US EPA ARCHIVE DOCUMENT

4-4-86

MEMORANDUM

SUBJECT: Peer Review of Trifluralin

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) 4/4/86

TO: Addressees

The Toxicology Branch Peer Review Committee met on January 29 and February 27, 1986 to discuss and evaluate the data base on Trifluralin with particular references to the oncogenic potential of the chemical.

A. Individuals in Attendence: —

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber — Theodore M. Farber

William L. Burnam — William L. Burnam

Reto Engler — Reto Engler

John A. Quest — John A. Quest

Bernice Fischer — Bernice Fischer

Louis Kasza — Louis Kasza

2. Reviewers: (Non-panel members responsible for data presentation; signatures indicate technical accuracy of panel report).

R. Bruce Jaeger

3. The following persons were not able to participate in the peer review. They however reviewed the document and concur with the overall conclusions.

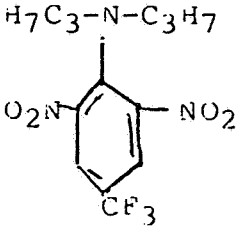
Anne Barton

B. Material Reviewed:

The material available for review consisted of a comprehensive summary of toxicology information on Trifluralin (Jaeger, memorandum dated 12/24/85), including tumor data on Trifluralin, metabolism and structure-activity information, historical control information, mutagenicity data, and previous Agency deliberations contained in Special Review PD 1/2/3 (dated 8/22/79) and PD 4 (dated 7/4/82).

C. Background Information:

Trifluralin is a dinitroaniline compound (a,a,a-Trifluoro-2, 6-dinitro-N,N-dipropyl-p-toluidine).



TRIFLURALIN

The Review Panel evaluated oncogenicity data from 2 mouse and 3 rat studies. The oncogenicity data are summarized below.

D. Evaluation of Oncogenicity Evidence for Trifluralin:

1. Mouse Oncogenicity Study of Trifluralin (NCI, 1978):

The NCI Administered Trifluralin in the diet of B6C3F1 mice for 78 weeks, after which they were observed for an additional 12 weeks on a control diet. There were 20 per sex in the control groups and 50 per sex in the dose groups of 2375 and 5000 ppm. However, it was determined that the technical trifulralin administered was contaminated with N-nitroso-di-n-propylamine (NDPA). Although hepatocellular carcinomas and alveolar/bronchiolar adenomas were observed in treated females, "the Agency concluded that the NDPA contamination of the Trifluralin used by NCI explains their findings of hepatocellular carcinoma in female mice". (PD 2/3 pg. 73, 1979).

Table 1. NCI Mouse

Tumor Site and Type	Sex	Matched Control	Pooled Control	2375 ppm	5000 ppm
Hepatocellular CA	F	0/20	0/60	12/47	21/44
Alveolar/Bronchiolar AD	F	0/19	0/59	6/43	3/30

2. Mouse Oncogenicity Study of Trifluralin (Eli Lilly, 1980).

Eli Lilly repeated the mouse oncogenicity study performed by NCI because of the contamination with NDPA. In a 2-year oncogenicity study, B6C3F1 mice were administered Trifluralin in the diet at concentrations of 0, 563, 2250 and 4500 ppm. There were 120 mice per sex in the control groups and 80 per sex per dose group. In this repeat study there was no evidence of oncogenic potential.

3. Rat Oncogenicity Study of Trifluralin (Eli Lilly, 1966).

Sprague-Dawley rats (25 per sex per group) were administered 0, 200 1000, or 2000 ppm Trifluralin in the diet for two years. There was no compound related oncogenic response. "The CAG concluded that this study showed no evidence of carcinogenicity and that the study was an adequate basis for safety evaluation". (PD 2/3, 1979).

4. Rat Oncogenicity Study of Trifluralin (NCI, 1978).

The NCI administered Triufluralin in the diet to Osborne Mendel rats for 78 weeks, after which they were observed for an additional 33 weeks on a control diet. There were 50 per sex per group administered 0, 3250 or 6500 ppm Trifluralin. However, it was determined that the technical trifluralin administered was contaminated with NDPA. Although it was negative in all dose groups, it was determined that "at the dose levels used in this experiment, the results are inadequate to demonstrate that Trifluralin is a carcinogen in Osborne Mendel Rats (NCI, 1978)". (PD 2/3, 1979).

The following Tables summarize the incidence of neoplasms of the kidney, urinary bladder and thyroid in this NCI study.

Table 2. NCI Rat

a. Primary Tumors of the kidney and urinary bladder

Dose (ppm)	0		3250		6500	
Sex	M	F	M	F	M	F
Number of rats examined	47	50	11	12	12	10
Papilloma of the bladder	3	0	1	1	1	0
Carcinoma of the renal pelvis	0	0	0	0	0	1
	3	0	1	1	1	1

° Additional tumors of the kidney in the NCI Study

Tubular - cell Adenoma	0	0	0	0	1	0
Lipoma	2	1	0	0	0	0
Hemangiosarcoma	1	0	0	0	0	0
Mixed, malignant	0	1	0	1	0	0
Hamartoma	0	1	0	0	0	0
	3	3	0	1	1	0

° Furthermore, the incidence of chronic inflammation of the kidneys was observed as follows:

40	24	4	5	10	7

The information presented here in the NCI study indicate that tumors of the urinary tract occurred with similar or greater frequency in the control as the dosed groups. However, a short-coming of the data are the few number of rats examined in the treated groups which compromises any valid comparisons between the control and dose groups. The data on thyroid tumors presented below in b. are equivocal and fail to demonstrate any possible compound effect.

b. Tumors of the thyroid

Dose (ppm)	0		3250		6500	
Sex	M	F	M	F	M	F
Numbr of rats	48	50	49	50	48	49
Foll. Cell Ad.	3	0	4	3	5	0
Foll. Cell Ca.	4	1	4	4	5	0
	7	1	8	7	10	0

5. Rat Oncogenicity Study of Trifluralin (Eli Lilly, 1980).

Fischer 344 rats (60 per sex per group) were administere Trifluralin in the diet for two years at concentrations of 0, 813, 3250 and 6500 ppm. The amount of NDPA present was determined to be less than 0.01 ppm.

Malignant neoplasms of the renal pelvis were increased in all dosed males, but significant in high dose only ($p<0.05$). There was also an increase in benign urinary bladder neoplasms in all dosed females; again significant in the high dose group only ($p<0.05$). These data are summarized in Table 3.

Table 3. Eli Lilly, Rat 1980

a. Tumors of the Transitional Epithelium of the Urinary Tract.

Dose (ppm)	0		813		3250		6500	
Sex	M	F	M	F	M	F	M	F
Number of rats	60	60	59	60	60	60	60	60
Papilloma of the bladder	0	0	1	0	1	1	1	3
Carcinoma of the bladder	0	0	0	0	0	0	0	2
Carcinoma of the renal pelvis	0	0*	2	0	3**	0	6	0
	0	0	3	0	4	1	7	5

* There was one adenoma of the convoluted tubule in Animal #51, R-97; note that R-87 and R-97 listed in various tables in this document refer to "study" R-87 and R-97 which were identical concurrent studies performed at Eli Lilly.

** There was also 1 fibrosarcoma in animal #204, R-87; and one adenoma of the convoluted tubule in animal #215, R-87.

NOTE: Urinary bladder hyperplasia was looked for histologically but, unlike the renal pelvis, there was no compound related increase in incidence. In fact, there was minimal response in all groups.

In addition to the urinary tract tumors, there was an increase in follicular cell adenomas and carcinomas of the thyroid, which were significant at the high dose in males ($p<0.05$). However, there was no significant increase or dose-related trend for females. These data are summarized below:

b. Follicular cell neoplasms of the Thyroid

Dose (ppm)	0		813		3250		6500	
Sex	M	F	M	F	M	F	M	F
Number of rats	60	60	59	60	59	60	60	60
Follicular Adenoma	1	0	0	1	3	0	10	1
Foll. papillary Ad.	2	0	0	0	2	0	0	1
Foll. cystadenoma	0	0	0	0	0	0	2	0
Foll. carcinoma	2	0	1	0	3	1	1	1
	5	0	1	1	8	1	13	3

There was no treatment related decrease in latency for kidney, urinary bladder or thyroid tumors.

The total number of tumor bearing animals is summarized below. As noted, there was no associated increase with dose.

Table 4. Tumor Bearing Animals (Eli Lilly, 1980 Fischer 344 Rat).

a. Benign Neoplasms

Dose	Sex		Combined
	M	F	
0	25/60	30/60	55/120
813	19/59	34/60	53/119
3250	21/60	21/60	42/120
6500	26/60	19/60	45/120

b. Malignant Neoplasms

Dose	Sex		Combined
	M	F	
0	22/60	14/60	36/120
813	15/59	17/60	32/119
3250	18/60	12/60	30/120
6500	19/60	4/60	23/120

c. Total Number w/Neoplasias

Dose	Sex		Combined
	M	F	
0	38/60	40/60	78/120
813	32/59	46/60	78/119
3250	34/60	32/60	66/120
6500	40/60	22/60	62/120

6. Historical Control Incidence from Eli Lilly

The following historical control data have been compiled by Eli Lilly from 24 separate oncogenicity studies started from 1976-1983 and using the Fischer 344 strain of rat.

a. Urinary Bladder Transitional Cell Hyperplasia

	Percent	Incidence
Male	0.71% (0-6.67)	5/704
Female	0.28% (0-3.57)	2/704

b. Urinary Bladder Transitonal Cell CA

	Percent	Incidence
Male	0	0/704
Female	0	0/704

c. Urinary Bladder Transitional Cell AD

	Percent	Incidence
Male	0	0/704
Female	0	0/704

d. Kidney Transitional Cell AD

	Percent	Incidence
Male	0.42% (0-3.45)	3/719
Female	0	0/721

e. Kidney Transitional Cell CA

	Percent	Incidence
Male	0	0/719
Female	0	0/721

f. Thyroid Follicular Cell AD

	Percent	Incidence
Male	1.68% (0-10.0)	12/713
Female	0.56% (0-3.57)	4/717

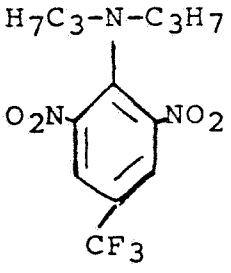
g. Thyroid Follicular Cell CA

	Percent	Incidence
Male	0.84% (0-6.67)	6/713
Female	0.14% (0-3.33)	1/717

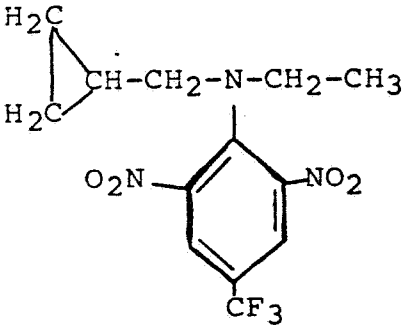
E. Additional Toxicology Data on Trifluralin

1. Strucuture - Activity correlations:

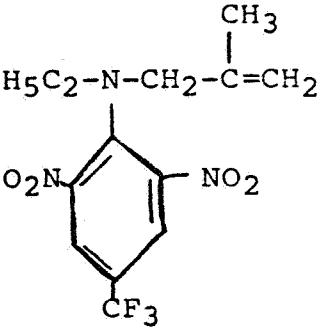
There are two other pesticide active ingredients, which are dinitroaniline compounds, that have structural similarity to trifluralin. They are profluralin and ethalfluralin:



Trifluralin



Profluralin



Ethalfluralin

Profluralin, is no longer being manfactured. Data submitted to the Agency demonstrated that, in rats, 3-35% of the administered dose is excreted in urine, with the remaining 65-70% eliminated in the feces. Subchronic 90 day feeding studies in rats and dogs demonstrated increased relative liver and thyroid weights; hemosiderosis; decreased RBC, Hgb and Hct; increased urinary bilirubin; increased incidences of progressive glomerulonephrosis, and dark brown pigment in convoluting tubules of the kidney. The NOELs were 200 and 600 ppm, respectively. Symptoms were those associated with hemolytic anemia. There were no long-term studies conducted.

Ethalfluralin demonstrated an oncogenic potential at $\geq$ 250 ppm when tested in Fischer 344 rats at dietary levels of 100, 250 and 750 ppm for 2 years. There was a dose related increase in fibroadenomas of the mammary gland at the mid and high doses in females. It was negative when tested in the B6C3F1 mouse at 100, 400 and 1500 ppm for 2 years. It was also a weakly positive mutagen in the bacterial cultures of S. typhimurium and E. coli, but negative in unscheduled DNA synthesis and L5178Y mouse lymphoma cell assays.

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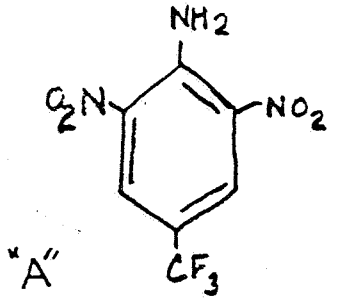
2. Metabolism

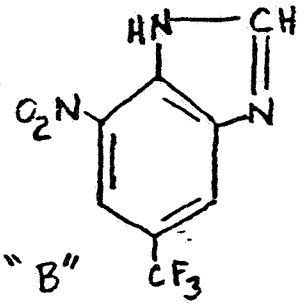
The collective data on profluralin, ethalfluralin and trifluralin demonstrate similar patterns of excretion, with approximately 25-30% of the administered dose eliminated via urine. They demonstrate hemolytic effects at higher doses, as evidence by decreased Hct, Hgb, and RBC. They all apparently cause adverse effects on the kidney at lower levels than hemolysis occurs, as evidenced by increased serum BUN and creatinine levels, with glomerulonephritis or CPN increased in severity and frequency at dose levels similar to those eliciting hemolytic effects.

a. Metabolism of Ethalfluralin

- 88-95% of the ^{14}C was excreted in urine and feces; with urine accounting for 24%;
- 31% of urinary activity due to unconjugated metabolites
- 14% of urinary activity due to glucuronide-conjugated metabolites
- No ethalfluralin detected in urine
- Urinary metabolite structures:



"A"



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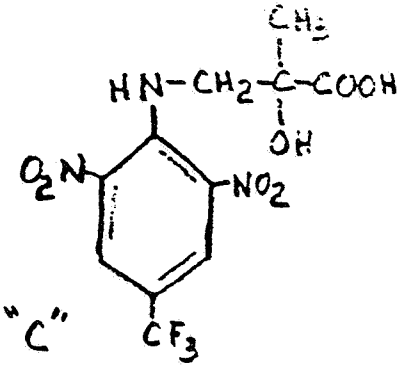
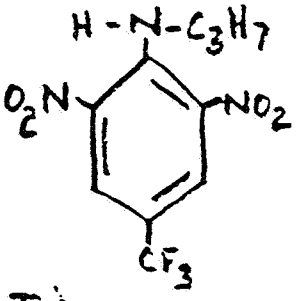


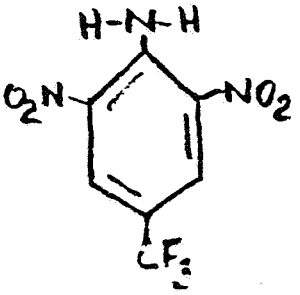
"C"

b. Metabolism of Trifluralin

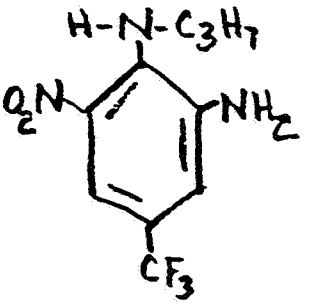
The metabolism data for trifluralin demonstrate the potential for identical metabolites to ethalfluralin to be formed in the rat. Metabolism data for trifluralin in rats and dogs, reviewed in 1967, demonstrated 80% of the administered dose was eliminated in feces and the remainder in urine. Of the 10 urinary metabolites chromatographed, only three were identified:



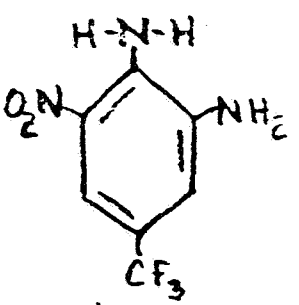
(I) Intermediate



(II)



(III)



(IV)

Two of these metabolites were administered in the diet to Harlan strain rats at 200 and 2000 ppm for 90-105 days. There were 10 M/10F per group. "Intermediate" metabolite I presented evidence of decreased Hgb at 2000 ppm. There was also hyaline degeneration of the convoluting tubules, predominantly in males, at $\geq$ 200 ppm. There was no incidence in controls. Metabolite II (also Ethalfluralin metabolite "A") demonstrated increased incidence of renal pathology with hyaline degeneration of the convoluted tubules, chronic pyelonephritis and calculi in the urinary bladder (one high dose male). There was a dose response, although some controls had very slight hyaline degeneration also. There was also "hypertrophy of the nuclei of some cells of the convoluted tubules". Severity was greater among treated males.

3. Chronic Progressive Nephrosis (CPN), Calculi and Sex Difference

The administration of trifluralin in the diet to Fischer 344 rats was associated with a significant increase in compound-related effects on the renal pelvis.

Table 6. Renal Pelvis CPN, Hyperplasia and Calculi in Fischer 344 Rat (Eli Lilly, 1980)

	Dose							
	0		813		3250		6500	
Sex:	M	F	M	F	M	F	M	F
No. of Rats:	60	60	59	60	60	60	60	60
Hyperplasia:		1	1	3	11	1	23	23
CPN:	37	27	50	23	51	21	54	50
Calculi:	3	9	8	27	20	35	43	43

The interpretation of kidney lesions in rats associated with the administration of a toxic substance is often confounded by the concomitant finding of "chronic progressive nephrosis", a common degenerative process in aging rats. J. E. Gray (CRC Critical Reviews in Toxicology, Sept. 1979) and K. Benirschke (Pathology of Laboratory Animals, Vol. I, 1978) discuss the pathogenesis of CPN and observe that it is the single most important renal disease in adult and aged rats, occurring earlier in life and with more serious manifestations in the male than in the female. The chief pathologic denominator is the presence of hyaline casts in dilated tubules. Although the pathogenesis of the lesion has not been established, two theories have been advanced. "One is that the initial lesions are due to obstruction of the tubules by protein casts, followed by cyst formation with degeneration of tubular cells. The other is that cysts result from hyperplasia of the tubular epithelial cells that occlude the lumen" (K. Benirschke). "There is evidence that a progressive degeneration is initiated and perhaps maintained by excretion of large amounts of urinary protein" (J. E. Gray). There is a dose relationship between the severity or grade of kidney degeneration and the amount of protein excreted in the urine, and this is offered as one reason why the onset and severity of CPN are greater in males than females at the same age interval. "Gray and Purmalis (Michigan State Univ. Vet., 25:83, 1965) monitored the progress of nephrosis in male and female Sprague-Dawley rats by periodic determinations of protein in 24 hour urine samples. They found that in rats from age 9 to 18 months protein excretion in female rats advanced from <1 to <5 mg/ml, while in males it advanced from <5 to <30 mg/ml urine. Histologic grading of kidney degeneration in the same animals demonstrated that nearly all female values were on the order of 1+, while males ranged from 1+ to 4+ for kidney degeneration.

The males excrete appreciably more protein than females, generally 3 to 5 times more protein per hour. Furthermore, the protein source in the male is not in the urinary bladder but in the kidney (Sellers, Goodman and Harmorston, Am. J. Physiol. 163:662, 1950). It has been suggested that the mechanism by which the kidney prevents the net passage of protein from blood to urine is just adequate in the female albino rat and not quite adequate in the normal male rat (Linkswiller et al., Am. J. Physiol., 168:504, 1952).

Subchronic studies recently conducted by Eli Lilly have been provided to the Agency which purport to demonstrate this increase in protein excretion with dose and also differences between sexes. Although these data have not undergone thorough Agency evaluation, prelimary examination by Toxicology Branch is supportive of Eli Lilly's conclusion regarding protein excretion.

F. Compound Related Effects on Survival, Body Weight and Food Consumption

The Committee examined the effects of compound ingestion on general health, survival, body weight gain, food consumption and efficiency of food utilization in the 1980 Fischer 344 Rat Study. There were no observed treatment related effects on appearance or behavior throughout the study. Survival was generally unaffected by treatment except for high dose females at 24 months.

Table 7. Survival
(60M/60F per group)

a. Males (Cumulative Mortality)

Dose (ppm)	Month 7	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	0	0	0	0	0	0 (100)	0	1 (98)	1	2	2	4 (93)	7 (88)	8 (87)	13 (78)
813	0	0	0	0	0	2 (97)	2	2	3 (95)	3	3	6 (90)	6	9 (85)	11 (82)
3250	1	1	1	1	1	2 (97)	3 (95)	3	3	3	4 (93)	8 (87)	10 (83)	13 (78)	17 (72)
6500	0	1	1	2	2	2 (97)	2	2	2	2	2	3 (95)	7 (88)	8 (87)	15 (75)

b. Females (Cumulative Mortality)

Dose (ppm)	Month 10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	0	0	0	0	0 (100)	2 (97)	2	2	5 (92)	6 (90)	7 (88)	8 (87)	9 (85)	13 (78)	17 (72)
813	0	0	0	1	2 (97)	2	2	2	2	2	4 (93)	6 (90)	8 (87)	12 (80)	15 (75)
3250	0	0	0	1	2 (97)	2	4 (93)	4	4	4	4	7 (88)	9 (85)	10 (83)	15 (75)
6500	0	0	0	0	0 (100)	1 (98)	1	3 (95)	3	5 (92)	6 (90)	9 (85)	11 (82)	16 (73)	29 (52)

( ) indicates % surviving

The mean body weight body weight data (combined from both studies R-87 and R-97), summarized below in Table 8. demonstrates no apparent compound related effect in either sex throughout the study at ≤ 813 ppm. However, at 3250 ppm there was > 10% decrease in body weight gain for males at 21 months and for females at 18 months. Both sexes demonstrated > 10% decrease at 6500 ppm at 18 months.

Table 8. Body Weight Gain (grams) (data combined from R-87 and R-97)

% in diet	Males Month 18	21	24	Females Month 18	21	24
0	476	477	448	316	335	328
813	472 (-0.8)	468 (-1.9)	431 (-3.8)	297 (-6.0)	312 (-6.9)	308 (-6.1)
3250	436 (-8.4)	420 (-11.9)	370 (-17.4)	259 (-18.0)	264 (-21.2)	255 (-22.2)
6500	396 (-16.8)	367 (-23.1)	309 (-31)	220 (-30.4)	206 (-38.5)	167 (-49.1)

( ) indicates % difference from control

In addition to the overall mean body weight changes, a comparison of body weight dosage versus efficiency of food utilization (EFU) can be found in Table 9. below. These data are reported separately for both studies, R-87 and R-97. These data demonstrate that significant decreases in body weight gain cannot be solely accounted for on the basis of EFU although there is certainly a strong influence which is both dose- and time-dependent.

Table 9. Body Weight Change vs. Efficiency of Food Utilization (EFU)

a. Study R-87

Males

Dose (ppm)	Month 0	3	6	9	12	15	18	21	24
0		17.9	10.9	8	6.4	5.4	4.6	3.9	3.3
813									*
		17.9	11	8	6.4	5.3	4.4	3.8	3
3280			*	*	*	*	*	*	*
		17.6	10.6	7.8	6.2	5.1	4.1	3.3	2.7
6500		*	*	*	*	*	*	*	*
		16.8	10.2	7.3	5.7	4.7	3.7	2.9	2.2

Females

Dose (ppm)	Month 0	3	6	9	12	15	18	21	24
0		12	7.4	5.6	4.8	4.3	3.8	3.5	3
813					*	*		*	
		12	7.3	5.5	4.6	4.2	3.8	3.4	2.9
3250		*	*	*	*	*	*	*	*
		11.2	6.9	5.1	4	3.6	3.2	2.8	2.4
6500		*	*	*	*	*	*	*	*
		10.6	6.2	4.5	3.5	3.0	2.5	1.8	1.2

* = significant decrease in b.wt. at $p \leq .05$
Numbers = EFU, amount of weight gain in grams per 100 grams of diet consumed.

b. Study R 97

Dose (ppm)	Males Month 0	3	6	9	12	15	18	21
0		17.5	10.8	8	6.2	5.3	4.5	3.8
813		17.7	10.9	8.1	6.2	5.3	4.4	3.7
3250			*	*	*	*	*	*
		17.2	10.4	7.6	5.8	4.9	4	3.3
6500		*	*	*	*	*	*	*
		16.6	10.0	7.2	5.5	4.6	3.7	2.9

Dose (ppm)	Females Month 0	3	6	9	12	15	18	21
0		11.5	7.3	5.5	4.6	4.3	3.8	3.4
813		11.4	7.2	5.4	* 4.3	* 4	* 3.5	* 3.2
3250		*	*	*	*	*	*	*
		11	6.8	5	3.9	3.5	3.2	2.8
6500		*	*	*	*	*	*	*
		10.9	6.5	4.7	3.6	3	2.6	2

* = significant decrease in b.wt. at $p \leq .05$
Numbers = EFU, amount of weight gain in grams per 100 grams of diet consumed.

In the following table, Table 10., food consumption is compiled for both studies, R-87 and R-97. These data demonstrate an initial rejection of food at 6500 ppm for both sexes for the first 7 weeks. After that, food consumption is reasonably comparable between control and treated groups, except for mid- and high-dose females at 18 months.

Table 10. Average Daily Food Consumption (Grams)

Study R-87 Dose (ppm)	Week 7	14	28	63	91 (3 mo)	182 (6 mo)	273 (9 mo)	364 (12 mo)	455 (15 mo)	546 (18 mo)	637 (21 mo)	728 (24 mo)
0 M	12.4	14.7	16.0	15.9	16.0	14.4	15.5	15.0	15.5	14.6	16.1	15.0
813	12.5	14.3	15.8	16.0	16.3	14.8	15.8	15.0	15.7	15.0	15.7	14.7
3250	11.0	13.6	15.4	15.5	15.8	14.4	15.7	15.1	15.4	14.2	14.8	13.6
6500	8.4	11.1	13.8	14.6	15.0	14.7	15.0	14.7	15.4	14.1	13.8	13.3
0 F	9.3	10.2	10.5	10.9	11	10.1	11.5	10.9	11.3	11.1	12.5	12.5
813	9.2	10.2	10.2	10.7	10.7	9.5	10.9	10.8	11.2	11.1	11.9	12.1
3250	8.4	9.3	9.3	9.3	9.9	9.4	10.0	9.7	10.7	10.0	11.3	10.9
6500	7.5	8.9	9.5	9.6	9.8	9.1	10.2	9.6	10.7	9.7	16.8	10.0
Study R-97 Dose (ppm)												
0 M	11.8	14.7	15.5	17.0	16.4	14.2	16.5	14.3	15.4	14.7	17.2	13.7
813	12.2	14.6	15.2	16.7	16.9	14.3	16.5	14.4	15.8	15.0	15.5	13.4
3250	10.6	14.1	15.3	16.2	16.4	14.5	16.1	14.5	15.2	14.4	15.1	13.1
6580	8.3	11.4	14.1	15.1	15.7	14.3	15.6	14.0	15.0	13.9	13.6	12.1
0 F	9.3	10.6	10.3	11.6	10.9	9.7	11.8	10.4	11.9	11.0	13.6	12.1
813	9.5	10.5	10.3	11.0	11.3	9.3	11.2	10.3	12.0	10.9	13.0	11.5
3250	8.3	9.6	9.5	9.7	9.9	8.9	10.4	9.4	10.5	10.0	11.2	11.3
6500	6.5	8.6	9.6	9.8	9.5	8.6	10.4	8.9	10.5	10.0	11.0	8.7

In summary, the above data demonstrate that trifluralin, administered in the diet for 2 years to Fischer 344 rats did not adversely effect behavior, appearance or survival, except at 24 months in high dose females. There were however significant decreases in body weight at doses $\geq$ 3250 ppm in both sexes. This body weight change in females is partially explained by decreased food consumption and EFU. However, in males this is not necessarily demonstrated except at the high dose near term. Although there is some demonstrated rejection or unpalatability of the diet, the body weight changes at the mid and high-doses in both sexes appears to be due primarily to compound ingestion and not compound rejection.

G. Correlation of CPN, Hyperplasia, Calculi and Neoplasia of the Kidney and Urinary Bladder.

The Agency took the position in PD 4 (July 1982) that there is no correlation between the role of micro-calcifications in the formation of urinary tract tumors associated with trifluralin treatment. The Agency concluded, therefore, that "trifluralin treatment is associated with the production of kidney and bladder tumors in rats", as well as thyroid tumors in male rats. Much of the support for this conclusion was based on evaluation of the data by Agency scientists, Dr. L. Kasza, as well as by an independent pathologist, Dr. R. R. Bates (Clement Associates, Inc.). The Committee finds no justification for differing with previous agency conclusions that "there is no [demonstrated] correlation between calculi formation and urinary tract tumors" in the 1980 Eli Lilly rat study. There is no consistent finding of calculi formation with a tumorigenic response. Further, a few urinary tract tumors were identified in the absence of any marked morphological change.

Examination of the data on target organ toxicity, evident from the biochemical, physiological and morphological changes displayed, demonstrate trifluralin has a marked effect on the reabsorptive function of the nephron, predisposing the animals to urinary tract tumors after continued long-term exposure to higher doses.

Although, there were insufficient clinical chemistry, hematological and urine analyses conducted, the changes in serum BUN, creatinine and glucose levels at all doses are clearly indicative of chronic renal failure, and in that regard bear close relationship with the human situation.

The available literature, as well as the data from the 1980 rat study, demonstrate sex differences associated with the severity and earlier onset of kidney degeneration. Such sex differences have been correlated to the greater amount of protein excreted by males compared to females. The possible increased rate of protein excretion, compound related effect on renal absorption, together with the anatomical differrences between the male and female urinary tract are reasonable explanations for the prevalence of transitional cell papillomas and carcinomas in the renal pelvis of the male, versus trans. cell papillomas and carcinomas of the bladder in the female. The calculi which were formed are clearly related to compound ingestion, but as noted in the available literature, the exact physiological process involved can only be surmized. Unfortunately, examination of urines was not performed in this study and therefore excretion of minerals, such as calcium, as well as protein, could not be confirmed. Recent data asserts these conclusions, but incomplete examination to date does not permit a firm conclusion.

There is also a dose related increase in morphological change in the kidney as evidenced by the increase in hyperplasia and severity of CPN, clearly evident at doses of $\geq$ 3250 ppm. In addition, the changes in thyroid weight and structure evidenced at higher doses, demonstrates a specific physiological response to renal dysfunction and not a primary tumorigenic repsonse to trifluralin ingestion.

Finally, there was no mutagenic response in any of the mutagenicity assays using trifluralin.

Based upon the foregoing examination of the available data and literature, trifluralin should be considered a nephrotoxic compound in the rat at doses less than 813 ppm and which, demonstrates the potential to elicit transitional cell papillomas and carcinomas of the urinary tract following prolonged exposures to higher doses, resulting from excessive pharmacological impairment of the reabsoprtive capabilities of the nephron.

The thyroid gland response at the high dose may be a compensatory pharmacological response and not a target organ tumorigenic effect. Evidence exists in the literature that an adverse efect on the kidney (demonstrated by the advanced degree of CPN) can effect iodide uptake and excessive loss of thyroxine resulting in thyroid hypertrophy or goiter. The thyroid morphological change may be a physiologic response resulting from renal dysfunction.

H. Weight of Evidence Considerations:

The Committee considerced the following facts regarding toxicology on trifluralin to be of importance in a weight of the evidence determination of oncogenic potential.

1. Trifluralin produced an increased incidence of malignant neoplasms of the renal pelvis in all dosed male rats, which was significant ($p < 0.05$) at the high dose.

2. Trifluralin produced an increased incidence of benign urinary bladder neoplasms in all dosed female rats, which was significant ($p < 0.05$) at the high dose.

3. Trifluralin produced an increase in follicular cell adenomas and carcinomas of the thyroid in male rats receiving $\geq$ 3250 ppm in the diet. The increase became significant ($p < 0.05$) at the high dose.

4. Trifluralin demonstratd a species specific effect on the urothelium of the kidney and urinary bladder in rats, but not mice. Ingestion of trifluralin in the diet of rats was associated with a dose-related increase of: (a) chronic progressive nephropathy (CPN), (b) hyperplasia of the urothelium, (c) formation of microscopic renal calculi, and (d) BUN and plasma creatinine.

5. Trifluralin was not oncogenic in the B6C3F1 mouse at doses up to 4500 ppm for 2 years.

6. Trifluralin was not oncogenic in long term Sprague-Dawley or Osborne-Mendel rat studies at doses up to 2000 and 6500 ppm, respectively.

7. There was no evidence of decrease time-to-tumor for neoplasms of the renal pelvis, urinary bladder or thyroid.

8. There was no evidence of mutagenicity in rat dominant lethal, L5178Y mouse lymphoma, Ames Salmonella typhimurium, Saccharomcyces cerevisciae, DNA repair assays, or SCE using chinese hamster cell.

9. Structure-activity relationship to other dinitroaniline pesticides demonstrated similar compound related effects on the renal pelvis and, at higher doses, effects on the thyroid and hemology as well as an oncogenic response in rodents. Metabolism data demonstrated a common urinary metabolite for trifluralin and ethalfluralin, which when isolated and administered separately, produced adverse effects on tne urothelium and formation of calculi.

10. Trifluralin was not teratogenic when administered to either rats or rabbits.

I. Classification of Oncogenic Potential:

The Committee concluded that the data available for Triflurlin provides limited evidence of oncogenicity in male and female rats. Criteria contained in the proposed EPA Guidelines (CFR, November 23, 1984) for classifiying a carcinogen in either Category B_2 or C were considered. Trifluralin met some of the criteria specified for the B_2 classification. That is, it produced tumors in at least 2 different sites; there was an increase in incidence of malignant or, combined malignant and benign tumors of the renal pelvis, and benign tumors of the urinary bladder; there was a dose-relationship; and the incidences were outside the historical control data for the Fischer 344 strain of rat, in both sexes.

Despite the above considerations, the Committee concluded that Category C (limited evidence of carcinogenicity) better characterizes the oncogenic potential of trifluralin for the following reasons: (1) the oncogenic responses observed were confined to one species and one strain; (2) the finding of malignant neoplasms of the renal pelvis occurred at all dose levels including control; (3) there were at least 2 other negative oncogenic rat studies and one (valid) negative oncogenic mouse study; (4) there was no demonstrated decrease time-to-tumor; (5) there were dose related increases in CPN, hyperplasia and urolith formation, evident at the lowest dose administered; (6) compound ingestion had significant effects on body weight gain, but not survival, at $\geq$ 3250 ppm in the diet to both sexes; and (7) there was no evidence of mutagenic activity in any of the assays performed. Because of these factors the Committee determined that there was insufficient evidence for the B_2 category and therefore, in conformity with the EPA Guidelines noted above, classified. Trifluralin as a Category C (possible human) carcinogen.

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