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

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JUL 16 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Peer Review of Terbutryn

From: Judith W. Hauswirth, Ph.D.
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769C)

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

The Toxicology Branch Peer Review Committee met on June 11, 1986 to discuss and evaluate the data base on Terbutryn. Particular attention was focused on the oncogenic potential of the chemical in Charles River CD rats.

A. Individuals in Attendance:

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

Theodore M. Farber	Theodore M. Farber
William Burnam	Wm J Burnam
Reto Engler	Reto Engler
Robert Beliles	Robert A. Beliles
Louis Kasza	Louis Kasza
Esther Rinde	Esther Rinde
John A. Quest	John A. Quest
Herbert Lacayo	Herbert Lacayo
William Dykstra	William Dykstra

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

Judith W. Hauswirth	Judith W. Hauswirth

3. Peer Review Committee Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Anne Barton

Diane Beal

Richard Hill

Stephen Johnson

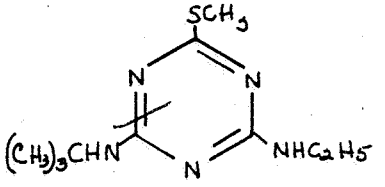
B. Material Reviewed:

The material available for review consisted of DER's on rat (Charles River CD) and mice (CD-1) oncogenicity studies of Terbutryn.

C. Background

Terbutryn is a selective herbicide registered for postemergence on winter wheat and barley; preemergence and preplant incorporated on grain sorghum; and preemergence and postemergence on fallow. It is structurally similar to several other triazine herbicides: simazine, cyanazine, propazine and atrazine.

A registration standard is presently being generated on terbutryn and it is also the subject of an NRDC reassessment and Data-Call-In.



Terbutryn
(2-t-butylamino-4-ethylamino-6-methylthio-s-triazine)

D. Evaluation of the Evidence:

1. Mouse Oncogenicity Study of Terbutryn:

a. Data Considered:

Two-hundred-forty male and 240 female Charles River CD-1 mice were initiated in this study. Sixty male and 60 female mice were placed in one of four groups: 0, 3, 1000, or 3000 ppm terbutryn in the diet. The study was conducted by International Research and Development Corporation (IRDC). The test material was terbutryn technical (ARS No. 2046/76; Batch No. FL-761552, white powder).

No treatment-related effects were seen on general behavior, appearance, body weight gain, food consumption or survival. No-treatment related non-neoplastic effects were noted in any of the male mice. Slight increases in amyloidosis were seen in various organs/tissues of female mice as well as an increase in brown pigment in the cervical lymph node. The incidence of these lesions is summarized in the following table.

Incidence of Non-Neoplastic Lesions in Female Mice

Non-neoplastic lesion	Group (ppm) 0	3	300	3000
Thyroid amyloidosis	5/58	0/0	0/0	13/57
Parathyroid amyloidosis	3/32	0/0	0/0	8/30
Adrenal amyloidosis	11/60	0/0	0/0	22/56
Stomach amyloidosis	1/60	0/3	0/9	6/60
Kidney amyloidosis	13/60	11/19	1?/19*	28/58
Lymph Node, Cervical brown pigment, reticulo-endothelial cells	8/57	0/1	0/2	26/55

* From the report it was difficult to decipher this number.

No neoplastic lesions were seen that could be considered to be treatment-related.

b. MTD Consideration:

The Committee concurred that there was no evidence of oncogenicity due to terbutryn seen in the CD-1 mouse; however, they felt that the MTD was not reached in this study. In a four week mouse study the NOEL was determined to be 3000 ppm based upon decreased weight gain, corneal opacity and eccentric pupils seen at 10,000 ppm. A more appropriate high dose for this study was considered to be 7500 ppm. The Committee decided that a repeat study was not required since 1) the highest dose tested was close to one half the expected MTD, 2) structurally related triazines have not been found to be oncogenic toward CD-1 mice (see the section on Structure Activity Relationship under E. Additional Non-oncogenic Data. of this report), and 3) there is no evidence that terbutryn is mutagenic.

2. Rat Oncogenicity Study of Terbutryn:

a. Data Considered:

Two hundred-sixty male and 260 female weanling Charles River CD rats were initiated in this study which was conducted by IRDC (Report dated March 27, 1980). They were placed in one of the groups as depicted in the table below.

Group (ppm)	Number of Rats/Group Male	Female
0	70	70
2	60	60
300	60	60
3000	70	70

Ten additional rats were placed in the control and high dose groups. Five of these rats of each sex were killed and necropsied at 12 months. The other five per sex of the high dose group were placed on control diet at the twelve month period and were killed and necropsied, along with the remaining 5 control rats, four weeks later. The test material was terbutryn technical (ARS No. 2046/76, Batch No. FL761552).

Survival was unaffected by treatment. Body weight gain for both males and females was significantly reduced in the high dose group (20% for males and 30% for females). Erythrocyte and hemoglobin values were significantly decreased at 18 months for all dosage groups and SGOT and alkaline phosphatase values were significantly elevated at the 3 and 6 month and at 12, 18, and 24 months in the high dose group, respectively.

Several variations in absolute and relative organ weights were seen at the highest dose tested. These included a statistically significant decrease in absolute spleen weights in male and female rats, increase in relative liver, kidney, brain and thyroid weights in males and females, increase in absolute and relative testes weights in males, increase in relative ovarian and adrenal weights in females and an increase in relative but decrease in absolute heart weights in male and female rats.

The incidence of neoplastic lesions seen in this study can be found in the following two tables.

Incidence of Mammary Tumors in Female Rats

	Control	Low	Mid	High	Sign. (p)[1]
# animals with adenomas[2]	6/57(10.5)[3]	9/58(15.5)	8/58(13.8)	13/55(23.6)	0.055
# animals with adeno-carcinomas[2]	15/57(26.3)	8/58(13.8)	7/58(12.1)	20/55(36.4)	0.170
# animals with fibro-adenomas[2]	12/57(21.1)	15/58(25.9)	20/58(34.5)	18/55(32.7)	0.120
# animals with adenomas and/or adenocarinomas[4]	18/57(31.6)	17/58(29.3)	13/58(22.4)	28/55(50.9)	0.03
# animals with adenomas and/or adenocarcinomas and/or fibroadenomas[4]	24/57(42.1)	29/58(50.0)	29/58(50.0)	34/55(61.8)	0.03

1 Derived from Fishers Exact test comparison of control and high dose groups.
2 These animals could have other types of mammary tumors as well.
3 Numbers in parentheses indicate percentage incidence.
4 There is no duplication of animals in these numbers. These are numbers for individual animals.

Incidence of Other tumors and Pertinent Non-Neoplastic Lesions in Male and Female Rats.

Organ/Tissue	Control	Low	Mid	High	Sign. (p)*
			Male		
Thyroid					
follicular cell					
adenoma	1/59(1.7)**	0/59(0)	0/60(0)	6/57(10.5)***	
carcinoma	0/59(0)	0/59(0)	1/60(1.7)	3/57(5.3)	
aden. + carcin.	1/59(1.7)	0/59(0)	1/60(1.7)	9/57(15.8)	0.007
hyperplasia	0/59(0)	0/59(0)	0/60(0)	2/57(3.5)	
Testes					
interstitial cell					
adenoma	13/59(22.0)	11/60(18.3)	14/60(23.3)	23/57(40.4)	0.026
hyperplasia	0/59(0)	2/60(3.3)	3/60(5.0)	0/57(0)	
			Female		
Liver					
hepatocellular					
adenoma	3/57(5.3)	2/60(3.3)	3/59(5.0)	12/56(21.4)	
carcinoma	2/57(3.5)	0/60(0)	0/59(0)	4/56(7.1)	
aden. + carcin.	5/57(8.8)	2/60(3.3)	3/59(5.0)	16/56(28.6)	0.006
focal cytomegaly	9/57(15.8)	13/60(21.7)	9/50(18.0)	18/56(32.1)	

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* Derived from Fishers Exact test comparison of control and high dose groups.
** Number in parentheses indicates the percentage incidence.
*** A thyroid adenoma was also seen in a male rat sacrificed at 12 months. This tumor is not included in this figure.

b. Historical Control Information:

Historical control data from IRDC on Charles River CD rats were available from the FDA files. These studies were initiated in the late 1970's and were designed with two control groups per study. The total number of studies the data were derived from was six. These data are summarized along with data from the control and high dose groups from the terbutryn study on the following page. Only these two groups were considered since only a high dose effect was apparent.

IRDC Historical Control Data Compared to Tumor Data Obtained in the Two Year Terbutryn Rat Study

Tumor Type	Historical Control Data Incidence	%	Range(%)	Terbutryn Data Control	High Dose
			Males		
Testes					
interst. cell and/or Leydig cell tumors	49/739	6.6	0-14	13/59(22.0)	23/57(40.4)
Thyroid					
follicular cell					
adenoma	14/731	1.9	0-3.6	1/59(1.7)	6/57(10.5)
carcinoma	2/731	0.27	0-1.8	0/59(0)	3/57(5.3)
combined[1]	16/731	2.19	-	1/59(1.7)	9/57(15.8)
			Females		
Liver[2]					
neoplastic nodules	15/750	2.0	0-8.6	3/57(5.3)	12/56(21.4)
hepatocellular carcinoma	3/750	0.4	0-1.7	2/57(3.5)	4/56(7.1)
combined*	18/750	2.4	-	5/57(8.8)	16/56(28.6)
Mammary Gland[3]					
benign tumors	415/733	56.6	31.8-82.6	14/57(24.6)	26/55(47.2)
malignant tumors	120/733	16.4	1.7-23.7	15/57(26.3)	20/55(36.3)
combined[1]	-	-	-	24/57(42.1)	34/55(61.8)

[1] The historical control data for liver and thyroid adenomas and carcinomas was combined with the assumption that no one animal had both an adenoma and a carcinoma. This assumption could be incorrect so that the combined numbers could be slightly higher than in actuality. The historical control data for mammary tumors was not combined for consideration since the likelihood of one animal having multiple types of mammary tumors is high. Individual animal data was used from the terbutryn study and, therefore, no animal was counted twice in the combined incidence data presented.

[2] In the terbutryn study the data represent the number of tumors diagnosed as adenomas. No neoplastic nodules were diagnosed in the study.

[3] In the terbutryn study the only benign mammary tumors were adenomas and fibroadenomas; the only malignant mammary tumors were adenocarcinomas.

In all cases, except for benign mammary tumors, the tumor incidence in the high dose group of the terbutryn study exceeded the upper range of the historical control data.

c. MTD Considerations:

The MTD was reached in this study as evidenced by a 20% depression in body weight gain in males and 30% in females at 3000 ppm. In addition, SGOT and alkaline phosphatase values were significantly elevated and hemoglobin and erythrocyte values were significantly decreased at several time points during the study in the high dose group.

E. Additional Non-Oncogenic Information:

1. Metabolism:

Metabolism studies have been conducted in the rat using both ring- and methylthio-^{14}C-labelled terbutryn. Eighty-five percent of the ring-labelled dose was excreted within 72 hours in urine and feces. Sixty-two percent of ^{14}C-methylthio-labelled terbutryn was recovered within 72 hours in expired CO_2.

	Percent of ^{14}C-label	
	Rat[a]	Rat[b]
CO_2	62.4	-
urine	11.1	39.7
feces	4.3	46.1
carcass	17.3	2.6
total	95.1	88.4

[a]methylthio-^{14}C-labelled terburyn
[b]ring-labelled ^{14}C-labelled terbutryn

Identified metabolites of terbutryn found in rat feces and urine are shown in Figure 1. The major pathways for metabolism are desulfuration, N-deethylation and S-demethylation.

2. Non-Oncogenic Toxicological Effects:

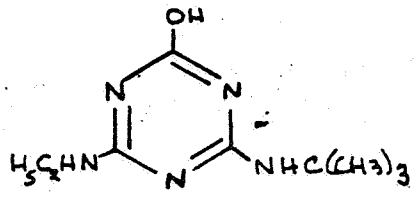
The acute oral LD_{50} for rats is 2.5 g/kg. The acute dermal LD_{50} for rabbits is >2000 mg/kg. Terbutryn induces corneal opacity.

Subchronic toxicity studies on terbutryn have not been done in the rat or dog.

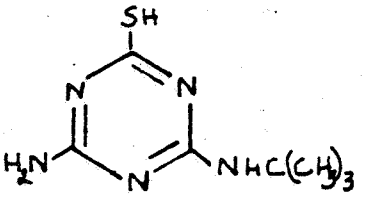
In a 6 month beagle dog study the NOEL was determined to be 10 mg/kg/day based upon mucosal thickening of various segments of the small intestine and submucosal lymphoid hyperplasia in the pyloric region of the stomach at 25 and 50 mg/kg/day. One dog placed in a four week recovery group also had submucosal lymphoid hyperplasia.

In a three generation reproduction study in Charles River-CD rats the NOEL was 300 ppm. At 3000 ppm, decreased mean body weights and food consumption values were found for the F_0, F_1 and F_2 parents as well as decreased pup

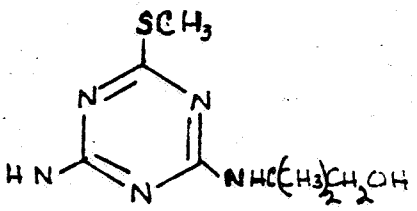
Figure 1. Metabolites of Terbutryn Identified in Rat Urine and/or Feces



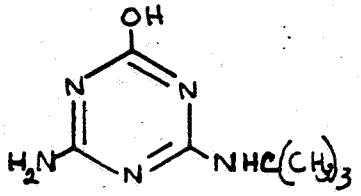
Urine 12% *
Feces 1% *



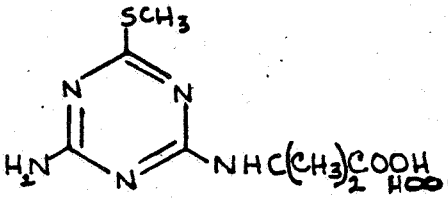
Urine 11% *



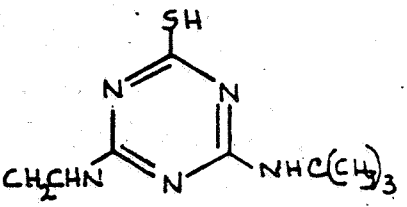
Urine 2% **



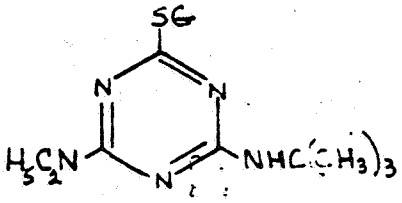
Urine 14%*
Feces 5%*



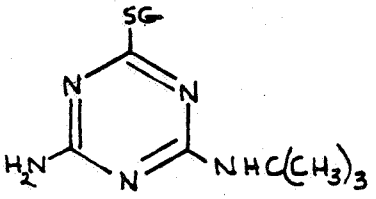
Urine 4%**



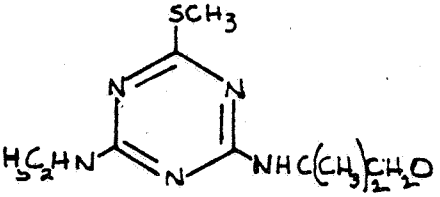
Urine 7%*



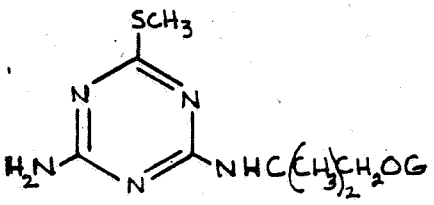
Urine 9%*



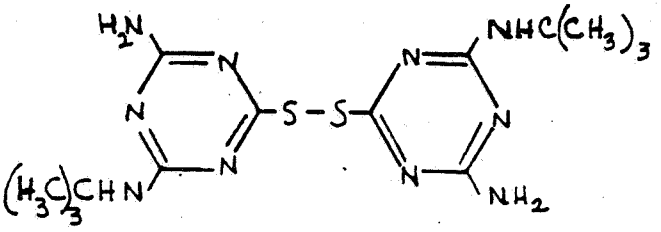
Urine 2% *



Urine 1% **



Urine 4% *
Urine 3% **



Urine 1% **

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* Isolated from rat given ring-labelled terbutryn
**Isolated from rat given Methylthio- labelled terbutryn

G = glucuronide

weights in all generations at lactation day 21.

Teratology studies on terbutryn have been conducted in rats and rabbits. Terbutryn was not teratogenic to either species. The NOEL for maternal toxicity in the rabbit was 10 mg/kg and 50 mg/kg for the rat, based upon decreased food consumption, increased food index and decreased body weight gain and stool changes in rabbits at 50 mg/kg and increased mortality, salivation, urine staining, blood discharge and weight loss at 500 mg/kg in the rat. The NOEL for fetotoxicity in the rat and rabbit was 50 mg/kg based upon reduced ossification and misalignment of the sternebrae and centrum vertebrae, reduced ossification of the metacarpals, proximal phalanges and distal phalanges of the forepaw and reduced ossification of the metacarpals and distal phalanges of the hindpaw in rats at 500 mg/kg and reduced ossification of sternebrae in rabbits at 75 mg/kg.

3. Mutagenicity

The available mutagenicity data was presented as summarized in the following table.

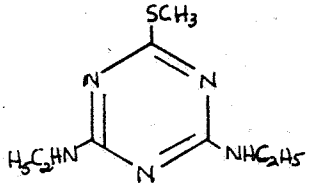
Test	Core Classification	Result	Comments
in-vivo cytogenetics in hamsters	acceptable	negative	no chromosomal aberrations in bone marrow up to 3000 mg/kg
Ames Salmonella	acceptable*	negative	negative up to solubility limit
Micronucleus	acceptable*	negative	negative at 3000 mg
Sister Chromatid Exchange	uninterpretable*	-	several reporting deficiencies

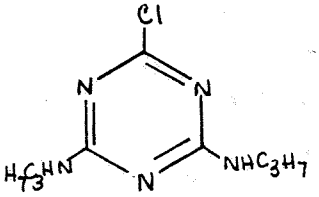
* These studies are still under review by Dynamac. The results given are from a preliminary screen.

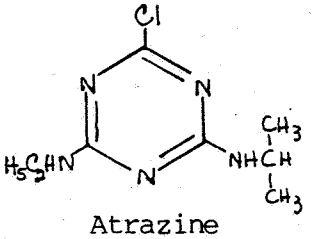
Terbutryn was not found to be mutagenic in any of the assays reported.

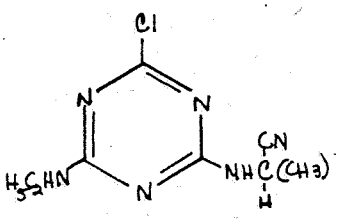
4. Structure Activity Relationship:

Terbutryn is structurally related to several other triazine herbicides, namely simazine, cyanazine, atrazine, and propazine.


Simazine


Propazine


Atrazine


Cyanazine

Oncogenicity studies have been conducted on all of these triazines but many of them are deficient and cannot be used for a weight-of-the-evidence evaluation.

a. Simazine:

Simazine is rapidly metabolized in the rat. Eighty-six percent of the labelled compound is excreted within 48 hours in the urine and feces. Characterization of metabolites has not been done. Oncogenicity studies are presently underway.

b. Cyanazine:

In rats, 87.84% of labelled cyanazine is eliminated within 4 days, 41.63% in urine and 47.21% in feces. Five and one third percent remains in the carcass. The major metabolic pathways in the rat and cow are dechlorination and deethylation.

Cyanazine did not produce chromosomal aberrations in bone marrow of mice. No other studies were available to evaluate the mutagenic potential of this compound.

Cynazine did not appear to be oncogenic to CD mice. Studies adequate to determine the oncogenic potential of cyanazine in rats were not available. A new study in the rat has just been initiated.

c. Atrazine:

Atrazine is rapidly eliminated in the rat. Sixty-seven to 72% of the label is excreted within 48 hours in urine and feces. Identification of metabolites has not been done.

Atrazine is not mutagenic in the Ames Salmonella assay or the rec assay using H 17 Rec^+ and M 45 Rec^- strains of Bacillus subtilis. These were the only assays available to judge the mutagenic potential of atrazine.

A study adequate to evaluate the oncogenicity of atrazine in mice has not been done. A 13 month interim report is available on a chronic toxicity/oncogenicity study of atrazine in Charles River Sprague-Dawley rats. An increased incidence of mammary gland adenocarcinomas was reported as follows: 0/22 (control), 1/5 (10 ppm), 1/1 (70 ppm), 0/5 (500 ppm) and 8/25 (1000 ppm). Preliminary data have also been reported for terminal sacrifice. The incidence of adenocarcinomas of the mammary gland was: 15/66 (control), 15/64 (10 ppm), 26/68 (70 ppm) 27/65 (500 ppm) and 35/64 (1000 ppm).

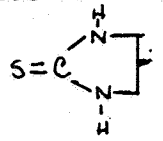
d. Propazine:

Forty-two percent of a ^{14}C-propazine dose was eliminated in the urine of rats and 28% in the feces.

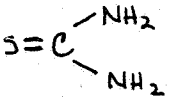
No mutagenicity information was available to the Committee for evaluation.

Propazine was negative for oncogenicity in the mouse (CD-1) but caused a statistically significant increase in mammary tumors in female CD rats. The number of mammary tumor bearing rats was 27/56 (0 ppm), 33/57 (30 ppm), 32/60 (100 ppm) and 39/55 (1000 ppm). The increase in tumors in the high dose group was significant at $p<0.05$.

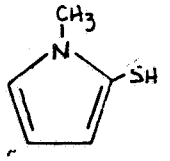
Terbutryn is also structurally similar to several thyroid inhibitors, namely thiourea, ethylene thiourea, thiouracil, propylthiouracil and methimazole, structures of which are shown below.



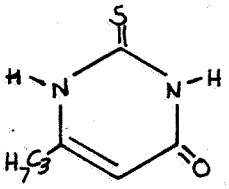
Ethylene Thiourea



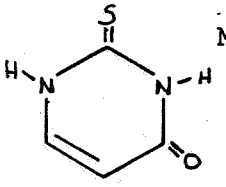
Thiourea



Methimazole



Propylthiouracil



Thiouracil

Chronic studies on thiourea have shown that it induces hepatomas and thyroid enlargement in rats. Thyroid neoplasia was not observed. In another study in rats thiourea was reported to induce malignant tumors of the face. Thiourea was negative for mutagenicity in the Ames Salmonella assay for tester strains TA 1530 and 1538 but positive in TA 100, negative for sex-linked recessive lethals in Drosophila and for UDS in rat hepatocytes, and positive for mutagenicity in S. cerevisiae D_6.

Ethylene thiourea induces hepatomas in two strains of mice (C57Bl/6XC3H/Anf and C57Bl/6X AKR) and thyroid tumors in Charles River CD rats. It has been reported to be weakly positive in the Ames Salmonella assay but these results were not reproducible. Ethylene thiourea has also been reported to be weakly mutagenic toward S. cerevisiae, and to cause an increase in chromosomal aberrations in the bone marrow of mice. It was negative for sex-linked recessive lethals in Drosophila and in the dominant lethal test in mice.

Methimazole, propylthiouracil and thiouracil all induce thyroid tumors in rats. Propylthiouracil also induces pituitary adenomas in mice and thiouracil induces hepatomas in C3H mice.

F. Weight of Evidence Consideration:

The Committee considered the following facts regarding the toxicology data on Terbutryn to be important in a weight-of-the-evidence determination of oncogenic potential.

1. When administered in the diet to female Charles River CD rats terbutryn induced a statistically significant increase in combined mammary gland adenomas and adenocarcinomas and in combined hepatocellular adenomas and carcinomas. In males, terbutryn induced an increase in combined thyroid follicular adenomas and carcinomas and in testicular interstitial cell adenomas.

2. The highest dose level of terbutryn administered to Charles River CD rats reached the maximum tolerated dose in both male and females. This was evidenced by a 20% body weight depression male rats and a 30% body weight depression female rats.

3. Historical control information from the performing laboratory (IRDC) provided additional evidence as to the biological significance of the

increased incidence of thyroid, testicular and liver tumors in the terbutryn treated rats. In all cases, except for benign mammary tumors, the tumor incidence in the high dose group of the terbutryn study exceeded the range of the historical control data.

4. Structure activity information on two structurally related triazine herbicides, propazine and atrazine, provided support for the association of mammary tumors with this class of chemicals. Propazine when administered in the diet to female CD rats, induced a statistically significant increase in mammary gland tumors and atrazine induced a statistically significant increase in mammary gland adenocarcinomas in female Sprague-Dawley rats. Furthermore, four stucturally similar thyroid inhibitors, ethylene thiourea, methimazole, propylthiouracil and thioruacil are known to induce thyroid neoplasia in rats.

5. Available information on the mutagenicity of terbutryn indicates that it is not genotoxic; however, only four assays were available for evaluation.

6. Terbutryn was not oncogenic to the CD-1 mouse.

G. Classification of Oncogenic Potential:

The Committee concurred that the classification of terbutryn, considering all of the available information, should be category C since the chemical produced 1) a marginal response in a tissue (mammary gland) known to have a high and variable background rate, 2) an increase in combined benign and malignant tumors (testicular, thyroid and liver) with an agent showing no response in a variety of short-term tests for mutagenicity (limited, but negative mutagenicity data were available), and 3) a tumor response only in one species. The Committee also considered a category B-2 classification for terbutryn since tumors were produced at multiple sites and since positive, but not conclusive, structure activity relationship (SAR) data were available on other triazines. The SAR data was not considered conclusive since, for propazine, historical control data on the mammary tumors seen in the study is still outstanding and, for atrazine, only a preliminary report on the incidence of mammary tumors was available and the final report has not been evaluated. In addition four thyroid inhibitors which are stucturally similar to terbutryn, are known to induce thyroid neoplasia. The Committee felt that a category C classification was most appropriate but that positive information in the area of mutagenicity for terbutryn and/or mutagenicity and oncogenicity for other structurally related triazines could raise terbutryn to category B-2 classification. In light of this possibility, the Committee decided that a quantitative estimation of the oncogenic potential for humans should be developed.

The dosage selection for both studies was criticized by the Committee. It appeared that the studies were designed such that a dose-response would not be seen except at the highest dosage level which is exactly what occured in the two year rat study.

There was also a discussion on the biological significance of the mammary tumors. The Committee felt that if this were the only tumor type seen, the weight-of-the-evidence would not support the positive oncogenicity of terbutryn because of the high and variable rate of these tumors. However, considering potential structure activity support for mammary tumors from propazine and atrazine oncogenicity studies in the same strain of rat, the Committee decided that in the

case of terbutryn these tumors were not only statistically but biologically significant, i.e. related to compound administration.

The appropriate combination of mammary tumors for statistical analysis was also considered. NTP in "Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation" does not recommend combining fibroadenomas and adenocarcinomas of the mammary gland. The Committee decided that the evidence for such a decision is equivocal and they elected to combine all mammary tumors for statistical analysis, in addition to combining adenomas and adenocarcinomas.

Questions arose to the possibility of a hormonal mechanism for the induction of the tumors seen in this study, especially in light of the Agency's developing policy on a threshold for thyroid neoplasia. The Committee concluded that sufficient information is not available on the mechanism of oncogenicity of terbutryn to consider a threshold for its neoplastic effects.