	8	7
Total Number W/Soft Tissue Variation	25	45	31	16	11	19	29	23	12	9

* Significantly different from the control at 0.05 level (for the males only).

Findings: Although there was a significantly greater incidence of unossified sternebra found in males of the 225 mg/kg group, no evidence of a dose-response relationship was observed. No other significant differences were observed between the fetuses of treated groups and the fetuses of control group.

007158

12. Total Number of Abnormal Fetuses

		Benefin (mg/kg)				
		0	50	225	475	1000
Number of feuses examined		185	179	182	167	198
		167	168	171	157	187
	Total	352	347	353	324	385
Number of abnormal fetuses		17	29	18	28	23
		0	1	7	2	5
		44	46	52	48	74
		25	45	31	16	11
	Total	86	121	108	94	113
Percent of abnormal fetuses		24.4 (86/352)	34.9 (121/347)	30.6 (108/353)	29.0 (94/324)	29.4 (113/385)

Evaluation and Conclusion:

1. The parameters which were unaffected by the treatment of Benefin in pregnant rats are clinical observation, maternal survival, postmortem examination, conception rate, fetal sex ratio, preimplantation loss, postimplantation loss, and the number or percent of live or resorbed fetuses. Urine staining, observed in the Benefin-treated groups from days 7 through 17 of gestation, were not dose-related.

2. The treatment-related decreases of maternal weights were observed in the treated pregnant female groups receiving 475 and 1000 mg/kg Benefin. These results were correlated well with the significant decreases of food consumption values in the same treated groups during this study.

3. Although there were no treatment-related increases of the number of abnormal fetuses (i.e., fetuses with skeletal and soft tissue variations and anomalies) noted in the Benefin-treated groups, the number of unossified sternebra was significantly greater for male fetuses of dams treated with 225 mg/kg Benefin when compared to the control group. The number of fetuses and litters with dark brown-red diffuse areas on the liver was also significantly greater in the 225 mg/kg group. However, the total number of fetuses with skeletal and soft tissue variations and anomalies in the treated and control groups accumulated in the Table on page 9 (i.e., total abnormal fetuses: control group, 87; 50 mg/kg group, 94; 225 mg/kg group, 89; 475 mg/kg group, 75; 1000 mg/kg group, 90) were less than the total number of abnormal fetuses given in the Table 7 (summary of fetal skeletal observations) on page 21 and in the Table 8 (summary of fetal soft tissue observations) on page 24. This discrepency should be clarified.

4. In addition, details regarding the visceral and skeletal examination techniques in this study must be provided in this report.

Since the submitted information in this report are inconclusive, the study is judged supplementary in the present form. Until the reporting deficiencies and data gaps cited in our conclusions #3 and #4 are clarified and resolved, this study is rated supplementary.

Classification of Data - Supplementary

Maternal Toxicity NOEL = 225 mg/kg

Developmental Toxicity NOEL = To be determined.

TS-769:CHEN:sll:X73710:7/26/86 Card Chen

REVIEWER

007158

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

MAY - 5 1988

006693

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Subject: Benefin: 1471-71 (Record Number 212720): Registrant's Reply to the Previous Toxicology Branch Review Comments Concerning the Rat Teratology Study with Benefin Caswell Number 130

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

To: Robert Taylor, PM 25
Herbicide-Fungicide Branch
Registration Division (TS-767C)

Thru: David Ritter, Acting Section Head
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Review of the Registrant's Response to the Previous TB Review Comments Concerning the Rat Teratology Study with Benefin (TB Memo 9/8/86 J. Chen)

1. High Incidence of Male Fetuses with Unossified Sternebrae at the 225 mg/kg/day Dose Level

Registrant's Response:

"... The occurrence of male fetuses with unossified sternebrae at the 225 mg/kg/day dose level was statistically greater than the concurrent control group but this finding did not occur in a dose-response manner across the benefin treatment groups. The proportion of female fetuses with unossified sternebrae at the 225 mg/kg/day dose level was similar to the proportion of male fetuses affected but was not statistically different from the concurrent control group. These data indicated that the occurrence of unossified sternebrae was not a sex-linked event. Hazleton historical control data from teratology studies conducted during the two-year period encompassing study 6180-101 reported minimum and maximum incidences of fetuses with unossified sternebrae of 18.4% and 29.4% respectively. The incidence observed at the 225 mg/kg/day dose level was well within the range for historical control fetuses. However, the concurrent control incidence for male fetuses was lower than all studies

conducted over the preceding two-year period. Based on these data, the occurrence of a statistically significant increase in male fetuses with unossified sternebrae at the 225 mg/kg/day dose level appeared to be the result of antypically low incidence in the concurrent control group and was not considered to be toxicologically significant. "

Reviewer's Comments:

The submitted historical control data provide adequate information for the spontaneous incidences of fetuses with unossified sternebrae in the rat teratology study. Registrant's explanation for the statistically increased incidences of fetuses with unossified sternebrae found at the 225 mg/kg/day dose level is considered to be reasonable.

2. High Incidence of Dark Brown-Red Diffuse Areas on the Liver at the 225 mg/kg/day Dose Level

Registrant's Response:

"... Contrary to the EPA's comments, the original report cited only the litter incidence of dark brown-red diffuse areas on the liver at the 225 mg/kg/day dose level to be statistically greater then the concurrent control group. The numbers of litters affected were 0, 0, 5, 1 and 4 from the 0, 50, 225, 475 and 1000 mg/kg/day dose levels respectively and did not indicate a pattern related to benefin treatment. It should be noted that no gross morphological changes in the liver were observed in conjunction with these dark brown-red areas. In the teratology segment of a rat multigeneration reproduction study with benefin, liver anomalies were not observed in fetuses from rats that received 0, 1000 and 5000 ppm benefin in the diet (ca. 0, 100 and 500 mg/kg/day) throughout gestation (Adams et al., 1973). Based on these data, the sporadic occurrence of fetuses with dark brown-red areas on the liver was not considered to be toxicologi al significant. "

Reviewer's Comments:

The provided information and explanation for the statistically increased litter incidences of dark brown-red diffuse areas of the liver at the 225 mg/kg/day dose level are considered to be reasonable.

[redacted]

007158

3. Tabulation Errors

Registrant's Response:

"... The numbers and percentages of abnormal and normal fetuses were derived from data in Table A-7 (Individual Fetal Skeletal Observations) on pages 54-67, and Table A-8 (Individual Fetal Soft Tissue Observations) on pages 68-73. Subsequent to receiving comments from the EPA, a review of study 6180-101 was conducted by Lilly scientists. Tabulation errors were discovered in the reported values for the numbers of fetuses examined and the numbers and percentages of abnormal and normal fetuses presented on page 9. Although the occurrence of these tabulation errors was regrettable, they were not of sufficient magnitude to affect the conclusions of this study. The corrected values for these parameters are shown below:

	Mg Benefin 54521/Kg				
	0	50	225	475	1000
No. of Fetuses Examined	353	349	353	324	385
No. of Abnormal Fetuses (%)	65(18)	93(27)	89(25)	74(23)	90(23)
No. of Normal Fetuses (%)	288(82)	256(73)	264(75)	250(77)	295(77)

In addition, a dose group heading for Table A-8 on page 73 of the original report was incorrectly identified as "225 mg Benefin 54521/kg (continued)"; the dose group identification should read "475 mg Benefin 54521/kg (continued). A report amendment correcting these errors was prepared by Hazleton Laboratories, Inc. and was submitted to EPA by Lilly Research Laboratories (Byrd., 1986)."

Reviewer's Comments:

The corrected Table (Total Number of Abnormal and Normal Fetuses) ia considered to be acceptable and should replace the same table summarized in the original Toxicology Branch review of this study (TB Memo 9/8/86 J. Chen).

4. Methodology for Visceral and Skeletal Examination Used by Hazleton Laboratories, Inc.

Registrant's Response:

" Copies of standard operating procedures for visceral and skeletal examination techniques were requested and received from Hazleton Laboratories, Inc. These standard operating procedures have been included in this document as Appendix C. "

Reviewer's Comments:

The provided details of visceral and skeletal examination techniques used by the Hazleton Laboratories, Inc. are considered to be acceptable (attached).

5. Registrant's Conclusion

" This document has addressed comments from the EPA review of a rat teratology study with Benefin. Additional information has been provided as requested by the EPA. Corrective action has been taken to rectify minor data errors. However, study 6180-101 still represents a valid assessment of the teratogenic potentital of Benefin. Data generated in study 6180-101 support a maternal toxicity NOEL of 225 mg/kg/day and a developmental toxicity NOEL of 1000 mg/kg/day (the highest dose level tested). "

6. Toxicology Branch Recommendation

Registrant's responses to the deficiencies citad in the previous Toxicology Branch Review of this study (TB Memo 9/8/86 J. Chen) appear to be justified. Therefore, the study is upgraded to Core Guideline.

Maternal Toxicity NOEL = 225 mg/kg/day
Maternal Toxicity LEL = 475 mg/kg/day (decreased maternal body weight)
Developmental Toxicity NOEL = 1000 mg/kg/day (HDT)

BENEFIN TOX REVIEW 007158

Page_____ is not included in this copy.

Pages 92 through 98 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

83-4 - Rat - Reproduction

007158

Reviewed by: John H.S. Chen John H.S. Chen 11/7/88
Section I, Toxicology Branch II (TS-769C,
Secondary reviewer: Quang Q. Bui
Section I, Toxicology Branch II (TS-769C) Quang Q. Bui

DATA EVALUATION REPORT

Study Type: Rat Reproduction

Tox. Chem. No.: 130

MRID No.: 00037676

EPA File Symbol:

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

Synonyms/CAS No.:

Study Number(s): R-0305, R-0795, R-0316 and R-0057

Sponsor: Division of Eli Lilly and Co., Indianapolis, Ind.

Testing Facility: Lilly Research Laboratories, Indianapolis, Ind.

Title of Report: A Multi-Generation Rat Reproduction Study with Benefin

Author(s): W.V. Owen, D.V.M, Ph.D.

Report Issued: 1973

Reproductive NOEL = Not Determined

Parental Toxicity NOEL = Not Determined

Levels tested: 0.1 and 0.5% of Benefin in the diet

Classification of Data: Supplementary

A new reproductive toxicity study in rats is required.

007158

Title of Study: A Multi-Generation Rat Reproduction Study with Benefin
Study No. R-0305, R-0795, R-0316, and R-0057

I. Materials and Methods:

1. Test Material

Technical benefin (Lot No. X-11424; 95.6% Purity) was fed as a dietary component to five successive generations of rats in this study (0, 0.1, or 0.5% of benefin in the diet). Detailed information for the preparation of test diet were not given.

2. Animals and Experimental Design

Weanling Harlan rats were obtained from Harlan Industries, Inc. Cumberland, Indiana. A total of of 120 females and 90 males were chosen for the study following an initial examination (40 females and 30 males per group). At study initiation, the F_0 animals (parental generation) were 9 weeks old. Throughout the course of the study, all animals were maintained in an environmentally controlled conditions. All animals were provided tap water and the Lilly Mill Diet and ad libitum. Parental animals of the same sex were group caged except during mating; females were individually housed following mating.

After 3 months of treatment, females were paired with males from the same dose group for 20 days to produce the F_{1a} litters. Ten females in each group were examined daily for the presence of copulatory plugs (gestation day zero) and those for which plugs were found were submitted for cesarean section (i.e., numbers of females subjected to C-section: 9, 8, and 6 for the control, 0.1%, and 0.5% dose group, respectively). The remainder of the pregnant females (30) were allowed to prodcue the F_{1a} litters. The dam were rested one week and then bred to a different male in the same dose group. Procedures same as described for the first mating trial (F_{1a}) with the exception that the progenies that were delivered (F_{1b} litter) were raised to become the parents of the F_1 generation. In this study, the F_0 females were continuously bred 6 times and the results of F_{1a} and F_{1b} were discussed in this report. The data from cesarean section were not discussed here because a separate rat teratology study with benefin has been previously reported (Hazleton Labs Study No. 6180-10).

F_{1b} (32 females and 20 males) pups were randomly selected and fed diets containing 0, 0.1, and 0.5% of benefin during the 60-day growth period. When the rats were approximately 3 months old, mating procedures were initiated as described for the F_0 generation with the exception that none of the females were designated for cesarean section. The females were allowed to deliver (F_{2a}) and raise the progenies to 21 days of age. At this time, the progenies were examined and sacrificed. The females were rested 1 week and bred with a different male from the same dietary level. The progenies from the second mating (F_{2b} litter) were raised to maturity and were designated as the F_2 parents.

F_{2b} pups (20 males and 20 females) were randomly selected and fed diet containing 0, 0.1, and 0.5% of benefin during the 60-day growth period. Mating procedures were the same as outlined for the F_1 generation parents. The progenies from the second mating were raised to maturity and become the parents of F_3 generation.

F_{3b} pups (20 males and 20 females) were randomly selected and fed diets containing 0, 0.1, and 0.5% of benefin during the 60-day growth period. Mating procedures were the same as outlined for the previous generations. The progenies from the second mating trial (F4b litter) were raised to become parents of F_4 generation.

II. Reported Results:

1. Parental Effects:

A. Mortality of Parents

Parents Generation	Dietary Level (%)					
	Males			Females		
	0	0.1	0.5	0	0.1	0.5
F_0	4	7	10	3	4	9
F_1	4	0	3	3	1	4
F_2	0	1	0	0	0	1
F_3	0	0	0	0	1	3

Results: A dose-related effects on parental mortality in F_0 males and females were observed, but, the cause of death was not evident. There were no treatment effects on parental mortality in F_1, F_2, and F_3 parents.

B. Parental Body Weights

Summary of mean parental body weight gains for the 60-day growth period:

Generation	Dietary Level (%)					
	Males			Females		
	0	0.1	0.5	0	0.1	0.5
F_0 - No. of Animals	29	29	27	39	40	38
Body Wt. Gain (g)	302	288	287	168	167	128
F_1 - No. of Animals	16	20	18	18	20	19
Body Wt. Gain (g)	340	342	311*	180	186	146*
F_2 - No. of Animals	20	20	20	20	20	20
Body Wt. Gain (g)	190	180	170*	329	351*	278*
F_3 - No. of Animals	20	20	20	20	20	20
Body Wt, Gain (g)	172	180	159	285	310	289

*Significantly different from control vlaues ($P \leq 0.05$; Dunnett's "t" test)

B. Parental Body Weights - continued

Results: Body weight gains for the F_1 and F_2 high-dose (0.5%) males and females were significantly lower ($P<0.05$) than that of the corresponding control groups after the 60-day growth period (premating). No significant reductions of body weight gain were observed in the other treatment groups (male or female) of F_0 and F_3 generations. However, the body weight data on gestation day and postpartum days were not included in the study.

C. Food Consumption

Mean food consumption values were recorded weekly during a 60-day growth period.

	Dietary Level (%)					
	Males			Females		
Generation	0	0.1	0.5	0	0.1	0.5
F_0 - No. of Animals	29	29	27	39	40	38
Daily Food Intake(g)	26.4	22.2	21.6	19.6	19.5	16.6
F_1 - No. of Animals	16	20	18	18	20	19
Daily Food Intake(g)	23	23	20	19.4	20.7	19.0
F_2 - No. of Animals	20	20	20	20	20	20
Daily Food Intake(g)	22.3	22.8	20.3	18.8	19.3	19.7
F_3 - No. of Animals	20	20	20	20	20	20
Daily Food Intake	19.3	21.3	20.1	18.6	16.3	17.6

Results: The only reduced mean food intake values were observed in the high-dose males and females of F_0 generation. However, the differences were not statistically significant when compared to that of the corresponding controls of the same generation.

D. Histological Examinations of Parental Animals

Incidence of Selected organ lesions in F_0 Parents

	Dietary Level (%)					
	Males			Females		
	0	0.1	0.5	0	0.1	0.5
No. Examined	29	29	27	33	40	38
Liver						
Fatty metamorphosis	3	5	13	3	2	2
Reproductive System						
Ruptured uterus	-	-	-	1	0	0
Endometritis, Cystic	-	-	-	0	1	2
Ovarian atrophy	-	-	-	2	0	0
Paraovarian inflam.	-	-	-	1	0	0
Ovarian Cysts	-	-	-	1	0	0
Focal atrophy of testis	1	0	0	-	-	-
Testicular degeneration	0	0	2	-	-	-

D. Histological Examination of Parental Animals - continued

Results:

1. The only change noted in F_0 parents was an increased incidence of fatty metamorphosis of the liver in the males of 0.5% dose group. This changes were related to the treatment. Other organ lesions observed were commonly found in laboratory rat colony. The author concluded that this finding was of no toxicological significance.

2. Histological examination of the dead F_1 parental animals indicated that no evidence of an effect on death rate or type of pathological conditions was found in control vs. benefin-treated rats.

3. Histological examination of F_3 parents indicated that no evidence of a treatment-related effect on body organs or tissues was observed.

2. Reproductive Data:

A. Summary of reproductive data of rats fed benefin:

Litter Generation	Dose Level (%)	No. Paired	Mated No.*	Mated %	Pregnant No.	Pregnant %	Total Pups/Live pups	Percent Viable
F_{0a}	0	40	39	98	35	90	312/306	98
	0.1	40	40	100	36	90	319/311	97
	0.5	40	38	95	37	97	323/316	98
F_{0b}	0	30	30	100	25	83	175/157	90
	0.1	32	32	100	29	91	254/235	93
	0.5	32	32	100	32	100	285/264	93
F_{1a}	0	20	18	90	17	94	167/157	94
	0.1	20	20	100	19	95	222/213	96
	0.5	20	19	95	17	89	163/158	97
F_{1b}	0	18	17	94	18	100	158/133	84
	0.1	20	19	95	17	89	181/163	90
	0.5	16	16	100	15	94	148/140	95
F_{2a}	0	20	20	100	16	80	190/185	97
	0.1	20	20	100	19	95	203/200	99
	0.5	20	20	100	16	80	151/141	93
F_{2b}	0	20	20	100	19	95	206/193	94
	0.1	20	20	100	18	90	209/203	97
	0.5	20	20	100	19	95	210/208	99
F_{3a}	0	20	20	100	19	95	206/187	91
	0.1	20	20	100	20	100	200/180	90
	0.5	20	20	100	20	100	200/194	97
F_{3b}	0	20	20	100	18	90	185/170	92
	0.1	20	20	100	20	100	193/185	96
	0.5	20	20	100	17	85	157/145	92

* No. of animals actually mated (discounting the animals died before mating)

007158

A. Summary of reproductive data of rats fed benefin - continued

Results: The percentage of females mating and bearing live pups were generally comparable in all groups for the F_0, F_1, F_2 and F_3 litters.

B. Summary of mean litter data of rats fed benefin

Litter Generation	Dose Level	Survival Index 4-day	12-day	21-day
F_{0a}	0	0.97(296/306)	0.93(286/306)	0.93(284/306)
	0.1	0.98(304/311)	0.97(301/311)	0.96(298/311)
	0.5	0.94(297/316)	0.87(276/316)	0.83(263/316)
F_{0b}	0	0.97(153/157)	0.84(132 157)	0.82(128 /157)
	0.1	0.97(229/235)	0.90(211/235)	0.84(197/235)
	0.5	0.94(249/264)	0.87(230/264)	0.85(223/264)
F_{1a}	0	0.96(150 /157)	0.88(138/157)	0.88(138/157)
	0.1	0.95(202/213)	0.92(198/213)	0.92(198/213)
	0.5	0.80(127/158)	0.77(144/158)	0.77(114/158)
F_{1b}	0	0.96(128/133)	0.92(122/133)	0.92(122/133)
	0.1	0.98(158/163)	0.94(152/163)	0.94(152/163)
	0.5	0.88(123/140)	0.87(122/140)	0.82(114/140)
F_{2a}	0	0.98(181/185)	0.98(181/185)	0.96(178/185)
	0.1	0.94(187/200)	0.91(181/200)	0.91(181/200)
	0.5	0.78(110/141)	0.78(110/141)	0.78(110/141)
F_{2b}	0	0.87(168/193)	0.86(166/193)	0.81(156/193)
	0.1	0.91(184/203)	0.87(177/203)	0.83(169/203)
	0.5	0.85(176/208)	0.80(166/208)	0.73(143/208)
F_{3a}	0	0.94(178/187)	0.93(173/187)	0.87(173/187)
	0.1	0.87(156/180)	0.82(147/180)	0.81(145/180)
	0.5	0.79(154/194)	0.66(128/194)	0.64(125/194)
F_{3b}	0	0.97(164/170)	0.94(160/170)	0.92(157/170)
	0.1	0.80(147/185)	0.77(140/185)	0.74(136/185)
	0.5	0.88(127/145)	0.85(123/145)	0.55(80 /145)

Results: A reduction in progeny survival was observed for mating trials at the 0.5% dose level in the F_0, F_1, F_2 and F_3 generations. But, the differences in survival index were not statistically significant at $P<0.05$ between the benefin-treated groups and the corresponding control groups for all generations.

B. Summary of mean litter data of rats fed benefin - continued

Litter Generation	Dose Level (%)	Group Mean Pup Wt. (g) 4-day	12-day	21-day	Litter Size Mean No. of Live-Born Per Litter	Percent of Males at 21-day Postpartum
F_{0a}	0	9.5	22	36.3	11.3	51
	0.1	9.5	20.9	35.3	10.7	51
	0.5	7.6	15.1*	24.3*	9.9	47
F_{0b}	0	9.8	21.4	38.1	10.5	55
	0.1	9.6	19.5	33.7	11.8	55
	0.5	8.0	16.5*	25.3*	10.6	47
F_{1a}	0	9.9	22.6	36.9	9.2	44
	0.1	9.0	20.3	32.4	11.2	54
	0.5	7.5	16.6	28.3	9.9	54
F_{1b}	0	9.9	24.8	38.3	7.8	41
	0.1	9.4	20.6	35.0	9.5	53
	0.5	8.1	18.9	26.3	9.3	42
F_{2a}	0	10.3	22.4	38.7	11.6	52
	0.1	9.3	21.0	35.6	10.5	51
	0.5	8.6	18.6	31.1	8.8*	50
F_{2b}	0	9.6	21.2	34.1	10.2	45
	0.1	9.2	19.5	32.3	11.3	60
	0.5	7.8	15.8	24.2	10.9	56
F_{3a}	0	9.2	21.6	36.2	9.8	44
	0.1	9.4	22.4	36.2	9.0	48
	0.5	8.6	19.4	33.2	9.7	46
F_{3b}	0	10.0	23.6	37.8	9.4	50
	0.1	9.7	22.4	35.4	9.7	49
	0.5	8.7	19.0	29.2	8.5	49

*Significantly different from the control $P<0.05$.

Results:

1. A statistically significant reduction in the mean pup weight values ($P<0.05$) was observed for the litters of F_0 generation (F_{0a} & F_{0b}) at the high-dose groups (0.5%). However, there were no significant differences in the mean pup weight values found between the litters of benefin-treated groups and the litters of corresponding control groups in other generations.

2. A statistically significant reduction in the mean number of live-born per pregnant female was also observed at the high-dose group ($P<0.05$) in the first mating of F_2 generation (F_{2a}). However, there were no significant differences in the mean group values of live litter size between the benefin-treated groups and the corresponding control groups in other generations.

3. Although the percent of males at 21-day postpartum was slightly lower in the high-dose groups than in the corresponding control groups of F_0 generation, the differences were not statistically significant at $P<0.05$. Sex ratios were unaffected by the treatment of benefin for all generations.

III. Study Author's Conclusions:

The study author concluded that " Benefin at levels at 0.1 and 0.5% did not interfere with the reproductive performance of rats. progeny survival and growth were adversely affected only at the benefin level at which parental growth retardation was also observed. The no effect level was 0.1%. "

IV. Reviewer's Assessment of Study Results:

1. Test Material Analysis:

The results of chemical analysis for the test diets containing 0.1% and 0.5% of benefin and findings for the homogeneity and stability of test diets are not presented in this report.

2. Parental Data:

There was a dose-related effect on mortality in F_0 males and females. Significant reduction in body gain was indicative of parental toxicity at 0.5% dose level for the males and females of F_1 and F_2 generations. Although significantly increased incidences of fatty metamorphosis of the liver were observed in the high-dose rats of F_0 generation, the findings are suggestive of adoptive changes rather than overt toxicological effects.

3. Reproductive Data:

The test material had no effect on mating, pregnancy rates, survival index, live litter size, or sex ratios. However, the reduction in pup weight is considered to be indicative of reproductive toxicity at 0.5% dose level for the litters of F_0 generation.

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

i. Two dose levels of benefin used in this study are not considered to be adequate to determine the dose-response toxic effects of benefin on reproductive systems in rats. At least three dose level froups should be used. The highest dose level should produce an observable toxicological effect in the test animal.

ii. The study records of examination for the clinical signs of toxicity, the date of delivery or abnormal behavior during esterous, gestation, or delivery relating to the reproduction of each females were not provided.

iii. The summary of mean parental body weight gains based on the 60-day growth period is not considered adequate. The body weight of each weanling recorded weekly was not included. The mean female body weight values for the periods of gestation days and postpartum days were also not given.

iv. because of the low fertility index indicated in the 3rd, 4th, 5th, and 6th mating trials of F_0 generation, the continuous breeding study is considered useless.

v. Complete necropsy and histopathology findings on the reproductive organs and tissues (i.e., vagina, uterus, ovaries, testes, epididymus, seminal vesicles, and prostate) for the randomly selected weanling of each sex from each test group in each generation (F_0 and F_1) were not presented.

5. Classification of Data: Supplementary (A new reproductive study in rats is required)

007158

Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen 4/1/88
Section I , Tox Branch (TS-769C)
Secondary reviewer: Kerry Dearfield 4/1/88
Toxicology Branch II/HED (TS-769C)

DATA EVALUATION REPORT

CHEMICAL: Benefin

Tox. Chem. No.: 130

AAID No. 160866

EPA File Symbol:

STUDY TYPE: Mammalian cells in culcure gene mutation assay in L5178Y Mouse Lymphoma Cells

ACCESSION NUMBER:

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

SPONSOR: Eli Lilly and Company, Greenfield, Indiana

TESTING FACILITY: Lilly Research Laboratories, Greenfield, Indiana

TITLE OF REPORT: The Effect of Benefin (EL-110, Compound 54521) on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells

AUTHOR(S): G.R. Koenig, T.J. Oberly, B.J. Bewsey, and G.S. Probst

STUDY NUMBER(S): 850612MLA2598 and 850724MLA2598

REPORT ISSUED: October 29, 1985

CONCLUSION(S) - Executive Summary:

Benefin technical was nonmutagenic in the in-vitro mouse lymphoma L5178Y cell assay (by measuring induction of resistance to trifluorothymidine) with or without metabolic activation at the concentrations tested.

Concentrations tested: 5, 10, 15, 20, 25, 30, 35, and 40 ug/ml without S9 mix; 0.5, 1, 10, 20, 30, 50, 80, and 100 ug/ml with S9 mix.

Deficiency: Toxicities for reported concentrations under non-activated conditions were not high enough.

Study: Unacceptable, but can be upgraded to acceptable upon receipt of mutation data for 30 and 35 ug/ml concentrations under non-activated conditions.

007158

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS

1. Test Material: Name: Benefin (EL-110, Compound 54521)
Description (e.g. technical, nature, color, stability):
Benefin technical
Batch #: 231EF4 Purity: 97.3%
Contaminants: if reported, list in CBI appendix
Solvent used: DMSO
Other comments:

2. Control Materials:
Negative: DMSO
Solvent/final concentration:
Positive: Non-activation (concentrations, solvent):
620 ug/ml, Ethylmethanesulfonate (EMS) in DMSO

Activation (concentrations, solvent):
2 ug/ml, 3-methylcholanthrene (3MC) in DMSO

3. Activation: S9 derived from Fischer 344 male
X Aroclor 1254 X induced X rat X liver
___ phenobarbital ___ non-induced ___ mouse ___ lung
___ none ___ hamster ___ other
___ other ___ other
If other, describe below
Describe S9 mix composition (if purchased, give details):

The enzyme mixture contained (4 ml) 45 mg sodium isocitrate, 24 mg NADP and 1 ml of rat liver S9.

4. Test Cells: mammalian cells in culture
X mouse lymphoma L5178Y cells
___ Chinese hamster ovary (CHO) cells
___ V79 cells (Chinese hamster lung fibroblasts)
___ other (list):

Properly maintained? (Y) / N (circle one)
Periodically checked for Mycoplasma contamination?
Y / (N) (circle one)
Periodically checked for karyotype stability?
Y / (N) (circle one)
Periodically "cleansed" against high spontaneous background?
(Y) / N (circle one)

007158

MAMMALIAN CELLS IN CULTURE GENE MUTATION

5. Locus Examined:
   X thymidine kinase (TK)
   selection agent: _____ bromodeoxyuridine (BrdU)
   (give concentration) _____ fluorodeoxyuridine (FdU)
   3 ug/ml trifluorothymidine (TFT)

   ___ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
   Selection agent: _____ 8-azaguanine (8-AG)
   (give concentration) _____ 6-thioguanine (6-TG)

   ___ Na^+/K^+ ATPase
   Selection agent: _____ ouabain
   (give concentration)

   ___ other (locus and/or selection agent; give details):

6. Test compound concentrations used:
   Non-activated conditions:
   5, 10, 15, 20, 25, 30, 35, and 40 ug/ml

   Activated conditions:
   0.5, 1, 10, 20, 40, 60, 80, and 100 ug/ml

B. TEST PERFORMANCE

1. Cell treatment:
   a. Cells exposed to test compound for:
      4 hours (non-activated) 4 hours (activated)

   b. Cells exposed to positive controls for:
      4 hours (non-activated) 4 hours (activated)

   c. Cells exposed to negative and/or solvent controls for:
      4 hours (non-activated) 4 hours (activated)

   d. After washing, cells cultured for 2 days (expression period) before cell selection

   e. After expression, cells cultured for 12 days in selection medium to determine numbers of mutants and for 12 days without selection medium to determine cloning efficiency

2. Protocol (brief description, or attach copy to appendix, if appropriate; include e.g. number of cell cultures; medium; incubation times; cell density during treatment; number of cells seeded for treatment and selection; subculture and feeding schedules, if necessary):

The procedures used were based on the method of Clive et al. (Mutation Res. 31: 17-29, 1975) with minor modifications of Amacher et al. (Mutation Res. 64: 391-406, 1979) and Oberly et al. (Mutation Res. 105: 439-444, 1982). The details of test procedures (Appendix B) were attached.

3. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; reported results, e.g. cytotoxicity and solubility):

The toxicity test was conducted with suspension cultures of TK competent L5178Y cells using 8 concentrations of benefin (i.e., 1, 10, 25, 50, 100, 250, 500, and 1000 ug/ml with and without metabolic activation). After a 4-hour exposure period, suspension growth of each treated cultures was monitored over a two-day period. In the nonactivated test, benefin concentrations of 50, 100, 250, 500, and 1000 ug/ml were excessive cytotoxic and destroyed the cell culture. In the test with metabolic activation, benefin concentrations of 250, 500, and 1000 ug/ml were also cytotoxic and destroyed the cell culture (Table 1 attached). Therefore, the highest concentration of benefin selected for the mutation assay with or without metabolic activation was determined to be 100 ug/ml and 40 ug/ml, respectively.

007158

MAMMALIAN CELLS IN CULTURE GENE MUTATION

4. Mutagenicity assay (reported results, e.g. induction of mutant colonies - individual colony counts and/or summary given; mutant frequencies per 10^6 survivors; positive and background mutant frequencies; inclusion of concentration levels used; number of cultures per concentration; levels of cytotoxicity obtained; appropriateness of cloning efficiencies; include representative table, if appropriate):

In this study, no statistically significant increases in mutant frequency (mutants/10^6 clonable cells) above the negative (solvent) controls were observed in the benefin-treated cultures either in the presence or absence of metabolic activation (i.e., The criterion for determining a positive response was based on a dose-related response in which the mutation frequency at two or more successive test concentrations was at least two-fold higher than the mutation frequency of the solvent-treated control). Therefore, benefin technical showed no evidence of mutagenic activity in this in-vitro gene mutation assay in cultured mouse lymphoma L5178Y cells with or without metabolic activation at the concentrations tested (Summary of results presented in Table 5 attached).

MAMMALIAN CELLS IN CULTURE GENE MUTATION

5. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

(A) The positive control compounds (EMS and 3-MC) induced significant increases in mutation frequency with respect to the corresponding negative controls by a mutation factor (mutation index) of at least 10.6 (i.e., EMS, 28.7; 3-MC, 10.6) indicating the assay was sensitive to known mutagens in the absence or presence of metabolic activation.

(B) The highest concentration without activation (25 ug/ml) that provided mutation data only had a 35% total survival (top range for acceptable is about 10-20% survival): without activation portion not scored high enough, i.e. no mutant frequencies scored at concentrations above this. Although 30 and 35 ug/ml concentrations had survivals of 9 and 8%, respectively, data from these would be useful to help determine if there is a trend or not. If this information could be obatined, then a repeat of the testing may not be necessary.

(C) Therefore, the study is unacceptable in the present form. However, it may be upgraded to acceptable upon receipt of mutation data for 30 and 35 ug/ml concentrations under non-activated conditions.

6. Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)

7. CBI appendix attached Y / (N) (circle one)

BENEFIN TOX REVIEW 007158

Page_____ is not included in this copy.

Pages 113 through 121 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

607158

SALMONELLA

3. Mutagenicity assay (reported results, e.g. induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate):

The mutagenicity of benefin in the Salmonella/mammalian activation gene mutation assay was evaluated using 5 strains of histidine dependent auxotrophic mutants of S. typhimurium (TA1535, TA1537, TA1538, TA98 and TA100) either in the presence or absence of S9 mix. Results for the non-activated mutation assay show that counts of revertant colonies for each tester strain treated with benefin were not different than the corresponding DMSO-treated controls at the concentrations tested (i.e., 62.5, 125, 250, 500 and 750 ug/plate). Results for the activated mutation assay show that counts of revertant colonies for each tester strains treated with benefin were also not different than the corresponding DMSO-treated controls at the concentrations tested (i.e., 25, 50, 100, 200, and 300 ug/plate). The strain specific control compounds (MNNG, 2NF, and 9-AmAc) and the positive control compound (2AA) to ensure the efficacy of the activation system have given the positive responses as expected (Results presented in Table 3 attached).

007158

SALMONELLA

4. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

(A) The spontaneous revertant colonies for each of the five tester strains of Salmonella typhimurium are found within the normal ranges of revert colonies recommended by the Salmonella/mammalian activation gene mutation assay (Ames et al., Mutation Res. 31: 347-364, 1975).

(B) The strain specific control compounds (MNNG, 2-NF and 9-AmAc) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given significant positive responses over the corresponding negative (solvent) control values (at least 10-fold increase in the number of revertant per plate over the average value of the solvent control for the respective strain). These positive control values demonstrated the sensitivity of the assay system with or without metabolic activation.

(C) Results indicate negative response under tested conditions. Acceptable.

5. Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)

6. CBI appendix attached Y / (N) (circle one)

BENEFIN TOX REVIEW 007158

Page_____ is not included in this copy.

Pages 124 through 128 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007158

Guideline Series 84: Mutagenicity

Reviewed by: John H.S. Chen 11/1/88
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Kerry Dearfield 11/3/88
Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: In-Vivo Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters

Tox. Chem. No.: 130

MRID No.: 160864

EPA File Symbol:

Test Material: Benefin (EL-110, Compound 54521; Lot No. 2312F4; 97.3% Purity)

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

Study Number(s): 850722SCE2598

Sponsor: Division of Eli Lilly and Company, Greenfield, Indiana

Testing Facility: Lilly Research Laboratories, Greenfield, Indiana

Title of Report: The Effect of Benefin (EL-110, Compound 54521) on the In-Vivo Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters

Author(s): G.R. Koenig, S.B. Neal and G.S. Probst

Report Issued: November 7, 1985

Conclusions:

Benefin did not induce Sister Chromatid Exchange (SCE) in bone marrow of Chinese hamsters at the dose levels tested.

Dose levels: 200, 300, 400, and 500 mg/kg

Classification of Data: Unacceptable
(Deficiencies: Sample sizes and MTD determination)

Title of Study: The Effect of Benefin (EL-110, Compound 54521) on the In-Vivo Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters. Study No. 850722SCE2598, November 7, 1985

I. Materials and Methods:

1. Test Materials

The test material, Benefin technical (EL-110, Compound 54521; Lot No. 231-F4; 97.3% Purity), was suspended in 10 percent aqueous acacia to the appropriate concentrations. Cyclophosphamide was dissolved in 10 percent aqueous acacia and served as positive control.

2. Test Animals

The study consisted of 15 female Chinese hamsters (Cricetulus griseus; 32-39 g). Animals were housed individually in shoe-box cages under an environmentally controlled conditions. Purina laboratory rodent chow and tap water were provided ad libitum. Number of animals in each test group was given below:

Dose Group	No. of Animals Female
Solvent control	2
Benefin 200 mg/kg	3
" 300 "	3
" 400 "	3
" 500 "	3
Cyclophosphamide (50 mg/kg)	1
Total	15

3. In-Vivo Assay for Sister Chromatid Exchange

The assay was conducted according to a modification of the method of Neal and Probst (Mutation Res. 113: 33-43, 1982). After 5 hours following the subcutaneous implantation of a Difco Bacto agar coated tablet of 5-bromodeoxyuridine (BrdUrd) into the shaved abodomen of hamsters according to the BrdUrd tablet method of Allen et al. (Cytogenet. Cell Genet. 18: 231-237, 1977) with the agar coating

modification of King et al. (Mutation Res. 97: 117-129, 1982), the animals were treated orally by a single dose of benefin technical or a positive control agent (a suspension in a volume not exceeding 10 ml/kg). Nineteen hours after chemical treatment, Velban (1 mg/kg, i.p.) was administered to arrest cells in metaphase and 2-hour thereafter the animals were sacrificed. Bone marrow, which was flushed from femurs of each animal, was suspended in 0.075 M KCl (37°C) for hypotonic treatment, fixed in methanol-acetic acid (3:1, v/v). Metaphase preparations were made by standard air drying techniques. The dried slides containing metaphase chromosomes were maintained in the dark for at least 24 hrs prior to staining by a modification of the fluorescence plus Giemsa technique of Perry and Wolff (Nature 251: 156-158, 1974). The fluorescene plus Giemsa staining technique used in this study included the following steps: Slides were stained with a solution of bisbenzimide (H33258; 1 ug/ml), rinsed in distilled water, and irradiated with UV light of 366 nm. Following the development of the fluorochrome-UV-light of reaction at 60° C in 10 X SSC (1.5 M sodium chloride in 0.15 M sodium citrate for 20 min.), the slides were stained with 3% Giemsa.

4. Criteria for A Positive Response

At least 25 differently stained metaphases of the second cell cycle with BrdUrd were scored per animal for sister chromatid exchange. A positive response for this study, the test material must induce a dose-related increase in SCE frequency in which at least two doses were statistically different from controls as determined by Dunnett's t-test ($P<0.05$).

II. Reported Results:

1. As shown in Table 2 (Cell Cycle Analysis of Metaphase Figures), the study authors concluded that "... Slight cytotoxicity, characterized by an increase in the number of first division metaphase figures, was evident in two of three animals receiving 500 mg/kg of benefin. This pertubation of the cell cycle was considered to be a slight cytotoxic of the compound."

2. In the groups treated with various doses of benefin technical (i.e., 200, 300, 400, and 500 mg/kg), No statistically significant difference of the number of SCE's was found in comparison with the negative (solvent) control value (Tables 1 and 3).

3. By contrast, the positive control, cyclophosphamide, induced a statistically positive response ($P<0.05$) for SCE induction when compared with the negative (solvent) control value (Tables 1 and 3).

III. **Reviewer's Discussion/Conclusions:**

1. The positive control compound, cyclophosphamide (50 mg/kg), adequately demonstrated the sensitivity of the bone marrow of Chinese hamsters for the detection of induced SCEs in the intact target animal.

2. The spontaneous number (mean) of SCEs/metaphase in the negative (solvent) control (1.3/metaphase) was found within the acceptable range of background SCE frequency for the Chinese hamsters.

3. However, the evaluation of the mutagenicity of benefin technical by measuring its ability to induce SCE formation in the bone marrow of Chinese hamsters cannot be accomplished due to the following reporting deficiencies:

   i. The sample sizes (number of animal as well as metaphase cell counted) appear inadequate for this study. Generally, at least five male and five female animals per experimental and control group should be used in this study. Rationale for choosing single sex and less number of animals in this study was not given.

   ii. Although a slight cytotoxicity, characterized by an increase in the relative number of first division metaphase figures and by a decrease in the relative number of second division metaphases (i.e., about 50% reduction in M_2 cells required for the indication of cytotoxicity in the absence of toxicity to the test animal), was observed in animals receiving 500 mg/kg of benefin (Table 2), other results of initial toxicity tests with benefin technical were not included in this report. Since there was no toxicity evidenced either by animal morbidity (including death LD_{50}) or target cell toxicity (mitotic index) at the 500 mg/kg group, it is questionable whether a maximum tolerated dose of benefin was chosen for this study.

4. Therefore, the submitted report is incomplete and unacceptable in the present form. However, the study may be upgraded on resolution of the reporting deficiencies.

BENEFIN TOX REVIEW

Page_____ is not included in this copy.

Pages 133 through 135 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007158

Guideline Series 84: Mutagenicity

Reviewed by: John H.S. Chen 9/26/88
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Kerry Dearfield 9/30/88
Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: DNA Repair Assay in Rat Hepatocytes Tox. Chem. No.: 130

MRID No.: 160865 EPA File Symbol:

Test Material: Benefin (EL-110, Compound 54521; Lot No. 2315F4; 97.3% Purity)

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

Study Number(s): 850716UDS2598 and 850723UDS2598

Sponsor: Division of Eli Lilly and Company, Greenfield, Indiana

Testing Facility: Lilly Research Laboratories, Greenfield, Indiana

Title of Report: The Effect of Benefin (EL-110, Compound 54521) on the Induction of DNA Repair Synthesis in Primary Cultures of Adult Rat Hepatocytes

Author(s): G.R. Koenig, L.E. Hill, and G.S. Probst

Report Issued: October 29, 1985

Conclusions:

Benefin did not cause DNA damage and inducible repair in the rat hepatocyte unscheduled DNA synthesis assay at the concentrations tested.

Concentrations tested: 0.5, 1, 5, 10, 50, 100, 500, and 1000 ug/ml (50 through 1000 ug/ml were found to be too toxic to be evaluated for UDS)

Classification of Data: Acceptable

007159

Title of Study: The Effect of Benefin (EL-110, Compound 54521) on the Induction of DNA Repair Synthesis in Primary Cultures of Adult Rat Hepatocytes. LRL Study No. 850716UDS2598 and 850723UDS2598, October 29, 1985

I. Materials and Methods:

1. Test Materials

The test material, Benefin (EL-110, Compound 54521; Lot No. 231EF4 ; 97.3% Purity) was dissolved in DMSO and diluted in serum-free media to the appropriate concentrations. N-methyl-N'-nitro-N-nitrosoquanidine (MNNG) and 2-acetyl-aminofluorene (2AAF) were also tested and served as positive controls.

2. Medium

Williams' Medium E buffered with 0.05 M HEPES and supplemented with 10% fetal bovine serum, 50 units/ml gentamicin and 100 units/ml each of penicillin and streptomycin.

3. Indicator Cells

Primary rat hepatocytes derived from the livers of a 200 g and a 195 g male Fischer rats were used in this study.

4. Preparation of Rat Hepatocytes

The procedure used for obtaining rat hepatocyte cultures was essentially that of Williams et al (In Vitro 13: 809-817, 1977). Each liver was perfused first with HBSS containing 0.5 mM EGTA and then with 0.05 M HEPES (pH 7.2) that contained 100 units/ml collagenase. Cells were detached by combing in fresh 0.05 M HEPES - collagenase medium followed by sequential filtration through 80 mesh nylon. The cells were counted, and seeded into 26 X 33 mm multiplates containing 10.5 X 22 mm plastic coverslips (2.33×10^4 cells/cm^2).

5. UDS Assay

The test material, Benefin, was tested at 8 selected dose levels ranging from 0.5 to 1000 ug/ml (i.e., 0.5, 1, 5, 10, 50, 100, 500, and 1000 ug/ml). The assay was conducted using two independent hepatocyte preparations. MNNG, at 1, 5, 10, and 20 ug/ml and

2AAF, at 0.05, 0.1, 0.5 and 1 ug/ml, were used as the positive controls. Each test material and control plates received ^{3}H-thymidine at a final concentration of 10 uCi/ml (2.5 hrs. after cell seeding). Cells were exposed to chemicals for 20 hours. After the exposure period, the cultures were washed and prepared for fixation. Hepatocytes grown on plastic coverslips were washed in serum-free WME, swelled in 1% sodium citrate and fixed in ethanol/acetic acid (3:1). The coverslips were removed, allowed to dry and glued with permount to glass slide, then stained with 1% aceto-orcein for 3 to 5 minutes. The stained slides were air-dried, and individually dipped into undiluted NTB-2 liquid photographic emulsion (East Kodak), developed with Kodak D-19 developer and fixed with Kodak fixer.

6. Scoring

The number of silver grain over the cell nucleus was counted using a semi-automated Artek Model 880 Colony Counter, adopted for oil immersion microscopy. Cytoplasmic background counts were determined by counting three nuclear-sized areas adjacent to the nucleus. The net nuclear grain count represents the difference between the gross nuclear grain count and the mean cytoplasmic background count. Nuclei of 20 morphologically unaltered cells, judged to be representative of the UDS responsiveness of the cell population and containing at least 4 grains, were counted for each treatment. Autoradiographic grain counts were conducted for the highest compound concentration that did not produce pronounced cytotoxicity and for all lower concentrations of the test compound.

7. Criteria for A Positive Response for UDS

A compound was judged to have induced a positive response for UDS when at least two successive concentrations of the test compound produced nuclear grain counts which exceeded those of the control by three standard deviation of the control value.

II. Reported Results:

1. Cytotoxicity was evident in cultures treated with benefin at concentrations of 1000, 500, 100, and 50 ug/ml. Chemical precipitation also occurred at 1000 and 500 ug/ml treatment. These observations were noted in both assays.

2. Control cultures treated with 1% DMSO, showed neither cytotoxicty nor induction of UDS. There was no indication of UDS observed from treatment with benefin at concentrations of 10, 5, 1, and 0.5 ug/ml in both assays (Table 1 attached).

3. A positive autoradiographic response for UDS was noted in cultures treated with either the MNNG or 2AFF. Cytotoxicity resulted from the treatment of MNNG and 2AAF at the 20 and 1 ug/ml treatments, respectively. This effect was noted in both assays (Table 1).

007158

III. Evaluation and Recommendation:

1. The nuclear labeling in the negative control (1% DMSO) was found within the normal range of net nuclear grain count per nucleus for performing the rat hepatocyte UDS assay as recommended by Williams (i.e., none of nuclei containing 1 or more net nuclear grains per nucleus; Williams, G.M., Chemical Mutagen Vol. 6, 61-79, 1979).

2. The positive control compounds, MNNG at 5 ug/ml and 10 ug/ml, and 2AAF at 0.05 ug/ml, 0.1 ug/ml, and 0.5 ug/ml, induced a significant increase in net nuclear grain count (i.e., MNNG: 13.77-41.75 grains/nucleus; 2AAF: 21.42-58.88 grains/nucleus; 1% DMSO: less than 1 grain/nucleus) demonstrating the sensitivity of the assay to detect a DNA-damaging response.

3. The test material has been tested to cytotoxicity level (i.e., cells unevaluable to UDS at 50, 100, 500, and 1000 ug/ml).

4. Since the mean net nuclear grain count per nucleus for the Benefin-treated hepatocytes was also found to be less than one at the dose levels tested (0.5 through 10 ug/ml), the test material, Benefin, did not cause any increase in net nuclear grain count over the negative solvent control value.

5. Therefore, the test material, Benefin, was inactive in the unscheduled DNA synthesis in primary hepatocytes at the dose levels tested (0.5, 1, 5, and 10 ug/ml). The study is acceptable.

12

TABLE 1. THE AUTORADIOGRAPHIC IDENTIFICATION OF UNSCHEDULED DNA SYNTHESIS IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES TREATED WITH BENEFIN. STUDIES 850716UDS2598 AND 850723UDS2598.

Compound	Concentration Tested µg/ml	Net Nuclear Silver Grains (Mean ± SD)[a] Study 850716	Study 850723
Benefin	1000	Toxic[b,e]	Toxic[b,e]
	500	Toxic[b,e]	Toxic[b,e]
	100	Toxic[b]	Toxic[b]
	50	Toxic[b]	Toxic[b]
	10	-1.06 ± 1.48	-1.18 ± 0.70
	5	-1.67 ± 0.97	-1.78 ± 2.42
	1	-0.92 ± 1.15	-1.76 ± 1.93
	0.5	-0.97 ± 0.93	-1.16 ± 2.28
MNNG	20	Toxic[b]	Tox/Pos[c]
	10	26.15 ± 8.81[d]	41.75 ± 8.25[d]
	5	13.77 ± 7.79[d]	14.74 ± 7.44[d]
	1	0.99 ± 2.17	0.53 ± 2.79
2AAF	1	Tox/Pos[c]	Tox/Pos[c]
	0.5	58.88 ± 16.86[d]	48.70 ± 10.06[d]
	0.1	27.90 ± 8.88[d]	21.42 ± 6.87[d]
	0.05	8.90 ± 5.46[d]	5.87 ± 4.63
DMSO (Four replicate cultures)	1%	-1.09 ± 0.92	-1.81 ± 1.56
	1%	-0.33 ± 0.88	-1.32 ± 1.25
	1%	-1.21 ± 1.77	-0.78 ± 1.43
	1%	-0.70 ± 1.10	-1.13 ± 1.15

[a]Represents counts from 20 morphologically unaltered nuclei from each treatment.
[b]Cytotoxic:cells unevaluable for UDS.
[c]Cytotoxic:surviving cells positive for UDS.
[d]Judged to be a positive response for UDS.
[e]Chemical precipitation.

81-6 - Guinea Pig - Dermal Sensitization

007158

9/30/88

Reviewed by: John H.S. Chen
Section I, Toxicology Branch II (TS-769C)
Secondary reviewer: Quang Q. Bui
Section I, Toxicology Branch II (TS-769C)

DATA EVALUATION REPORT

Study Type: Skin Sensitization in Guinea Pigs

Tox. Chem. No.: 130

MRID No.: 144283

EPA File Symbol:

Test Material: Technical Benefin (Compound 54521; Lot No. X35746; 98.2% Purity)

Synonyms/CAS No.:

Study Number(s): GL796

Sponsor: Division of Eli Lilly and Company, Greenfield, Indiana

Testing Facility: Lilly Research Laboratories, Greenfield, Indiana

Title of Report: A Guinea Pig Sensitization Study of Benefin (Compund 54521)

Author(s): G.R. Koenig and C.L. Mattingly

Report Issued: March 13, 1984

Conclusions:

Benefin technical (Compound 54521; 5% in ethanol) is a skin sensitizer

Classification of Data: Core Guideline

007158

Title of Study: A Guinea Pig Sensitization Study of Benefin (Compound 54521). LRL Study Report No. GL796

I. Materials and Methods:

1. Test Material

The test material, Benefin (Compound 54521; Lot No. X35746; 98.2% Purity), was dissolved and diluted in ethanol to the appropriate concentration (5%). Dinitrochlorobenzene(DNCB; Lot No. 83F-0036) dissolved in ethanol was used as the positive control.

2. Test Animal

Twelve female albino guinea pigs of the Hartley strain (10 to 14 weeks old; 330.5 - 24.8 grams) per group were used in this study. The six treatment groups were identified as follows: I. Induction control: 95% ethanol at doses of 0.2 ml; II. Challenge control: 95% ethanol at doses of 0.2 ml; III. Positive induction control: 0.1% dinitrochlorobenzene at doses of 0.2 ml; IV. Positive challenge control: 0.1% DNCB(0.2 ml) V. Induction by treatment: 5% benefin in 95% ethanol at doses of 0.2 ml;VI. Challenge by treatment: 5% benefin in 95% ethanol at doses of 0.2 ml. These animals were housed in groups of three in suspended cages with wire floors under an environmentally controlled conditions. They had free access to water and a Purina guinea pig diet No. 5025.

3. Treatment

The protocol employed in this study was the modified Buehler topical patch method described by Buehler (Archives of Dermatology 91: 171-177, 1965). The guinea pigs were prepared for treatment by clipping the hair in the nuchal area with Oster clippers and swabbing the exposed skin with acetone. Each induction treatment was to the nuchal area, which was occluded with a 1.5 inch square patch held in place with adhesive tap for 6 hours. The challenge dose was administered to a previously untreated area in the center of the back of each animal, which was prepared for treatment by clipping the hair and swabbing the exposed skin with acetone. The challenge application site was treated and occluded for 6 hours as described for induction.

II. Reported Results:

1. Response to Induction: Treatment sites of all guinea pigs treated with the 95% ethanol vehicle (Group I) were negative throughout the induction

phase of the study (Table 1 attached). During DNCB induction (Group III), severe erythema and slight edema developed at the treatment sites of all guinea pigs after the sixth application (Table 1). Following the sixth application of 5% benefin very slight to severe erythema and very slight edema developed at the treatment sites of 12 and 11 pigs, respectively, and persisted to the end of induction (Table 2 attached).

2. Response to Challenge: There were no signs of sensitization or dermal irritation in any of the animals challenged with 95% ethanol (Group II)(Table 3 attached). Animals receiving induction and challenge with DNCB (Group IV) demonstrated a positive sensitization response of very slight to moderate erythema and very slight to slight edema 24 hours after challenge (Table 3). Observations conducted 48 and 72 hours after challenge confirmed a positive response. Seven of 12 animals receiving induction and challenge with 5% benefin (Group VI) demonstrated a positive sensitization response of very slight edema and/or very slight to slight erythema 24 hours after challenge (Table 4 attached). Observations conducted 48 and 72 hours after challenge confirmed a positive response.

III. Reviewer's Conclusions:

1. Evidence of delayed contact hypersensitivity was seen in 9 of 12 test animals (Guinea pigs bearing hypersensitive response were: Nos. 251, 252, 255, 257, 258, 259, 260, 261, and 262).

2. The average skin reaction scores for the negative control group (95% ethanol) were found to be 0 for all the challenge sites at 24, 48, and 72 hours post-treatment.

3. The average skin reaction scores for Benefin-treated group were found to be 1.3, 2.3, and 2.0 for the challenge sites at 24, 48, and 72 hours post-treatment, respectively.

   (Skin reaction scores were based on the combined scores for erythema and edema observed at the anterior and posterior site of each animal)

4. Based on the results reported, benefin is classified as a sensitizer.

5. Classification of Data: Core Guideline

BENEFIN TOX REVIEW 007158

Page_____ is not included in this copy.

Pages 144 through 147 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.