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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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SUBJECT: Health Effects Division (HED) Peer Review Committee
Draft Document on AMITROLE

FROM: Esther Rinde, Ph.D. E.R. 11/20/89
Manager, HED Carcinogenicity Peer Review
Science Analysis Coordination Branch
Health Effects Division (H7509c)

TO: Addressees

Attached for your review is a DRAFT document for AMITROLE. Due to the large data base on this chemical, and the complexity of the issue, this document is very long (41 pages). Therefore, I am allowing a longer comment period than usual; please provide your comments on this draft and return to me by - DEC. 15, 1989.

ADDRESSEES

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
K. Baetcke
R. Beliles
M. Copley
K. Dearfield
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W. Sette
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Y. Woo
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G. Paynter
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DRAFT

MEMORANDUM

SUBJECT: DRAFT Peer Review of Amitrole

FROM: Esther Rinde, Ph.D.
Science Analysis and
Coordination Branch
Health Effects Division (H7509c)

TO: James Yowell
Product Manager #25
Registration Division (TS-7505c)

The Health Effects Division Peer Review Committee met on July 26, 1989 to discuss and evaluate the weight-of-the-evidence on Amitrole with particular reference to its oncogenic potential.

A. Individuals in Attendance:

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

Penelope A. Fenner-Crisp _____
Reto Engler _____
Edwin R. Budd _____
Marcia Van Gemert _____
Marion Copley _____
Julie Du _____
George Ghali _____
Richard Hill _____
John Quest _____
Esther Rinde _____
Lynnard Slaughter _____

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

Caroline Gregorio/
Pam Hurley _____
Robert Zendzian/
Roger Gardner _____

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

William L. Burnam _____
Robert Beliles _____
Karl Baetcke _____
Kerry Dearfield _____
Richard Levy _____
William Sette _____

4. Other Attendees: (Observers)

O.E. Paynter (HED)
Bernice Fisher (HED)
Hugh Pettigrew (HED)
Linda Kutney (HED)

B. Material Reviewed:

The material available for review consisted of data summaries prepared by Caroline Gregorio, "Amitrol - Assessment of Oncogenic Potential" by Dr. Paynter (11/23/88), Publications: "Review - Thyroid Follicular Cell Carcinogenesis" by Dr. Hill et al. [Fund. Appl. Tox. 12 629-697 (1989)] and "Goitrogens and Thyroid Follicular Cell Neoplasia: Evidence for a Threshold Process" by Dr. Paynter et al.; Tables and statistical analysis by Burt Litt. The material reviewed is attached to the file copy of this report.

C. Background Information:

Amitrole, a Special Review chemical, was evaluated by the Toxicology Branch (1985) as a B2 Carcinogen based on liver tumors in mice and thyroid tumors in rats.

It is a non-selective herbicide used on non-crop sites: rights of way, marshes, drainage ditches, ornamentals; around agricultural, domestic and recreational areas.

FDA cancelled all crop uses in 1971.

The original reviewer (Dr. Caroline Gregorio) provided a comprehensive summary of the existing data base from which the following pages 3 through 29 are excerpted. (No DER's were available for any of the studies at the time of the Peer Review Meeting; the original studies were subsequently retrieved by Dr. Gregorio to complete the data gaps).

D. Evaluation of Oncogenicity Evidence for Amitrole:

I. Thyroid/Pituitary

1. Keller, J.G.; Hazleton (1959)
Study: 2-Yr Chronic Feeding/Onco - Rats
Strain: Charworth Farms rats
Testing Lab: Hazleton for American Cyanamid
Total Rats In Study: 35/sex/dose (interim sacrifice at 13, 26, 52 weeks)
Classification: Supplementary

Rats were given dietary levels of 0, 10, 50, and 100 ppm (equivalent to 0, 0.5, 2.5, and 5.0 mg/kg/day) for two years; another group, 500 ppm (equivalent to 25 mg/kg/day) was treated for 19 weeks and then placed on control diet due to poor weight gain; the weight loss was reversible, no pathology was reported for this group.

This study suffers from serious conduct problems, particularly in the area of the histological examination and presentation. Not all animals were examined, many were autolyzed and those which were examined were not well reported by the pathologist. The other significant problem concerning the interpretation of these data was the extremely poor reproduction of the hard copy from microfiche; entire sections, such as the Material and Methods, Results, Discussion and Conclusion were either totally missing or totally unreadable. Interim reports for the 13, 26 and 52 week sacrifices were also missing.

However, the data do show an increased incidence of thyroid neoplasia (adenoma and carcinoma combined) in both the high dose (100 ppm equivalent to 5.0 mg/kg/day) males and females at the terminal sacrifice (Table 5). It should be noted that the following table represents only those animals reportedly sacrificed at 104 weeks; the interim sacrifices were not available for evaluation.

Table 5. Neoplastic Changes in the Thyroid at 104 Weeks
Keller, J.G.; Hazleton, 1959)

	MALES				FEMALES			
Dose (ppm)	0	10	50	100	0	10	50	100
# Examined	2	1	5	11	3	8	10	15
Adenoma	0	0	1	6	0	0	1	5
Carcinoma	0	0	0	1	0	0	0	2
Total	0	0	1	7	0	0	1	7

1. Keller, J.G.; Hazleton (1959) (contd.)

High dose males and females (100 ppm) were observed to have an increase in thyroid hyperplasia (cell type not described) at 68 weeks however this trend was not seen at 104 weeks (Table 1). It should be noted that there was no detailing of the type of cellular involvement, e.g. follicular cell or parafollicular cell.

Table 1. Non-Neoplastic Changes in the Rat Thyroid[a]

	MALES							
	68 Weeks				104 Weeks			
Dose (ppm)	0	10	50	100	0	10	50	100
# Examined	3	3	3	3	2	1	5	11
Hyperplasia	0	0	1	3	0	0	1	0

	FEMALES							
	68 Weeks				104 Weeks			
Dose (ppm)	0	10	50	100	0	10	50	100
# Examined	3	1	2	3	3	8	10	15
Hyperplasia	0	0	0	3	0	0	1	1

[a] Registrant submitted individual animal data.

2. Johnson, Food Drug Research Lab (1981)
   Study: Lifetime Pulse Feeding/Onco - Rat
   Strain: Fischer 344
   Testing Lab: Food Drug Research Lab for Union Carbide
   Total Rats In Study: 75/sex/dose (interim sac. at week-24, 36, 60)
   Classification: Supplementary

Group A, control animals received no test compound, Group B rats were fed amitrole in their diet at a constant level of 5 ppm during weeks 1-39 and 100 ppm during weeks 40-115 for males or 40-119 for females. Rats in Group C, D, E received amitrole in their diet at pulsed levels (alternate 4 weeks periods) of 1, 3, and 100 ppm, respectively, during weeks 40-115 for males or 40-119 for females. On alternate months, Groups C, D, and E were fed basal diets without amitrole. For convenience, these doses will be referred to as follows: Group A = control; Group B = 5-100 ppm (equivalent to 0.25-5 mg/kg/day); Group C = 1-20 ppm (equivalent to 0.05-1.0 mg/kg/day); Group D = 3-60 ppm (equivalent to 0.15-3.0 mg/kg/day); Group E = 10-200 ppm (equivalent to 5.0-10 mg/kg/day).

Although individual and/or group mean body weights were not included in the report, the author reported that body weights for all treatment groups were similar to control animals. Increased thyroid weights (both absolute and relative to body weight) were observed for Group B and Group E males and females at the 60 week and terminal sacrifice times.

The thyroid function assay was not well performed in this study. The authors did not assay for TSH levels and thyroid hormones T_3 and T_4 were only assayed through week 104 instead of termination, which was 115 weeks for males and 119 for females. The lack of assay at termination is significant since most of the thyroid neoplasia was observed at terminal sacrifice. Contary to what would be expected based on thyroid hormone assays described in other amitrole studies (Babish, 1977) and observed in other antithyroid compounds, in this study thyroid hormone T_3 was elevated throughout the study for all treatment groups when compared to controls while T_4 values showed transient differences in mean values without any relationship to dose or time (Table 6).

Table 6. Summary of Thyroid Hormone Results (FDRL, Johnson; 1981)

Group[b]	Week 44		Week 60		Week 84		Week 104	
	T_3	T_4	T_3	T_4	T_3	T_4	T_3	T_4
Males								
A	122	7.0	119	6.9	85	7.8	67	3.6
B	173*	7.8	217	6.3	64	3.7	251*	6.9
C	124	5.8	128	6.4	78	1.9	101	5.5
D	143	6.2	187	7.8	132	3.1	59	4.7
E	298*	10.0*	320*	7.7	148	7.7	62	5.2
Females								
A	125	3.9	66	5.8	37	3.2	65	3.6
B	122	3.6	120	5.8	72	1.4	190	4.7
C	104	2.9	95	5.5	109	2.6	62	3.2
D	131	2.7	75	5.7	121	2.5	69	3.4
E	212*	4.9	275*	7.5	214	6.4	70	4.7

Group A = Control; Group B = 5-100 ppm; Group C = 1-20 ppm; Group D = 3-60 ppm; Group E = 10-200 ppm.
* "Significantly different from control, $p < 0.05$."

The authors reported an increased incidence of thyroid follicular cell hyperplasia in all treatment group males and females from week 60 of the study to termination, with the exception of the Group C females which only displayed the increase at the end of the study (Table 7).

Table 7. Thyroid Follicular Cell Hyperplasia (FDRL, Johnson, 1981)

Dose Groups	24 Wk	37 Wk	60 Wk	115/119 Wk[a]	Totals
-Male Rats					
A (Control)	0/5	0/5	0/5	0/60	0/75
B (5-100 ppm)	0/5	0/5	3/6	38/58	41/74
C (1- 20 ppm)	0/6	0/5	1/6	12/57	13/74
D (3- 60 ppm)	0/5	0/5	5/5	29/55	34/75
E (10-200 ppm)	0/5	0/5	5/6	25/60	31/76
-Female Rats					
A (Control)	0/4	0/6	0/6	0/52	0/68
B (5-100 ppm)	0/5	0/7	4/6	40/56	44/74
C (1- 20 ppm)	0/5	0/6	0/5	7/54	7/70
D (3- 60 ppm)	0/5	0/9	5/6	25/50	30/70
E (10-200 ppm)	0/5	0/9	2/5	31/56	33/74

[a]Males terminated at 115 weeks; females terminated at 119 weeks.

Treatment groups B, D and E male and female animals were reported to have an increased incidence of thyroid follicular cell adenomas and a slight increase in malignant thyroid follicular cell neoplasia (Table 8).

Table 8. Thyroid Follicular Cell Neoplasia (FDRL, Johnson; 1981)

Dose Groups	Males				Females			
	24 Wk	37 Wk	60 Wk	115 Wk	24 Wk	37 Wk	60 Wk	119 Wk
-Adenoma								
A (Control)	0/5	0/5	0/5	1/60	0/4	0/6	0/6	0/52
B (5-100 ppm)	0/5	0/5	2/6	49/58	0/5	0/7	0/6	48/56
C (1- 20 ppm)	0/6	0/5	0/6	1/57	0/5	0/6	0/5	1/54
D (3- 60 ppm)	0/5	0/5	0/6	16/55	0/5	0/9	0/6	5/50
E (10-200 ppm)	0/5	0/5	0/6	48/60	0/5	0/9	0/5	42/56
-Carcinoma								
A (Control)	0/5	0/5	0/5	0/60	0/4	0/6	0/6	0/52
B (5-100 ppm)	0/5	0/5	0/6	3/58	0/5	0/7	2/6	3/56
C (1- 20 ppm)	0/6	0/5	0/6	0/57	0/5	0/6	0/5	0/54
D (3- 60 ppm)	0/5	0/5	0/6	0/55	0/5	0/9	0/6	1/50
E (10-200 ppm)	0/5	0/5	0/6	3/60	0/5	0/9	0/5	2/56

3. Bayer AG (1979), Steinhoff (1979; 1983)
Study: Lifetime Feeding Carcinogenicity - Rat
Strain: Wistar Rat
Testing Lab: Bayer AG (West Germany)
Total Rats In Study: 75/sex/dose
Classification: Supplementary

Wistar rats were given dietary levels of 0, 1, 10, and 100 ppm (equivalent to 0, 0.05, 0.5, and 5.0 mg/kg/day) for their lifetime (maximum of 1,021 days). Body weight gain was reported to be similar for all treatment and control groups throughout the study (no individual or group mean weights were provided).
The author's also reported a "slight reduction" in survival for the 100 ppm males and females (average survival was 980 days for control animals versus 940 days for the 100 ppm treatment animals.

An increase in incidence of thyroid "cysts" was observed in the 100 ppm treatment males an females when compared to controls (Table 9). Additionally, the individual animal histology did not describe other non-neoplastic alterations generally associated with thyroid neoplasia, e.g., colloid depletion and/or hyperplasia. No other significant non-neoplastic lesions were identified.

Table 9. Thyroid "Cyst" Observations[a] (Bayer AG,1979; Steinhoff, 1979)

	MALES				FEMALES			
Dose (ppm)	0	1	10	100	0	1	10	100
# Examined	73	75	75	74	75	73	74	74
"Cysts"	1	1	1	43	1	5	2	27

[a] Registrant submitted individual rat data.

Analysis of the individual animal data showed an increased incidence in"thyroid tumors" for the high dose (100 ppm) males and females when compared to controls (Table 10). Differentiation between "malignant" or "benign" was not specified and there was no description of the cell type of tumor, e.g., follicular cell or parafollicular type.

Table 10. Thyroid "Tumor" Observations[a] (Bayer AG,1979; Steinhoff, 1979)

	MALES				FEMALES			
Dose (ppm)	0	1	10	100	0	1	10	100
# Examined	73	75	75	74	75	73	74	74
"Tumor"[b]	8	8	5	35	7	13	9	43

[a] Registrant submitted individual rat data.
[b] Individual animal data did not differentiate between malignant or benign tumor.

Additionally, an increased incidence of "pituitary tumors" were observed in all treatment male and female groups (Table 11). Again, no differentiation between "malignant" or "benign" was specified in the individual animal data.

Table 11. Pituitary "Tumor" Observations[a] Bayer Ag, 1979; Steinhoff, 1979)

	MALES				FEMALES			
Dose (ppm)	0	1	10	100	0	1	10	100
# Examined	73	74	75	74	73	74	73	75
"Tumor"[b]	4	10	10	13	14	22	18	41

[a] Registrant submitted individual rat data.
[b] Individual animal data did not differentiate between malignant or benign tumor.

In the published article (Steinhoff, 1983), the authors reported that "the percentage accumulation of radioiodine in the thyroid of 100-ppm male and female rats showed an increase at the majority of the test times. These elevated amounts of radioiodine were due not to rises in concentration but to an increase in physiologically active thyroid tissue. The proportional plasma iodine remained very largely constant, apart from a few transient variations."

4. Cox (1978); Food Drug Research Laboratories (1978)
Study: 2-Year Inhalation Study - Rat
Strain: Fischer 344
Testing Lab: FDRL for Union Carbide
Total Rats In Study: 75/sex/dose
Classification: Invalid

This study had serious conduct problems associated with the accuracy of the dose generated throughout the study and the reporting of the results. Although the data suggest that there is an increased incidence of thyroid neoplasia, these results are impossible to verify based on the information provided. For example, the target doses were grossly exceeded due to technical problems. In fact the variation in the administered dose is so great it is impossible to accurately quantify what the doses really were. Other associated problems with respect to the apparent high concentration of the test compound, suggest that the atmosphere could have been so dense that the test compound could have become lodged on the skin and likely could have been "eaten" by the animals, thereby adding the potential of dermal and oral exposure. Therefore, the significance of the results of this study are ambiguous at best and render any conclusion(s) as pure speculation.

The design of the study indicated that amitrole was to be intermittently administered to Fischer 344 rats by inhalation at levels of 0, 0.05 or 0.50 mg/L for 5 hrs/day, 5 days/week during weeks 1-13, 40-52 and 78-90. Chamber concentrations were highly variable as analyses ranged from 15.8 +/- 9.3 to 32.2 +/- 13.7 mg/L for the 0.05 mg/L group and 97.9 +/- 60.3 to 376.4 +/- 262.0 mg/L for the 0.50 mg/L group throughout the study. Control values were not assayed. Since the exact dose cannot be quantified, the terminology "low dose" or "high dose" will be used to describe the various reported results.

The authors reported that survival was significantly lower for high dose males and females when compared to control rats; all remaining rats were sacrificed at week 51. In addition, body weight and food consumption were also significantly lower for high dose animals when compared to controls.

Thyroid hormones T_4 and T_3 were assayed and reported to show significantly lower T_3 for the low dose group at 78, 91 and 104 weeks and for the high dose group at 13 weeks. T_4 levels were also lower for the 0.5 mg/L group at 13 weeks.

The authors also reported significantly higher thyroid weights at each sacrifice interval for both low and high dose male and female groups when compared to controls.

4. Cox;Food Drug Research Laboratories '78 (contd.)

Increased incidence of thyroid hyperplasia was observed in both treatment groups when compared to controls (Table 12). The pathology section did not provide separate information for interim sacrifice and final sacrifice data.

Table 12. Thyroid Hyperplasia (Cox, 1978; FDRL, 1978)

Dose (mg/L)	Males	Females
Control	2/68	0/75
Low Dose	52/75	51/75
High Dose*	61/72	63/75

*Due to poor survival, all remaining rats were sacrificed at week 51.

An increased incidence in thyroid neoplasia was observed for the low dose males and females when compared to controls (Table 13). Differentiation between the cell type of thyroid tumor was not specified, e.g., follicular cell or parafollicular type.

Table 13. Thyroid Neoplasia (Cox, 1978; FDRL, 1978)

Dose (mg/L)	Males			Females		
	Adenoma	Carcinoma	Total	Adenoma	Carcinoma	Total
Control	0/69	0/69	0/69	0/75	0/75	0/75
Low Dose	14/75	3/75	17/75	4/75	2/75	6/75
High Dose*	2/72	0/72	2/72	5/75	0/75	5/75

*Due to poor survival, all remaining rats were sacrificed at week 51.

5. Innes (1969)

Study: 18-Month Oncogenicity Screening Study - Mice
Strain: (C57BL/6 X C3H/Anf)F1 and (C57BL6 X AKR)F_1
Testing Lab: Bionetics Research Laboratories of Litton Industries
Total Mice In Study: 18/sex/dose
Classification: Supplementary

Amitrole was used as a positive control in the screening of 120 compounds for tumorigenicity. Mice were given (by stomach tube) 1000 mg/kg (6667 ppm) amitrole from day 7 to day 28 of age followed by 2192 ppm (equivalent to 329 mg/kg) in the diet for 18 months.

All amitrole treated animals either died or were sacrificed *in extremis* between 53 and 60 weeks on test. The early death of all the amitrole treated animals in this study indicate that the doses selected exceeded the Maximum Tolerated Dose (MTD) for these strains of mice.

The authors reported in a footnote of the article that "carcinoma of the thyroid were found in 64 [of 72] mice" treated with amitrole. No further data were provided in the article.

II. Thyroid and Liver

1. Innes (1969)

Study: 18-Month Oncogenicity Screening Study - Mice
Strain: (C57BL/6 X C3H/Anf)F1 and (C57BL6 X AKR)F1
Testing Lab: Bionetics Research Laboratories of Litton Industries
Total Mice In Study: 18/sex/dose
Classification: Supplementary

Amitrole was used as a positive control in the screening of 120 compounds for tumorigenicity. Mice were given (by stomach tube) 1000 mg/kg (6700) amitrole from day 7 to day 28 of age followed by 2192 ppm (equivalent to 329 mg/kg) in the diet for 18 months.

All amitrole treated animals either died or were sacrificed *in extremis* between 53 and 60 weeks on test of a designed 126 week study. The early death of all the amitrole treated animals in this study indicate that the doses selected exceeded the Maximum Tolerated Dose (MTD) for these strains of mice.

The authors reported that "hepatomas" were observed in 67 (of 72) mice treated with amitrole (Table 14). No further description of the liver neoplasia was presented in the study.

Table 14. "Hepatomas" (Innes, 1969)

Strain	Male	Female
C57BL/6 x C3H/Anf	16/18	18/18
C57BL/6 x AKR	16/18	17/18

2. Napalkov (1962)

Study:	Chronic Exposure - Rats
Strain:	"Albino mongrel rats"
Testing Lab:	Not Identified
Total Rats In Study:	Not Identified
Classification:	Invalid

Albino mongrel rats (100-120 gm) were given 125 mg (25000 ppm) amitrole by subcutaneous injection "usually twice a week" or by the drinking water as an "average of 20 to 25 mg (4000 - 5000 ppm) preparation per day" or 250 (50000 ppm) or 500 mg/day (100000 ppm) amitrole in the feed; no concurrent control group was discussed in the report. Animals were sacrificed at varying time intervals (from 152 to 691 days on test) "depending on the condition of the animal."

This study is vastly incomplete in the reporting. For example, the author states that the results in this report are based on the outcome of "several other experiments previously reported"; the data of these other experiments were not further described. The test material was not identified, e.g. source of the material is unknown; purity, etc. not discussed. The number of rats used in each dosing regime was not identified; no concurrent control animals were reportedly used in any of the experiments.

The primary focus of the study was to describe the oncogenic potential of amitrole. However, the reported pathology did not distinguish between benign or malignant types of neoplasia.

The reporting of the experimental design and the results are ambiguous at best and therefore, do not permit the reviewer to make final conclusions regarding the accuracy of the conclusions.

The author reported thyroid and liver neoplasia in all treatment regimes described in the study (Table 16). Again, the significance of these findings are very questionable.

Table 16. Liver and Thyroid Neoplasia[b] (Napalkov, 1962)

Treatment	# Examined	Liver	Thyroid
125 mg (subcutaneously)	NS[a]	5	5
20-25 mg (drinking water)	NS	6	3
250 mg (diet)	NS	8	2
500 mg (diet)	NS	10	5

[a] NS = Not Specified
[b] Author does not distinguish between malignant or benign.

III. Liver

1. Vesselinovitch (1983)

Study: Perinatal Carcinogenicity - Mice
Strain: $B6C3F_1$ mice
Testing Lab: University of Chicago
Total Mice In Study: Varying from 45 to 100
Classification: Supplementary

The purpose of this study was to research the use of neonatal and infant mice as potentially sensitive animals to chemical carcinogenicity. This study "highlights the incidence of hepatocellular adenomas and hepatocellular carcinomas observed in B6C3F1 mice following prenatal, preweaning and postweaning administration of benzidine-2HCl, safrole, amitrol, ethylnitrosurea (ENU) and diethylnitrosamine (DEN)."

Mice were fed 500 ppm amitrole (equivalent to 75 mg/kg/day) ad libitum as follows:

Group 1 - "pregnant females from the 12th day of gestation to delivery" (placentally in utero),

Group 2 - "mothers with litters from delivery to weaning" (preweaning through the mother's milk,;

Group 3 - "offspring from weaning through 90 weeks" (postweaning through the diet).

Non-treated controls were sacrificed at 52, 90 or 142 weeks.

The authors reported that "amitrol was ineffective in inducing liver neoplasia in prenatal and infant male mice [Group 1 and 2]. Only adult males [Group 3] responded to protracted amitrol treatment with development of benign and malignant liver tumors. The adult females [Group 3], however, showed only marginal neoplastic response" (Table I5).

It is unclear as to which of the non-treated control groups (those sacrificed at 52, 90 or 142 weeks) were used to make the above conclusions regarding the prenatal (Group 1)) and preweaning (Group 2) exposure scenarios. Without the benefit of identically sacrificed control animals it is impossible to make such comparisons and the author's conclusions are not adequately supported by the provided data.

Table 15. Liver Neoplastic Observations* (Vesselinovitch 1983)

Group	Number Examined	Adenoma	Carcinoma	Total	Total (%)
Males					
1[a]	74	4	2	6	8
2[b]	45	6	4	10	22
3[c]	55	9	11	20	36
Controls[d]	100	0	0	0	0
Controls[e]	98	1	0	1	2
Controls[f]	100	4	3	7	14
Females					
1[a]	83	0	0	0	0
2[b]	55	0	0	0	0
3[c]	49	5	4	9	18
Controls[d]	99	0	0	0	0
Controls[e]	96	0	0	0	0
Controls[f]	100	1	0	1	1

* Numbers taken from authors Table 1 and Table 4.
[a] Transplacental *in utero* exposure (gestation to weaning).
[b] Delivery to weaning via mother's milk (delivery to weaning, mothers).
[c] Weaning through 90 weeks via the diet (offspring).

[d] Sacrificed at 52 weeks.
[e] Sacrificed at 90 weeks.
[f] Sacrificed at 142 weeks.

ONCOGENICITY - NEGATIVE STUDIES

A. Mice

1. Bayer AG (1979), Steinhoff (1979; 1983)
Study: 18-Month Feeding/Oncogenicity - Mice
Strain: NMRI mice
Testing Lab: Bayer AG
Total Mice In Study: 75/sex/dose
Classification: Supplementary

Mice were fed 0, 1, 10 or 100 ppm (equivalent to 0, 0.15, 1.50 or 15.0 mg/kg/day) amitrole for 18 months. The authors reported that survival, body weights and food consumption were similar for all treatment and control groups throughout the study (no individual animal or group mean data were presented in the report).

Increased thyroid weights were observed in the 10 ppm male treatment group when compared to controls at final sacrifice only. The high dose (100 ppm) male and female thyroid weights were reportedly increased throughout study.

Evidence of liver toxicity was observed in treated females. A dose related increase in fatty degeneration and necrosis of the liver was observed in treated females when compared to controls (Table 17). A slight increase in liver necrosis was observed in the 10 and 100 ppm males (Table 17).

Table 17. Liver Non-Neoplastic Observations[a] (Bayer AG, 1979; Steinhoff, 1979)

	Fatty Degeneration		Necrosis	
Dose (ppm)	Males	Females	Males	Females
0	12/73	2/73	2/73	3/73
1	9/70	5/70	2/70	6/70
10	16/72	10/72	5/72	10/72
100	12/70	14/71	9/70	15/71

[a] Registrant submitted individual animal data.

A slight non-significant increase in incidence of hepatocellular neoplasia was observed for high dose females (100 ppm) when compared to controls (Table 18). Other reported neoplastic lesions were similar in incidence between treated and control groups.

Table 18. Liver Neoplastic Observations[a] (Bayer AG, 1979; Steinhoff, 1979)

	MALES				FEMALES			
Dose (ppm)	0	1	10	100	0	1	10	100
# Examined	73	70	72	70	73	70	72	71
Adenoma	7	3	5	3	2	0	0	2
Carcinoma	2	0	1	1	0	0	1	3
Total	9	3	6	4	2	0	1	5

[a] Registrant submitted individual animal data.

In the published article (Steinhoff, 1983), the authors reported results for thyroid function tests performed (percent accumulation of iodine in the thyroid, iodine concentration in the thyroid and protein-bound iodine). In this report, it was stated that there was "increased percentage accumulation of radioiodine throughout the study in the male 100 ppm group". Increases in the concentration of iodine in the thyroid were observed at 3 and 9 months but continued to decline, returning to the control level at 18 months. Transient variation in assayed protein-bound hormonal iodine was observed in the high dose (100 ppm) male and female mice.

B. Hamster

1. Bayer AG (1979), Steinhoff (1979;1983)
Study: 18-Month Chronic Feeding/Oncogenicity - Hamster
Strain: Golden Hamster
Testing Lab: Bayer AG
Total Hamsters In Study: 75/sex/dose
Classification: Supplementary

Hamsters were fed 0, 1, 10 or 100 ppm (equivalent to 0, 0.15, 1.50 or 15.0 mg/kg/day) amitrole for 2 years. The authors reported that both male and female hamsters in the high dose group (100 ppm) had lower body weights when compared to controls from the 13th or 14th month until termination at 24 months (no individual or group mean weights were provided in the report).

The provided histopathology revealed no evidence of treatment related increase of tumors of any type, including the thyroid, liver, pituitary.

Five male and female hamsters per treatment group were additionally used for thyroid function tests performed at 3, 6, 9 12 and 18 months (Steinhoff, 1983). Iodine concentration in the thyroid and protein-bound iodine measurements were unaffected by amitrole treatment in any group when compared to controls.

E. Additional Toxicology Data on Amitrole:

1. Metabolism:

I. Rats

1. Oral Exposure

a. Fang (1964): Wistar rats were fed 1 mg C^{14}-amitrole (per rat) via stomach tube. The expired air, urine, feces and tissues were analyzed for radioactivity during a three day period following dosing. During the first 24 hours, 70-95.5% of the radioactivity was found in the urine; a small variable amount of activity was found in the feces. After absorption, amitrole was distributed throughout most body tissues. The maximum radioactivity was found in liver and kidney; within three to four hours of dosing, the tissue levels began decreasing.

Paper chromatography revealed both unchanged amitrole and one unidentified metabolite in rat liver slices taken at various times following dosing.

b. Franco and Municio (1975): Male Wistar (number unspecified) rats "were treated with amitrole [unspecified amount] during 8 days by the method described elsewhere." The authors reported that "unaltered amitrole and three metabolites are present in the urine of treated animals." The metabolites were not identified or quantified.

2. Inhalation Exposure

a. MacDonald, Hazleton (1976): Rats (5/sex; Charles River Ltd.) were exposed by inhalation to an estimated dose of 25.8 ug/L for "whole body" or 49.2 ug/L for "head only" radiolabelled amitrole for one hour. Blood samples were taken at specified intervals and urine, feces and carcasses were examined for radioactivity. The results were reported as follows:

"Head Only"
- blood plasma half life was estimated to be 20 hours,
- approximately 75% of the radioactivity was found in urine,
- level of radioactivity is "substantially lower in females",
- no appreciable quantities of radioactivity found in the carcasses.

"Whole Body"
- blood plasma half life estimated to be 23 hours,
- major route of excretion was urine,
- no appreciable quantities of radioactivity found in feces and carcasses.

b. Turner, Hazelton (1976): As a supplement to the "whole body" and "head only" inhalation metabolism study (discussed above), metabolites in the urine and feces were identified by using chromatography:

Urine - 60% was presumed to be amitrole unchanged,
- 15-20% was retained at the origin,
- 5-8% was unidentified.

Feces - 56% was presumed to be amitrole unchanged,
- 25% was retained at the origin.

3. Dermal Exposure

a. Shah (1977): This preliminary study in female New Zealand white rabbits (3 animals/pesticide) was designed to obtain a comparative rate of dermal penetration of 5 radiolabelled pesticides, including amitrole. The pesticides were "applied in 0.1 ml of acetone containing 1 mgr of non-radioactive pesticide per kilogram body weight". Blood samples were taken at specified intervals up to 24 hours following treatment. Urine and feces were collected and "various organs" removed and assayed for radioactivity. After 24 hours, the site of application was swabbed with cotton and acetone.

The authors reported that "after 15 minutes, the order of penetration into blood was aminotriazole > carbaryl = parathion > malathion > DDT > dieldrin." Although the percent of dose was not reported, "appreciable quantities of aminotriazole was found in the urine, feces and gall bladder."

The amount of amitrole remaining at the site of application was estimated to be "fifty percent or more".

b. Puhl, Hazleton (1985): Young male Charles River rats (20/dose) were dermally given 0.10, 1.0 or 10 mg/kg ^{14}C-amitrole. Following application, urine and fecal samples were collected and whole carcasses were analyzed at 0.5, 1, 2 4 and 10 hours following dosing.

The results indicate that significantly high percentages of the applied dose (as calculated by urine, feces and carcass) remained on the skin: 18-25% at 0.1 mg/kg, 13-24% at 1.0 mg/kg, and 3.5-4.5% at 10 mg/kg.

The absorption rate was calculated to be less than 0.1% of the applied dose.

II. Mice

1. Oral Exposure

a. Tjalve (1975): Male and female mice (7/sex; "C57/Bl" strain) were injected intravenously with 5 uCi of C^{14} amitrole and sacrificed from 5 minutes to 5 days following treatment. Whole body radiography showed a "high accumulation of radioactivity in tissues with rapid cell turnover such as the bone marrow, the spleen, the thymus, the lymph nodes and the gastrointestinal mucosa." The authors reported the following for liver and thyroid:

Liver - "the radioactivity in the liver is irregularly distributed, being highest in the peripheral parts of the liver lobules around the portal spaces"; "radioactivity was also present in the mitochondrial and microsomal fractions",

Thyroid - "a moderate accumulation of radioactivity was found in the thyroid.

III. Humans

1. Oral Exposure

a. IARC Monographs (1974): 39-yr old woman showed no signs of intoxication following the ingestion of a commercial preparation containing 30% amitrole and 56% diuron. It was reported that 50% of the estimated dose was eliminated in urine within a "few hours" of exposure. Unchanged amitrole was found in the urine; no metabolites were identified. .

2. Dermal Exposure

a. Dynamac (1982): Five male "spraymen" were exposed to amitrole for 10 working days (5-days/week, 8-hour work days) and five males not exposed to the amitrole spraying were considered to be controls.

The medical monitoring reportedly found "no remarkable findings based on palpating the thyroids of the control or exposed subjects." The results of the thyroid function tests showed slightly higher TSH levels, slightly lower T_4 levels with basically no change in T_3 levels through the two week follow up period (Table 1). The authors reported that all the thyroid function values "were within normal limits."

Table 1. Mean Thyroid Function Results (Dynamac, 1982)

	T3	T4	TSH
Pretreatment			
Control	30	9.7	2.7
Exposed	31	8.6	2.4
Posttreatment			
Control	31	9.8	2.5
Exposed	32	8.2	3.1

SUBCHRONIC STUDIES

A. Rats

1. Oral Exposure

a. Jukes and Shaffer (1960): Rats (number and strain unspecified) were given 15, 30, 60 or 120 ppm amitrole (equivalent to 0.75, 1.5, 3.0, or 6.0 mg/kg/day) in the diet for 2 weeks. Thyroid enlargement and pronounced lowering of radioiodine uptake were reported at 3.0 and 6.0 mg/kg/day doses; no effects were reported for the 0.75 and 1.5 mg/kg/day groups.

b. Fregley (1968): Male rats (10/dose; Blue Spruce Farms strain) were given 0.25 and 0.05 ppm amitrole (equivalent to 0.01, 0.003 mg/kg/day amitrole) in the diet for 11 weeks or 0, 2, 10 and 50 ppm (equivalent to 0.1, 0.5 or 2.5 mg/kg/day) for 13 weeks. Mean body weights for all dose groups were similar to control values throughout the study.

Assessment of thyroid function revealed decreased radioiodine uptake and decreased protein-bound iodine in the 0.5 and 2.5 mg/kg/day test groups when compared to controls. Microscopic examination of the thyroids of these test group animals also revealed disturbances in follicle size and depletion of colloid when compared to control rats. Additionally, thyroid weights were significantly increased in the 2.5 mg/kg/day dose group.

No histological differences were noted in the thyroids of the 0.1 mg/kg/day group, however, decreased radioiodine and protein-bound iodine were observed.

No thyroid function or histological effects were reported for 0.01 and 0.003 mg/kg/day doses.

c. Babish, et.al.; Food Drug Research (1977): Male rats (20/dose; Sprague-Dawley) were given 0, 30, 100 and 300 ppm (equivalent to 0, 1.5, 5 or 15 mg/kg/day amitrole) in the diet for 4 weeks followed by 4 weeks of non-treated control diet.

During the 4 week treatment period, the 100 and 300 ppm treatment groups were reported to have significantly depressed body weights and food consumption which returned to normal for the 100 ppm group by the third week of the recovery phase; the 300 ppm group weights never returned to normal values although food consumption was comparable to controls during the recovery period.

In the amitrole-treatment period, serum T_3 and T_4 were significantly depressed by the end of the first week in the 300 ppm group and by the second week for the 100 ppm group. Slight depression was reported in the 30 ppm group was observed by the end of the fourth week. All treatment groups showed T_3 and T_4 values comparable to controls by the third week of the recovery period.

The T_3/T_4 ratio was increased for all treatment groups by the third week of the amitrole-treatment phase and returned to comparable control values by the third week of the recovery period.

d. Bagdon et.al. (1956): Male rats (10/dose; "albino rats") were given 0, 50, 250 and 1250 ppm amitrole (equivalent to 0, 2.5, 12.5 and 62.5 mg/kg/day) in the drinking water for 15 weeks (106 days).

A dose related decrease in mean body weight, food consumption and water intake was reported throughout the study. Microscopic examination of the liver and thyroid were reported (Table 2).

Table 2. Microscopic Examination (Bagdon, 1956)

	Thyroid[a]
Dose (ppm)	
0	-normal
50	-enlarged; follicles contain colloid
250	-hyperplasia; most follicles lack colloid
1250	-hypertrophy; hyperplasia; no follicles with stored colloid
	Liver
Dose (ppm)	
0	-scattered vacuolization (one animal)
50	-"no liver lipid" (one animal)
250	-slight fatty infiltration (one animal)
1250	-"moderate increase in hepatic cell fat (3 (animals)

[a] The report did not indicate the number of animals displaying the noted histopathological diagnosis.

e. Vidone et.al. (1958): Male "albino" rats (10/dose) were given amitrole in the diet as at 500 ppm (equivalent to 35 mg/kg/day as calculated by the registrant) for 32 days, 1000 ppm (equivalent to 75 mg/kg/day as calculated by the registrant) on alternate days for 32 days with control diet on other days, or Control (equivalent to 0 mg/kg/day).

Mean body weight and food consumption decreased for both the 500 and 1000 ppm treatment groups when compared to controls. "At autopsy, thyroid glands of the animals of the 500 ppm group appeared quite hyperemic and considerably enlarged. Thyroids of the animals fed 1000 ppm on alternate days appeared only slightly hyperemic and of approximately the same size, by inspection, as those of the controls."

f. Tsuda (1974): Female Wistar rats (number unspecified) were given 0, 2500 or 5000 ppm (equivalent to 0, 125 or 250 mg/kg) amitrole in the drinking water for 16 weeks.

The author reported that initially the thyroids showed "deformation of the thyroidal follicular epithelium, decreased colloid, dilation of the endoplasmic reticulum and decreased peroxidase activity." Finally, "at termination less than 50% of the treated animals survived and microscopic evaluation of the thyroid revealed atypical proliferation" described as "malignant adenoma".

g. Tsuda (1976): Female outbred Wistar rats (10-40 rats/treatment type) were subjected to the following treatments for 70 weeks: 2500 ppm amitrole in the drinking water (equivalent to 125 mg/kg/day) (Group 1), partial thyroidectomy and administration of 2500 ppm amitrole in the drinking water (Group 2), partial thyroidectomy plus autoimplantation of resected thyroid tissue plus administration of 2500 ppm amitrole in the drinking water (Group 3), no amitrole in the drinking water (control Group I) or partial thyroidectomy and no amitrole in the drinking water (control Group 2), or partial thyroidectomy plus autoimplantation of resected thyroid tissue without amitrole in the drinking water (control Group 3). The results are listed below (Table 3).

Table 3. Summary of Pathologic Findings in Rat Thyroids (Tsuda 1976)

Group	# Rats	# Survive	# Goiter	Invasion of Tissues	# Adenoma
Treatment with 2500 ppm Amitrole in Drinking WAter					
1	40	26	26 (100%)	19 (73.1%)	3 (11.5%)
2	30	14	14 (100%)	14 (100.0%)	1 (7.1%)
3	30	10	10 (100%)	10 (100.0%)	1 (10.0%)
Control Groups					
1	10	7	0 (0%)	0 (0%)	0 (0%)
2	10	7	0 (0%)	0 (0%)	0 (0%)
3	10	8	0 (0%)	0 (0%)	0 (0%)

The author indicated that although there was evidence of follicular tissue invasion in all treatment groups exposed to amitrole, there was no metastasis to other organs, "but such metastasis may well be possible." as the data suggest that invasive growth was more readily seen in the partial thyroidectomy treatment group. The papillary adenomas that were observed were reported to be "similar to those induced in rat thyroids by ^{131}I or x-ray".

f. Strum and Karnovsky (1971): Male Sprague-Dawley rats were given 0.04% amitrole in the drinking water. Two animals were sacrificed at 3 days, two animals were sacrificed at weekly intervals from 1 to 9 weeks and one animal was maintained for sacrifice at 6 months.

After 3 days of exposure to amitrole in the drinking water, the thyroid gland was not enlarged. Following one week of administration, the gland was reported to be twice its normal weight and marked structural changes were evident as well as decreased colloid in the follicles.

The authors noted that a few functional follicles were still present in the thyroid from the animal which had been on the compound for six months (in a thyroid that increased to 10 times its normal size) "which indicates that the thyroid continued to attempt to offset the antithyroid action of amitrole."

Peroxidase activity was also monitored and was reported to show decreased activity at one week and continued to decrease over the 6 month study.

2. Dermal Exposure

NO AVAILABLE DATA

3. Inhalation Exposure

NO AVAILABLE DATA

B. Mice

1. Oral Exposure

NO DATA AVAILABLE

2. Dermal Exposure

NO DATA AVAILABLE

3. Inhalation Exposure

NO DATA AVAILABLE

EVIDENCE OF REDUCED THYROID HORMONE SYNTHESIS

A. Rats

1. Alexander (1959):

1.a. Male Sprague-Dawley rats (7 animals) were given 0.04% amitrole in the drinking water for 12, 20 or 37 days. Liver, kidney and plasma catalase was measured as well as thyroid to serum inorganic radioiodine ratios.

The author reported that the "liver and kidney catalase activities were inhibited by 50% but red cell catalase was unaltered." Microscopic examination of the thyroid showed "a loss of colloid and hyperplasia". Increased thyroid weights were reported in all examined animals treated for 12, 20 and 37 days of treatment.

1.b. Male Sprague-Dawley rats were injected with 1 mg of amitrole followed by in one hour by radiolabelled iodine; control animals were injected with isotonic saline solution. Animals were sacrificed at 30, 60 and 240 minutes following treatment. Iodine uptake and serum inorganic radioiodine concentrations were measured.

The author reported that "amitrole appears to inhibit thyroid iodine uptake and the organic binding of radioiodine without affecting the iodide trap."

2. Strum and Karnovsky (1971): Male Sprague-Dawley rats were given 0.04% amitrole in the drinking water. Two animals were sacrificed at 3 days, two animals were sacrificed at weekly intervals from 1 to 9 weeks and one animal was maintained for sacrifice at 6 months.

Peroxidase activity was also monitored and was reported to show decreased activity at one week and continued to decreased over the six month study.

3. Tsuda (1975): Female Wistar rats (number unspecified) were given 0, 2500 or 5000 ppm (equivalent to 0, 125 or 250 mg/kg) amitrole in the drinking water for 16 weeks.

The author reported decreased peroxidase activity. He also reported that "at termination less than 50% of the treated animals survived and microscopic evaluation of the thyroid revealed atypical proliferation" described as "malignant adenoma".

4. Hoshino (1960): Male albino rats were injected interperitoneally with amitrole (1000 mg/kg) once and sacrificed after 3 hours or injected with amitrole (1000 mg/kg) every two days and sacrificed on day 30 of the experiment; the control group was reportedly "not treated with amitrole." The results revealed that "repeated injections of amitrole were able to maintain a prolonged low level of liver catalase activity" (Table 4).

Table 4. Liver Catalase Values (Hoshino, 1960)

Dose	# Animals Examined	Mean Catalase Values (Ko/mgm)
Control	10	$(43.01 \pm 8.01) \times 10^{-2}$
One Injection	10	$(4.11 \pm 0.43) \times 10^{-2}$
Multiple Injection	10	$(3.32 \pm 0.51) \times 10^{-2}$

B. Mice: NO DATA AVAILABLE

E. 2. Mutagenicity

The data base for genotoxicity is summarized in Appendix E of Hill et al. (1989) as follows: "There is limited evidence for the genotoxicity of Amitrole. This effect is probably not mediated through mutagenic mechanisms: there is no indication of the production of chromosomal mutations and, at best, the point mutagenic evidence is inconclusive. There are indications, however, that under some circumstances amitrole produces DNA-damageing effects. These results are augmented by confirmed positive responses in in vitro transformation. Thus, there is support for amitrole having a weak DNA-interactive or genotoxic effect that probably does not involve mutation per se."

E. 3. Developmental and Reproductive Effects

2-Generation Reproduction

a. Gaines (1973): Sherman rats were given 0, 25, 50 mg/kg amitrole in the diet for 55 days and then mated. The author reported that body weights and food consumption were reduced, thyroids were enlarged, and liver and kidney weights were reduced in parents of both treatment groups. The number of offspring, mean body weights and survival were reduced in the offspring of both treatment groups. No malformations were reported.

In another experiment, Sherman rats (10/sex) were given 1.25 and 5 mg/kg amitrole in the diet for 61-137 days. Thyroid hyperplasia was observed "in all rats" treated with 5, 25 and 50 mg/kg.

E. Developmental Effects

a. Gaines (1973): No developmental effects were reported at birth through weaning in Sherman rats (number unspecified) dosed with 20 and 100 mg/kg/day amitrole from day-7 through day-15 of gestation.

b. Tjalve (1975): NMRI mice (number of females/dose unspecified) were given 0, 500, 1000, 2500 or 5000 ppm amitrole (equivalent to 0, 75, 150, 375 or 750 mg/kg/day) in the drinking water from the 6th to 18th day of gestation. A "marked decrease in the weight gain of the dams and a pronounced under development of fetuses" (immature skeletons with decreased ossification) was observed in the 1000, 2500 and 5000 ppm treatment groups when compared to controls; an increased incidence of resorption was also reported for the 5000 ppm group.

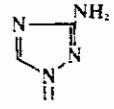
One abnormal fetus (hydrocephalus) was reported in the 5000 ppm treatment group; no other reported abnormalities in the other groups.

c. Hazleton (1988): New Zealand white female rabbits (18/dose group) were artificially inseminated and administered 0, 1.0, 1.5 or 2.0 g/kg amitrole to 10% of the body surface during gestation days 7-19.

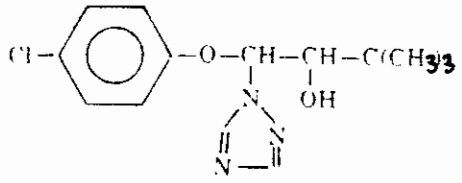
The no observed effect level for maternal toxicity was 1.0 g/kg based on significantly lower body weight on gestation day 20. The no observed effect level for developmental toxicity was 1.0 g/kg based on lower gravid uterine weight, significantly lower fetal body weights, and a statistically significantly greater number of total resorption at 2.0 g/kg. Skeletal anomalies, including unossified and absent talus were reported; visceral anomalies, including "left carotid arises from left innominate", were also observed at the 2.0 g/kg dose level.

E. 4. Structure-Activity Correlations

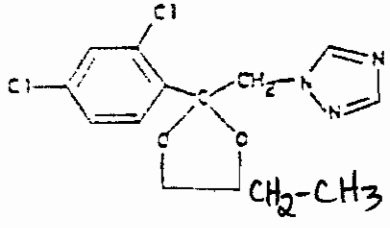
Amitrole, a triazole, is related to Baytan, Propiconazole and Etaconazole, all of which are associated with liver tumors in mice.



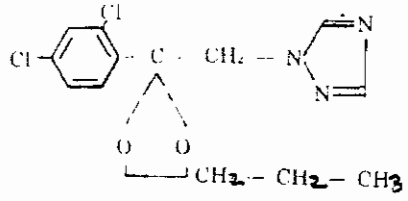
Amitrole



Baytan



Etaconazole



Propiconazole

F. Weight of Evidence Considerations:

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

I. Rat Studies

1. Keller; Hazleton '59: Amitrole administered in the diet (0,10,50 or 100 ppm) to Charworth Farms rats was associated with thyroid adenomas and carcinomas in treated rats. High dose (100 ppm) male and female rats were reported to have an increase in thyroid hyperplasia (cell type not specified) at 68 wks., which was not seen at 104 wks.

Although serious conduct problems were identified in this study, the data did show increased incidences of thyroid neoplasia.

2. Johnson, Food Drug Research '81,: Amitrole administered in the diet (0-0 (A), 1-20 (C), 3-60 ((D), 5-100 (B), or 10-200 (E) ppm (see text)) to Fischer 344 rats was associated with increased incidences of follicular cell thyroid neoplasia (mainly adenoma) in Groups D, B and E in both sexes.

There were increases in the incidences of follicular cell hyperplasia in both sexes in all treatment groups. Increases in thyroid organ weights were observed for Group B and Group E males and females. Thyroid hormone T3 was elevated throughout the study for all treatment groups compared to controls while T4 values were variable (these results conflict with what has been described in other Amitrole studies, in which hormone levels were reported).

DRAFT

F. Weight of Evidence (contd.)

I. Rats (contd.)

3. Bayer '79, Steinhof '79 '83: Amitrole administered in the diet (0,1,10 or 100 ppm) to Wistar rats was associated with increased incidences of thyroid "tumors" at 100 ppm in both sexes and in pituitary "tumors" in all treated male and female groups.

Percentage accumulations of radioiodine were reportedly increased in male and female rats at 100 ppm at "the majority of the test times"; thought to be due to increases in physiologically active thyroid tissue - proportional plasma iodine remained fairly constant throughout the study.

4. Cox '78, Food Drug Research '78: Amitrole administered by inhalation (0,0.05 (14.6 ppm) = "low dose", or 0.50 mg/L (146 ppm) = "high dose" - due to poor study design, the exact doses could not be accurately determined) to Fischer 344 rats was associated with increased incidences of thyroid adenomas at the low dose and slight increases at the high dose in both sexes. There were no increases in liver neoplasia or hyperplasia.

Thyroid weights were significantly higher compared to controls at each sacrifice interval in both treated groups of both sexes. Thyroid hyperplasia was increased in both treatment groups in both sexes. T3 levels were decreased at the high dose and T4 levels were decreased at the low dose.

This study was considered invalid because of serious problems associated with the accuracy of the doses and the reporting of the results. It was also speculated that exposure was not confined to the inhalation route.

5. Napalkov '62: Amitrole administered subcutaneously (25000 ppm), in the drinking water (4000 - 5000 ppm) or in the feed (50000 or 100000 ppm) to "Albino mongrel rats" was associated with thyroid and liver neoplasia in all treatment groups. No concurrent controls were mentioned.

This study was considered invalid because of incomplete reporting, failure to discuss the source and purity of the test material and absence of concurrent controls.

II. Mouse Studies

1. Innes '69: Amitrole administered by stomach tube (6667 ppm) followed by dietary feeding (2192 ppm) - as a positive control for screening 120 compounds to C57 mice - was associated with carcinoma of the thyroid in 64 of 72 treated animals. "Hepatomas" were also observed in 67 of 72 animals.

2. Vesselinovitch '83: Amitrole administered (500 ppm) to B6C3F1 mice: prenatal, preweaning and in the diet to offspring, postweaning through 90 weeks, was associated with increases in liver adenomas and liver carcinomas in postweaning males and what the author's called a "marginal neoplastic response" in postweaning females. (Author's conclusions regarding the prenatal and preweaning mice could not be substantiated without knowing which control groups were used for comparisons.)

3. Bayer '79, Steinhoff '79 '83: Amitrole administered in the diet (0,1,10 or 100 ppm) to NMRI mice was negative for oncogenicity. There was no evidence of treatment related induction of tumors of any type including the thyroid, liver and pituitary. Thyroid weights were increased throughout the study in both sexes at 100 ppm.

III. Hamster Study

1. Bayer '79, Steinhoff '79 '83: Amitrole administered in the diet (0,1,10, or 100 ppm) was negative for oncogenicity. There was no evidence of treatment related induction of tumors of any type including the thyroid, liver and pituitary. Thyroid iodine concentrations and protein-bound iodine were not affected in any treatment group vis a vis the controls.

G.. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered. The deliberations of the Peer Review Committee included the consideration of two classifications for Amitrole: Group B2 and Group C. The arguments for each are presented below.

I. Group B2

Amitrole was considered for classification as a Group B2 carcinogen, based on the total weight-of-evidence: both malignant and benign tumors in 2 species, both sexes, (by different routes) at 2 sites, with supporting data from structurally related chemicals.

Thyroid adenomas both sexes of rats (Johnson FDR '81 (feeding), Cox FDR '78 (inhalation)

Thyroid "tumors" both sexes of rats and pituitary "tumors" in both sexes (Bayer '79, Steinhoff '79 '83 (feeding)

Thyroid adenomas and carcinomas in both sexes of rats (Keller Hazleton '59 (feeding). This study had serious conduct problems.

Thyroid and liver tumors in rats (Napalkov '62). Author did not specify sex or grade of tumors; the study had serious reporting deficiencies.

Thyroid carcinomas and hepatomas in mice (Innes '69)

Liver adenomas and carcinomas in mice (Vessilinovitch '83)

There is limited evidence for the genotoxicity of Amitrole.

Amitrole is structurally related to Baytan, Etaconazole, Propiconazole (all are liver tumorigens in the mouse).

II. Group C

Since the induction of certain follicular cell tumors of the thyroid gland has been attributed to an hormonal imbalance of the normal thyroid-pituitary feedback control, it seemed appropriate to consider this mechanism in regard to the thyroid tumors associated with the administration of Amitrole. Thus, it has been shown that a block in the early steps in the progressive events: reduced thyroid hormone concentrations---elevated TSH levels---cellular hypertrophy/hyperplasia---nodular hyperplasia, and finally---neoplasia (resulting from long-term thyroid-pituitary imbalance) may also block the subsequent steps including tumor development. It has been postulated that the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists.

If this mechanism is operative here, and threshold consideration is applied to the thyroid tumors, it was also argued that the evidence for Amitrole could be considered as belonging in the Group C classification, based on the mouse liver tumors only (liver tumors in the rat were seen only in the Napalkov study, which had serious reporting deficiencies).

A discussion of how the Agency Draft Policy on the use of the threshold model may be applied to Amitrole is presented below.

The following guidance is given in the Agency's DRAFT Policy Document (Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988):

> "Studies over the last several decades in multiple laboratories and using a number of different treatment regimens (eg., iodine deficiency) have demonstrated the significance of long-term thyroid-pituitary hormonal imbalance in thyroid carcinogenesis. A consistent progression of events is noted: reduction in thyroid hormone concentrations, elevation in TSH levels, cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia. Hyperplasia and sometimes neoplasia of the pituitary may also be seen.. A block in any of the early steps acts as a block for subsequent steps including tumor development, and cessation of treatment at an early stage in the progression results in regression toward normal thyroid structure and function. Based on these observations ..... the Agency concludes that:
>
> 1. thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary feedback under conditions of reduced circulating thyroid hormone and elevated TSH levels:
>
> 2. the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists; and
>
> 3. models that assume thresholds may be used to assess the risks of thyroid follicular cell tumors where there is evidence of thyroid-pituitary hormonal imbalance."

Two basic questions must be addressed before this policy is applied.

> "The first is a qualitative issue which addresses whether it is reasonable to presume that the neoplasms are due to thyroid-pituitary imbalance. A corollary issue is the extent to which other carcinogenic mechanisms can be discounted. The second question concerns the procedures to be employed in estimating the risks of these agents."
>
> "The answers to the first question allow one to assign chemicals producing thyroid tumors to one of three categories. The assignation is based upon knowledge as to whether the chemical disrupts thyroid-pituitary feedback, whether tumors other than thyroid follicular cell (and relevant pituitary) tumors are found, and whether mechanisms other than thyroid-pituitary imbalance may apply to the observed tumor response."

1. DETERMINATION OF WHETHER NEOPLASMS ARE DUE TO THYROID-PITUITARY IMBALANCE

The document goes on to describe the 3 factors which should be considered in making this determination (answering the first question, or "qualitative issue"). These are addressed as they apply to Amitrole (with citations for the appropriate studies) as follows:

FACTOR I. Consideration of whether the thyroid tumors associated with administration of Amitrole can be attributed to disruption of the thyroid-pituitary hormonal balance. (In addressing this factor, the Policy states, 6 indicators should be considered.)

1. Goitrogenic activity in vivo:

Thyroid Follicular cell hypertrophy and/or hyperplasia was observed in both sexes of rats in the '59 Keller Hazleton, the Johnson FDR '81, the Bayer '79, Steinhof '79 '83 long-term feeding studies and in the Cox FDR '78 inhalation study.

2. Clinical chemistry changes (eg., reduced thyroid hormone and increased TSH serum concentrations).

In the Johnson FDR '81 study, T3 levels were actually elevated in all treatment groups and T4 levels were variable; TSH levels were not reported. In the Cox FDR '78 study, lowered T3 and T4 levels were reported, but TSH levels were not reported. The Keller Hazleton '59, the Bayer '79 Steinhof '79 '83 did not report any T3,T4 or TSH levels.

3. Specific evidence of reduced hormone synthesis (eg., inhibited iodine uptake) or increased thyroid hormone clearance (eg., enhanced biliary excretion).

There was no evidence of either in any of the long-term studies in which there were thyroid tumors; however, in sub-chronic studies, Jukes and Shaffer '60 reported thyroid enlargement and decreased radio-iodine uptake and Fregley '68 reported reduced radio-iodine uptake.

Other Sub-Chronic studies:

In male Sprague Dawley rats, Alexander '59 (amitrole in drinking water) reported reduced liver and kidney catalase activities but unaltered red cell catalase; thyroid hyperplasia and increased thyroid weights. The author also reported (amitrole by injection) that "amitrole appears to inhibit thyroid iodine uptake and the organic binding of radioiodine without affecting the iodide trap."

3. Specific evidence of reduced hormone synthesis (contd.)

Amitrole inhibits thyroid peroxidase, which is responsible for the conversion of iodide to the iodinating species and the subsequent iodination and coupling of the tyrosyl residues, involved in thyroid hormone synthesis (Hill, et al. 1989).

Strum and Karnovsky '71 reported reduced peroxidase activity in male Sprague-Dawley rats given amitrole in the drinking water.
Tsuda '75 reported decreased peroxidase activity in female Wistar rats given amitrole in the drinking water.
He also reported "at termination less than 50% of the treated animals survived and microscopic evaluation of the thyroid revealed atypical proliferation" described as "malignant adenoma".

Hoshino '60 reported prolonged low levels of liver catalase activity in male albino rats repeatedly injected interperitoneally with amitrole.

4. Evidence of progression (eg., hypertrophy/hyperplasia, nodular hyperplasia - neoplasia)

Keller Hazleton '59, Johnson FDR '81, Cox FDR '83.

5. Reversibility of lesions after exposure is terminated:

Babish FDR '77 - reduced T3, T4 reverted to normal after cessation of exposure in male rats (females not tested) - Subchronic Study.

6. SAR to other thyroid tumorigens

Similarity to thiourea. <u>However, also related to Baytan, Etaconazole, Propiconazole, which are mouse liver tumorigens</u>.

Based on the overall judgment of the 6 indicators in Factor I, it may be concluded that there is suggestive evidence that the thyroid tumors in the rat associated with administration of Amitrole may be due to a disruption in the thyroid-pituitary status.

<u>FACTOR II</u>. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

There is limited evidence of DNA-damageing effects and <u>in vitro</u> transformation; no indication that Amitrole produces chromosomal mutations and results of point mutation tests are inconclusive.

FACTOR III. Evaluation of neoplasms in addition to thyroid follicular tumors, including pituitary tumors.

Bayer '79 Steinhof '79 '83 reported thyroid neoplasms in both sexes, pituitary neoplasms in female rats.

In the mouse: Innes '69 reported Carcinoma of thyroid (both (?) sexes) and Hepatomas both sexes. Vesselinovitch '83 - Liver tumors (A & C) in both sexes (only tumor).

In the rat: Napalkov '62 (subcu in DW or in feed) reported thyroid and liver tumors.

The relationship between the liver and thyroid effects was discussed in light of the recognition that most organs in the body are responsive to thyroid hormone and presupposing that neoplastic development may be enhanced under conditions of reduced circulating thyroid hormone; however, the data to support this hypothesis were not available. (Innes study did not report hormone levels, nor did either Napalkov or Vessilinovitch).

In 2 negative studies: Bayer '79 Steinhoff '83 (mice); Bayer '79 Steinhoff '83 (Hamster) - transient changes in iodine-binding, thyroid function and weight were reported, but there were no tumors of any type.

Conclusions: As indicated above, based on the overall judgment of the 6 indicators in Factor I, it may be concluded that there is suggestive evidence that the thyroid tumors in the rat associated with administration of Amitrole may be due to a disruption in the thyroid-pituitary status. However, this is less clear from consideration of Factors II and III.

2. PROCEDURES TO BE EMPLOYED IN ESTIMATING RISKS

Guidance given in the EPA DRAFT policy for proceeding with the quantitation of risk is as follows:

> "1. Threshold considerations should be applied in dose-response assessments for those chemical substances where (a) only thyroid tumors (and relevant pituitary tumors) have been produced; (b) the tumors can be attributed to a disruption in thyroid-pituitary hormonal homeostasis; and (c) potential mechanisms other than thyroid-pituitary imbalance (eg., genotoxicity) can be disregarded.
> 2. Special attention should be given to chemicals (a) that have induced thyroid tumors (and relevant pituitary tumors) that may be due to thyroid-pituitary imbalance, and (b) where there is also evidence of either a genotoxic potential or the induction of neoplasms at sites other than the thyroid (or pituitary). Generally, those cases will be approached using various principles laid out in the EPA Guidelines for Carcinogen Risk Assessment. A strong rationale must be articulated for handling these agents otherwise.
> 3. For those chemicals producing thyroid tumors that do not seem to be acting via thyroid-pituitary hormonal inhibition, dose-response assessments will be performed in accordance with the EPA Guidelines for Carcinogen Risk Assessment."

Applying these 3 procedures to Amitrole:

Procedure 1.
While criterion (b) may be met, neither (a) (there were also liver tumors) nor (c) (there is evidence, albeit limited, that Amitrole is genotoxic) are satisfied.

Procedure 2.
Seems most appropriate for Amitrole.

Procedure 3.
Accepting the overall conclusions reached in the qualitative assessment, this procedure does not appear to apply.

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The Peer Review Committee agreed that 3 methods should be used for deriving the quantitation of risk for Amitrole:

1. Probit applied to the thyroid tumors based on threshold assumption (as previously performed by B.Litt).
2. RfD Method (thyroid tumors based on threshold assumption)
3. Conventional, multistage model (based on the liver neoplasia).

The Committee is invited to vote for either the Group B2 or C Classification and to provide assistance in further development of the appropriate supporting arguments.

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Chemical:	**Amitrole**
PC Code:	**004401**
HED File Code	**21200 PEER REVIEW**
Memo Date:	**11/20/1989**
File ID:	**00000000**
Accession Number:	**412-01-0126**

HED Records Reference Center
03/21/2001