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

OFFICE OF PREVENTION,
PESTICIDES, AND TOXIC SUBSTANCES

TXR No.: 0054224

MEMORANDUM

DATE: May 11, 2006

SUBJECT: AMITROLE: Fifth Report of the Cancer Assessment Review Committee
PC Code: 004401

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Robert Mitkus, Toxicologist (RAB1)
Health Effects Division (7509P)

James Tompkins
Herbicide Branch, Registration Division (7505P)

The Cancer Assessment Review Committee met on March 21, 2006 to re-evaluate the carcinogenic potential of Amitrole. Attached please find the Final Cancer Assessment Document.

cc: J. Pletcher
Y. Wo.

MAY 24 2006

EVALUATION OF THE CARCINOGENIC POTENTIAL OF **AMITROLE (FIFTH REVIEW)**

PC Code 004401

FINAL
May 11, 2006

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

AMITROLE CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION:

Robert Mitkus, Toxicologist

DOCUMENT PREPARATION:

Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated.)

Lori Brunsman, Statistician

William Burnam, Chair

Marion Copley

Kit Farwell

Abdallah Khasawinah

Tim McMahon

Jess Rowland

Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist

OTHER ATTENDEES: PV Shah (HED/RAB1), Whang Phang (HED/RRB1), Lisa Austin (HED/RAB1)

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EXECUTIVE SUMMARY

On March 21, 2006 the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to re-evaluate the carcinogenic potential of amitrole. This was the fifth peer review of this compound.

Amitrole was reviewed by the Cancer Peer review Committee four times in the past (in 1985, 1989, and twice in 1990) and was classified as a B2 Carcinogen (probable human carcinogen; old classification system). This B2 Carcinogen classification was based on thyroid tumors in both sexes of multiple strains of rats, pituitary tumors in rats, and liver tumors in two strains of mice. Supporting data included SAR based on triazoles (mouse liver tumors) and a stronger relationship to heterocyclic aromatic amines. Also, there was limited evidence of genotoxicity for amitrole. It was also concluded at the time that while the amitrole data in the rat were suggestive of a disruption in the thyroid-pituitary axis, the data were neither clear nor complete nor consistent, and did not support the use of a threshold model. Consequently, a low dose extrapolation multi-stage mode based on the thyroid tumors in the rat (Johnson 1981) was used for quantification of human risk (Q1*).

Since that time, significant strides have been made in the scientific understanding of the mode of action of many goitrogenic compounds, including amitrole (Hurley et al. 1998; IARC 2001), which is a thyroid peroxidase inhibitor in rodents. Also since that time, the Agency finalized its guidance document for thyroid follicular cell carcinogens (1998), as well as its Guidelines for Carcinogen Risk Assessment (2005). In June 2005, the registrant, Nufarm Americas, Inc. (U.S. affiliate of CFPI Agro, S.A., France), submitted a scientific justification for re-classification of the cancer potential of amitrole in order to modify use constraints listed in the 1996 RED for amitrole.

The present CARC document presents the re-evaluation of the carcinogenic potential of amitrole. A full-blown mode of action (MOA) analysis is not necessary for amitrole since its mode of action in rodents is well established and has been published elsewhere (Hurley et al. 1998; IARC 2001). However, the evidence required to make an assessment of the mode of action of thyroid follicular cell carcinogens in animals has been re-presented from the 3rd and 4th Peer Review of Amitrole using the framework published in the USEPA document, Assessment of Thyroid Follicular Cell Tumors (1998). In addition, the CARC re-evaluated the corrected liver tumor incidence data in mice (Bayer NMRI mouse study; Steinhoff 1979), as well as the results of a published paper on apparent perinatal carcinogenesis (Vesselinovitch 1983). Mutagenicity and SAR data were also re-evaluated within the MOA framework.

The CARC concluded the following:

Carcinogenicity

Rat

-Treatment-related thyroid follicular cell tumors were seen in male and female rats (multiple strains) based on evidence provided collectively from several studies (Keller 1959; Johnson et al. 1981; Steinhoff et al. 1979; and Tsuda et al. 1976). As a group, the submitted carcinogenicity studies provide sufficient information to assess the carcinogenic potential of amitrole and support the published fact that amitrole is a thyroid carcinogen, specifically of follicular cells, in rats. This decision was determined by HED's 3rd and 4th Peer Review of Amitrole and re-confirmed by the present CARC.

-Significantly increased incidences of pituitary tumors were also observed in rats in a lifetime feed study conducted by Steinhoff et al 1979. The presence of these tumors is also consistent with a disruption of the thyroid-pituitary hormonal homeostasis.

Mouse

No treatment-related tumors were seen in male or female mice (Innes et al. 1969; Steinhoff and Boehme 1979; Vesselinovitch 1983). Following a correction and re-evaluation of the liver tumors seen in the Steinhoff and Boehme 1979 study, the CARC concluded that the liver tumors were not treatment related. This decision reverses the previous CPRC decision on liver tumors. The CARC also concluded that the studies by Innes 1969 and Vesselinovitch 1983 did not contribute to the weight-of-evidence.

Mutagenicity

Based on the overall weight of the evidence found in the genotoxicity database (published by Hill et al. 1989 and IARC 2001), amitrole is not considered to be a mutagen or to cause thyroid follicular cell carcinogenesis through a mutagenic mode of action. This conclusion is the same as that reached by Hurley et al. (1998), IARC (2001), and USEPA (2005).

Structure Activity Relationship

Hill et al. (1998) contended that amitrole shared a *functional* similarity only, to certain thionamides and aromatic amines that inhibited thyroid peroxidase. This supports the established mode of action for amitrole.

Mode of Action

The mode of action of amitrole-induced thyroid tumorigenesis has been published previously by Hurley et al. (1998), and involves perturbation of thyroid-pituitary function. The CARC concluded that evidence for an antithyroidal mode of action in rodents, using the framework published in Assessment of Thyroid Follicular Cell Tumors (USEPA 1998; so-called "Purple book") was established. Amitrole is considered to be nonmutagenic and does not mediate thyroid carcinogenesis through a mutagenic mode of action (IARC 2001).

Amitrole induces thyroid tumors in rodents by inhibiting the activity of thyroid peroxidase leading to decreased thyroid hormone levels and increased TSH. In addition, the increases in cell growth *in vivo* (e.g. increases in thyroid weights and thyroid hypertrophy and hyperplasia) progressing to thyroid follicular cell tumors were seen in the presence of thyroid/pituitary hormone changes. Decreased iodine uptake by the thyroid points to an intrathyroidal site of action. Decreases in thyroid hormone production have been shown to be reversible upon cessation of treatment with amitrole, which strengthens the accepted mode of action.

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March 2005), the CARC classified amitrole as **"Not Likely To Be Carcinogenic To Humans At Doses That Do Not Alter Rat Thyroid Hormone Homeostasis".** This decision was based on the following: i) Treatment-related thyroid follicular cell tumors were seen in male and female rats (multiple strains) based on evidence provided collectively from several studies (Keller 1959; Johnson 1981; Steinhoff 1979; and Tsuda 1976); ii) No treatment-related tumors were seen in male or female mice (Steinhoff and Boehme 1979); iii) Amitrole is considered to be nonmutagenic and does not mediate thyroid carcinogenesis through a mutagenic mode of action; iv) The overall weight-of-the-evidence was considered sufficient to indicate that amitrole induced thyroid follicular tumors through an antithyroidal mode of action; v) Rats are substantially more sensitive than humans to the development of thyroid follicular cell tumors in response to thyroid hormone imbalance. The quantification of carcinogenic potential is not applicable.

I. INTRODUCTION

On March 21, 2006 the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to re-evaluate the carcinogenic potential of amitrole. This was the fifth peer review of this compound.

II. BACKGROUND

Amitrole (3-amino-1,2,4-triazole) is a non-selective herbicide currently used at various non-food crop sites, including rights of way, marshes, drainage ditches, and ornamentals, as well as around agricultural, domestic, and recreational areas. In 1971, the use of amitrole on all food crops was banned due to concerns that it was carcinogenic in animal studies. Amitrole was considered a B2 carcinogen after data review by the Toxicology Branch in 1985. On July 26, 1989 the Cancer Peer Review Committee (CPRC) of the Health Effects Division (HED) met (2nd review) to evaluate the weight of the evidence regarding the carcinogenic potential of amitrole. Data Evaluation Records (DERs) for most of the animal cancer studies were provided to the committee at that time. The committee concluded that additional review of the hazard database was necessary before making a decision as to cancer classification. On August 1 and November 21, 1990, the HED CPRC met for a 3rd and 4th time, respectively, to evaluate the carcinogenic potential of amitrole. At the 3rd meeting, the CPRC classified amitrole as a B2 (probable human carcinogen; old classification system). At the 4th meeting, CPRC (today known as the CARC) members evaluated published papers by Wynford-Thomas et al. (1982) that strengthened the association between rat thyroid tumors and disruption of the thyroid-pituitary axis. However, the committee reaffirmed the classification decided at the 3rd peer review. The unit risk to humans was calculated (Q_1* approach) based on an increased incidence of combined thyroid follicular cell tumors observed in a lifetime pulse feeding study in rats (Johnson et al. 1981). Since that time, significant strides have been made in the scientific understanding of the mode of action of many goitrogenic compounds, including amitrole (Hurley et al. 1998; IARC 2001), which is a thyroid peroxidase inhibitor in rodents. Also since that time, the Agency finalized its guidance document for thyroid follicular cell carcinogens (1998), as well as its Guidelines for Carcinogen Risk Assessment (2005). In June 2005, the registrant, Nufarm Americas, Inc. (U.S. affiliate of CFPI Agro, S.A., France), submitted a scientific justification for re-classification of the cancer potential of amitrole in order to modify use constraints listed in the 1996 RED for amitrole.

The purpose of the present document is to present the evidence for a re-evaluation of the carcinogenic potential and cancer classification of amitrole. Due to the comprehensive analyses presented in the "3rd and 4th Peer Review of Amitrole" (Memo, Esther Rinde, 1991), that report is a primary source for the current document. In fact, except where indicated, summaries and tumor incidence data in this document are taken directly (please note quotation marks) from the 3rd and 4th Peer Review of Amitrole, albeit with minor formatting changes. The current document also updates the information contained in the 3rd and 4th Peer Review of Amitrole with the following: 1) data received since 1990; 2) corrections to the 3rd and 4th Peer Review of Amitrole; 3) re-analyses of data contained in the 3rd and 4th Peer Review of Amitrole; and 4) re-

interpretation of the thyroid tumor database based on the increased understanding of thyroid tumorigenesis that has developed over the last decade. A full-blown mode of action analysis is not necessary for amitrole since its mode of action in rodents is well established and has been published elsewhere (Hurley et al. 1998; IARC 2001). However, the evidence required to make an assessment of the mode of action of thyroid follicular cell carcinogens in animals has been re-presented from the 3rd and 4th Peer Review of Amitrole using the framework published in the USEPA document, Assessment of Thyroid Follicular Cell Tumors (1998). Hurley et al. (1998) used the exact same framework in their published paper.

After examining the arguments contained in: 1) the submitted justification by the registrant, 2) Hurley et al. (1998), 3) IARC (2001), and 4) the data presented in the "3rd and 4th Peer Review of Amitrole" (Memo, Esther Rinde, 1991), the Registration Action Branch 1 (RAB1) of HED requests that the existing cancer classification for amitrole-induced thyroid tumors be updated to reflect the now well-established scientific understanding of thyroid tumorigenesis, the new EPA Guidelines for Carcinogen Risk Assessment (2005), and HED Hot Sheet #23 (2003). In addition, based on a correction to and re-analysis of submitted liver tumor incidence data in mice (Bayer NMRI mouse study; Steinhoff 1979), as well as a re-evaluation of the results of a published paper on apparent perinatal carcinogenesis (Vesselinovitch 1983), RAB1 recommends that the existing cancer classification for amitrole-induced liver tumors also be re-examined.

III. EVALUATION OF CARCINOGENICITY STUDIES

It should be noted that none of the carcinogenicity studies on its own – DERs for many, but not all, of these studies were included as part of the package submitted to the previous CPRC – was considered "acceptable" according to Agency standards at the time of the HED CPRC 3rd and 4th Peer Review of Amitrole (1991). This conclusion would likely be obtained today as well, given the guideline requirements for conduct of rodent carcinogenicity studies (1998). However, the HED CPRC 3rd and 4th Peer Review of Amitrole viewed all the studies, when taken together, as providing sufficient information to assess the carcinogenic potential of amitrole. Although adequacy of dosing for each study was not explicitly assessed by the 3rd and 4th Peer Review of Amitrole, the carcinogenic potential of amitrole in the rat thyroid is so well accepted that this dimension of the assessment is not emphasized here.

A. Rat

1. 2-Year Chronic Feeding (MRID 00082176)

Author: Keller JG (1959)[1]
Strain: Charworth Farms
Testing Lab: Hazleton Laboratories for American Cyanamid Co.
Total Rats in Study: 35/sex/dose (interim sacrifices at 13, 26, and 52 weeks)
Classification: Supplementary

The following text and tables are taken directly from the HED CPRC "3rd and 4th Peer Review of Amitrole":

"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 of data.** Not all animals were examined, many [tissue samples] 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."

Neoplastic lesions

"Statistical analysis of the data by the Exact Trend Test (conducted in the knowledge that interpretation is extremely limited) indicate that there are statistically significant dose-related positive **trends** in the incidence of thyroid gland tumors.

"There are also numerical increases, in both sexes at the terminal sacrifice, in the incidence of thyroid adenoma at the mid (50 ppm) and high dose (100 ppm) and in thyroid adenoma/carcinoma combined at the high dose (Tables 1a and 1b). However, there are **no significant pairwise comparisons** between any dose group and control by the Fisher Exact Test. It should be noted that the following tables represent only those animals reportedly sacrificed at 104 weeks; the interim sacrifices were not available for evaluation."

[1] Briefly summarized in Hodge HC, Maynard EA, Downs WL, Ashton JK, Salerno LL. (1966). Tests in mice for evaluating carcinogenicity. Toxicol Appl Pharmacol 9(3): 583-596.

Table 1a. Male Thyroid Gland Tumor Rates[+] with Fisher Exact Test and Exact Trend Test Results.

Dose (ppm)	0	10	50	100
Adenoma	0/2 (0) p=0.0524(ns)	0/1 (0)	1/5 (20)	6/11 (55)
Carcinoma	0/2 (0)	0/1 (0)	0/5 (0)	1/11 (9)
Combined Adenoma/carcinoma	0/2 (0) p=0.0240*	0/1 (0)	1/5 (20)	7/11 (64)

Table 1b. Female Thyroid Gland Tumor Rates[+] with Fisher Exact Test and Exact Trend Test Results.

Dose (ppm)	0	10	50	100
Adenoma	0/3 (0) p=0.0180*	0/8 (0)	1/10 (10)	5/15 (33)
Carcinoma	0/3 (0) p=0.1667(ns)	0/8 (0)	0/10 (0)	2/15 (13)
Combined Adenoma/carcinoma	0/3 (0) p=0.00234**	0/8 (0)	1/10 (10)	7/15 (47)

[+] Number of tumor-bearing animals/Number of animals at risk.
( ) Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p<0.05$ and ** denotes $p<0.01$.

Non-neoplastic lesions

"There are statistically significant dose-related positive trends in thyroid hyperplasia (cell type not described) in both sexes at 68 weeks (Tables 2a and 2b); however, this trend was not seen at 104 weeks (Table 2c). It should be noted that there was no detailing of the type of cellular involvement, e.g. follicular cell or parafollicular cell [C-cell]."

Table 2a. Male Thyroid Gland Hyperplasia Rates[+] at 68 weeks with Exact Trend Test Results.

Dose (ppm)	0	10	50	100
Thyroid gland Hyperplasia	0/3 p=0.00606**	0/3	1/3	3/3

Table 2b. Female Thyroid Gland Hyperplasia Rates[+] at 68 weeks with Exact Trend Test Results.

Dose (ppm)	0	10	50	100
Thyroid gland Hyperplasia	0/3 p=0.0119*	0/1	0/2	3/3

Table 2c. Non-Neoplastic Changes in the Rat Thyroid[a] at 104 Weeks.

	Males				Females			
	104 Weeks				104 Weeks			
Dose (ppm)	0	10	50	100	0	10	50	100
# Examined	2	1	5	11	3	8	10	15
Hyperplasia	0	0	1	0	0	0	1	1

[+] Number of tumor-bearing animals/Number of animals at risk.
[a] Registrant submitted individual animal data.
* denotes $p<0.05$ and ** denotes $p<0.01$.

2. Lifetime Pulse Feeding (MRID 00132445)

Authors: Johnson W, Becci P, and Parent R (1981)
Strain: Fischer 344
Testing Lab: Food and Drug Research Laboratories for Union Carbide Agricultural Products Co., Inc.
Total Rats In Study: 75/sex/dose (interim sacrifices at weeks 24, 37, and 60)
Classification: Supplementary

The following text and tables are taken directly from the HED CPRC "3rd and 4th Peer Review of Amitrole":

"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 10 ppm, respectively, during

weeks 1-39 and 20, 60, and 200 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). [Note: 10-200 ppm is equivalent to 0.05-10 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."

Neoplastic lesions

"Treatment groups B, D and E show **statistically significant increases in the incidences of thyroid follicular cell adenoma and combined adenoma/carcinoma in the pairwise comparison for both sexes**. There are also statistically significant positive trends for adenoma and carcinoma, individually and combined, in both sexes" (Tables 3a through 3c).

Table 3a. Thyroid Follicular Cell Neoplasia.

Dose Groups	Males				Females			
Week:	24	37	60	115	24	37	60	119
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

Table 3b. Male Thyroid Gland Tumor Rates[+] with Cochran-Armitage Trend Test and Fisher Exact Test Results.

Dose Group:	A	C	D	E	B
Follicular Cell Adenoma	1/60 (2) p=0.0000**	1/57 (2) n.s.	16/55 (29) p=0.0000**	48/60 (80) p=0.0000**	49/58 (84) p=0.0000**
Follicular Cell Carcinoma	0/60 (0) p=0.0098**	0/57 (0)	0/55 (0)	3/60 (5) n.s.	3/58 (5) n.s.
Combined	1/60 (2) p=0.0000**	1/57 (2) n.s.	16/55 (29) p=0.0000**	51/60 (85) p=0.0000**	52/58 (90) p=0.0000**

Table 3c. Female Thyroid Gland Tumor Rates[+] with Cochran-Armitage Trend Test and Fisher Exact Test Results.

Dose Groups:	A	C	D	E	B
Follicular Cell Adenoma	0/52 (0) p=0.0000**	1/54 (2) n.s.	5/50 (10) p=0.0254*	42/56 (75) p=0.0000**	48/56 (86) p=0.0000**
Follicular Cell Carcinoma	0/52 (0) p=0.0359*	0/54 (0)	1/50 (2)	2/56 (4)	3/56 (5) n.s.
Combined	0/52 (0) p=0.0000**	1/54 (2) n.s.	6/50 (12) p=0.0118*	44/56 (79) p=0.0000**	51/56 (91) p=0.0000**

[+] Number of tumor-bearing animals/Number of animals at risk.
( ) Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p<0.05$ and ** denotes $p<0.01$.

N.B. Trend test was performed on groups A, C, D and E only.

Non-neoplastic lesions

"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 Tables 4a through 4c). Statistical comparisons conducted for termination data only indicate statistical significance for both pair-wise comparisons and for trend."

Table 4a. Thyroid Follicular Cell Hyperplasia

Dose Groups	Wk 24	Wk 37	Wk 60	Wk 115/119[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.

Table 4b. Male Thyroid Gland Hyperplasia Rates[+] with Cochran-Armitage Trend Test and Fisher Exact Test Results.

Dose Group.	A	C	D	E	B
Follicular Cell Hyperplasia	0/60 (0) p=0.0000**	12/57 (21) p=0.0001**	29/55 (53) p=0.0000**	25/60 (42) p=0.0000**	38/58 (66) p=0.0000**

Table 4c. Female Thyroid Gland Hyperplasia Rates[+] with Cochran-Armitage Trend Test and Fisher Exact Test Results.

Dose Group:	A	C	D	E	B
Follicular Cell Hyperplasia	0/52 (0) p=0.0000**	7/54 (13) p=0.0073**	25/50 (50) p=0.0000**	31/56 (55) p=0.0000**	40/56 (71) p=0.0000**

[+] Number of animals with hyperplasia/Number of animals at risk.
( ) Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p<0.05$ and ** denotes $p<0.01$.
N.B. Trend test was performed on groups A, C, D and E only.

3. **Lifetime Feeding Carcinogenicity (MRID 00061351)**
Authors: Steinhoff D, Boehme K, Lorke D (1979)[2]
Strain: Wistar
Testing Lab: Bayer AG (West Germany) for Union Carbide Agricultural Products Co.
Total Rats In Study: 75/sex/dose
Classification: Supplementary

The following text and tables are taken directly from the HED CPRC "3rd and 4th Peer Review of Amitrole":

"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 authors 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 and females when compared to controls (Tables 5 and 6). 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.

"Analysis of the individual animal data showed a **statistically significant increase in the incidence of "thyroid tumors" for the high dose (100 ppm) [5 mg/kg/day] males and females** when compared to controls, as well as statistically significant positive trends in both sexes (Tables 5 and 6). 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 cell type."

[2] Published in the open literature as Steinhoff D, Weber H, Mohr U, Boehme K. (1983). Evaluation of amitrole (aminotriazole) for potential carcinogenicity in orally dosed rats, mice, and golden hamsters. Toxicol Appl Pharmacol 69(2):161-9.

Table 5. Male Thyroid Gland "Cyst" and "Tumor" Rates[+]

Dose (ppm)	0	1	10	100
Cyst Rates	1/73 (1) p=0.0000**	1/75 (1) n.s.	1/75 (1) n.s.	43/74 (58) p=0.0000**
Tumor Rates	8/73 (11) p=0.0000**	8/75 (11) n.s.	5/75 (7) n.s.	35/74 (47) p=0.0000**

Table 6. Female Thyroid Gland "Cyst" and "Tumor" Rates[+]

Dose (ppm)	0	1	10	100
Cyst Rates	1/75 (1) p=0.0000**	5/73 (7) n.s.	2/74 (3) n.s.	27/74 (36) p=0.0000**
Tumor Rates	7/75 (9) p=0.0000**	13/73 (18) n.s.	9/74 (12) n.s.	43/74 (58) p=0.0000**

[+] Number of tumor-bearing animals/Number of animals at risk.
( ) Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p<0.05$ and ** denotes $p<0.01$.
NB. Registrant submitted individual rat data. Individual animal data did not differentiate between malignant or benign tumors.

"Additionally, there was a numerical **increase in the incidence of "pituitary tumors"** in all treatment male and female groups, **which was statistically significant in both sexes at the high dose level,** with a statistically significant positive trend for females (Tables 7a and 7b). Again, no differentiation between "malignant" or "benign" was specified in the individual animal data." In addition, "cysts" were also not differentiated from "tumors" in the study. However, the numerical increase in the incidence of "pituitary tumors" in males and females that was reported in the "3rd and 4th Peer Review of Amitrole" was still observed when these data were published in the open literature (Steinhoff et al. 1983) and tumor types were differentiated (Table 8).

Table 7a. Male Pituitary Gland "Tumor" Rates[+] (Steinhoff et al. 1979)

Dose (ppm)	0	1	10	100
Tumor Rates	4/73 (5) p=0.0523	10/74 (14) p=0.0831	10/75 (13) p=0.0874	13/74 (18) p=0.0197*

Table 7b. Female Pituitary Gland "Tumor" Rates[+] (Steinhoff et al. 1979)

Dose (ppm)	0	1	10	100
Tumor Rates	14/73 (19) p=0.0000**	22/74 (30) p=0.0973	18/73 (25) n.s.	41/75 (55) p=0.0000**

[+] Number of "cyst" or "tumor" bearing animals/Number examined
( ) Percent
Note: Significance of trend by Cochran-Armitage Test denoted at Control. Significance of pairwise comparison with control by Fisher Exact Test denoted at Dose level.
* denotes $p<0.05$; ** denotes $p<0.01$; n.s. denotes $p>0.10$.

Table 8. Pituitary Neoplastic Observations (Steinhoff et al. 1983)

	MALES				FEMALES			
Concentration (ppm)	0	1	10	100	0	1	10	100
# Animals with Tumors	36	41	44	53	59	67	60	71
Benign	4	9	10	10	14	20	15	36
Malignant	0	1	0	3	1	2	4	5

4. Tsuda et al. (1976[3]; MRID 00052654)

A fourth study was performed in rats (Wistar) exposed for 70 weeks to 0 or 2500 ppm amitrole in drinking water. In the 3rd and 4th Peer Review of Amitrole, this study was reported as a subchronic study. The study did measure carcinogenic activity, however, and tumors and tissue invasion were observed in treated animals by the end of the study. Summary text and tables taken directly from the "3rd and 4th Peer Review of Amitrole" are presented below. Although not critical to this analysis, it should be noted that the published paper indicates that there were 15 survivors in group 3; however, thyroid autografting was successful in only 10/15 of these survivors.

[3] Tsuda H, Hananouchi M, Tatematsu M, Hirose M, Hirao K. (1976). Tumorigenic effect of 3-amino-1H-1,2,4-triazole on rat thyroid. J Natl Cancer Inst 57(4):861-4.

The following text and tables are taken directly from the HED CPRC "3rd and 4th Peer Review of Amitrole":

"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 250 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 1) 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 9].

Table 9. Summary of Pathologic Findings in Rat Thyroids

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.'"

5. Unacceptable studies

Two studies performed in the rat were determined to be unacceptable. According to the 3rd and 4th Peer Review of Amitrole, a 2-year inhalation study (Becci 1983; MRID 00127930) was performed in Fischer 344 rats. However, because the chamber concentrations of amitrole could not be verified and both dermal and oral exposures also inadvertently took place, no conclusions could be made from the study.

A second unacceptable study was performed by Napalkov (1962; MRID 00052656) in the former Soviet Union. According to the 3rd and 4th Peer Review of Amitrole, albino mongrel rats were administered amitrole by two separate routes of exposure, i.e., oral (feed or drinking water) and subcutaneous injection. Due to serious deficiencies in reporting of the experimental design and results, including non-use of concurrent controls and non-reporting of the number of animals tested per dose, the study is uninterpretable.

B. Mouse

1. 18-month Feeding Carcinogenicity (MRID 00043595)
Author: Innes (1969)[4]
Strain: (C57BL/6xC3H/Anf)F_1 and (C57BL6xAKR)F_1
Testing Lab: Bionetics Research Laboratories of Litton Industries for Union Carbide Agricultural Products Co.
Total Rats In Study: 18/sex (one dose only)
Classification: Supplementary

The following text and table are taken directly from the HED CPRC "3rd and 4th Peer Review of Amitrole":

"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 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 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 10). No further description of the liver neoplasia was presented in the study."

Table 10. Incidence of Hepatomas (# mice with hepatomas / # of mice necropsied)[1]

Strain	Male	Female
(C57BL/6 x C3H/Anf)F_1	16/18	18/18
(C57BL/6 x AKR)F_1	16/18	17/18

[1] The relative risk of hepatomas was increased ($P<0.01$) in both sexes and strains, relative to controls

[4] Published in the open literature as Innes JR, Ulland BM, Valerio MG, Petrucelli L, Fishbein L, Hart ER, Pallotta AJ, Bates RR, Falk HL, Gart JJ, Klein M, Mitchell I, Peters J. (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J Natl Cancer Inst 42(6):1101-14.

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

It should be noted that in Innes et al. (1969) both metastasizing and non-metastasizing liver tumors were referred to collectively as "hepatomas"; however, metastasizing liver tumors were reported as rare in the study. In addition, the intended study length was 18 months (72 weeks), rather than 126 weeks (as reported in the "3rd and 4th Peer Review of Amitrole"). Nevertheless, **100% mortality 12-19 weeks before study completion likely supports the conclusion that an MTD was exceeded.**

2. 18-month Feeding Carcinogenesis Study (MRIDs 00061348; 41317901, 41462501)

Authors: Steinhoff D, Boehme K (1979)[5]
Strain: NMRI
Testing Lab: Bayer Ag Institute of Toxicology for Union Carbide Agricultural Products Co.
Total Rats In Study: 75/sex/dose
Classification: Supplementary

The following text and table are taken directly from the HED CPRC "3rd and 4th Peer Review of Amitrole":

"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).

Neoplastic lesions

"A slight non-significant increase in the incidence of hepatocellular neoplasia was observed for high dose females (100 ppm) when compared to controls. Although this study was previously considered as negative, statistical analysis indicated that there are statistically significant positive trends for hepatocellular carcinoma and combined adenoma/carcinoma in females. Other reported neoplastic lesions were similar in incidence between treated and control groups."

Non-neoplastic lesions

"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

[5] Published in the open literature as Steinhoff D, Weber H, Mohr U, Boehme K. (1983). Evaluation of amitrole (aminotriazole) for potential carcinogenicity in orally dosed rats, mice, and golden hamsters. Toxicol Appl Pharmacol 69(2):161-9.

compared to controls. A slight increase in liver necrosis was observed in the 10 and 100 ppm males (statistically significant at high dose level)".

Updated Analysis of Liver Tumor Data

In the registrant's current petition for reclassification of amitrole, it was brought to HED's attention that the incidences of liver tumors in NMRI mice were not correctly reported in the "3rd and 4th Peer Review of Amitrole". Therefore, a **new analysis** of the incidences of benign, malignant, and combined benign/malignant primary liver tumors was performed based on a review of the individual animal data contained in the original study report.

The number of liver tumors observed across treatment group was re-counted, as were the number of animals examined, based on information contained in the original study submission (MRID 00061348). The re-analyzed liver tumor incidences are reported in Table 11. **A dose-dependent increase in benign (adenomas), malignant (carcinomas), or combined benign/malignant liver tumors was not evident in either sex.** Statistical analysis was not warranted since tumor incidences in each treatment group were less than or at control levels.

In addition, the recalculated liver tumor incidences for both hepatocellular adenomas and carcinomas were below the historical control values for NMRI mice from 1983-1993 reported by Carmichael et al. (1997)[6] for mouse studies conducted for 18 months (n=2 studies). The values are 14% and 4% for adenomas in male and females, respectively, and 7% and 1% for carcinomas in male and females, respectively. It is not clear from Carmichael et al. (1997) whether the reported numbers are means or upper limits for the historical control range.

[6] Carmichael NG, Enzmann H, Pate I, Waechter F. (1997). The significance of mouse liver tumor formation for carcinogenic risk assessment: results and conclusions from a survey of ten years of testing by the agrochemical industry. Environ Health Perspect 105(11):1196-203.

Table 11. Recalculation of Liver Tumor Incidences [a,b] (New Analysis)

	MALES				FEMALES			
Concentration (ppm)	0	1	10	100	0	1	10	100
# Animals Examined [c]	75	73	73	72	73	73	74	74
Benign [d]	6 (8)	3 (4)	3 (4)	2 (3)	2 (3)	0 (0)	0 (0)	1 (1)
Malignant [e]	1 (1)	0 (0)	2 (3)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Combined	7 (9)	3 (4)	5 (7)	3 (4)	2 (3)	0 (0)	0 (0)	1 (1)

[a] Based on the individual animal data provided in the study report (pp. 77-92, MRID 00061348)
[b] Incidence in parentheses
[c] Calculated based on the following information:
1) "the tumors and tumor-suspect organs and tissues were preserved for histological examination for all animals" (p.5, MRID 00061348), i.e., 75 animals/sex/dose
2) "Thirteen animals could not be examined histologically owing to autolysis; these included two female animals of the 0-ppm group, two male animals and two female animals of the 1-ppm group, two male and one female animal of the 10-ppm group, and three male and one female animal of the 100-ppm group" (p. 6, MRID 00061348).
[d] Also correctly tabulated on p. 26, MRID 00061348
[e] Also correctly tabulated on p. 24, MRID 00061348

3. Vesselinovitch (1983[7]; no MRID)

The following text and table are taken directly from the HED CPRC "3rd and 4th Peer Review of Amitrole":

"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, amitrole, ethylnitrosourea (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),

[7] Vesselinovitch SD. (1983). Perinatal hepatocarcinogenesis. Biol Res Pregnancy Perinatol 4(1):22-5.

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 12).

"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 12. Liver Neoplastic Observations* (3rd and 4th Peer Review)

Group	Number Examined	Adenoma	Carcinoma	Total	Total (%)
Males					
1[a]	74	4 (5)	2 (3)	6	8
2[b]	45	6 (13)	4 (9)	10	22
3[c]	55	9 (16)	11 (20)	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)	0	0
2[b]	55	0 (0)	0 (0)	0	0
3[c]	49	5 (10)	4 (8)	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 Tables 1 and 4 (Vesselinovitch 1983); tumor incidences for treated groups in parentheses
[a] Transplacental in utero exposure
[b] Delivery to weaning via mother's milk
[c] Weaning through 90 weeks via the diet
[d] Sacrificed at 52 weeks
[e] Sacrificed at 90 weeks
[f] Sacrificed at 142 weeks

Updated Study Interpretation

As the HED CPRC "3rd and 4th Peer Review of Amitrole" correctly pointed out, for groups 1 and 2 the data presented do not support the author's conclusions. This is so not only because data for same-aged controls for groups 1 and 2 were not provided, but also because it is unclear from the data whether the tumor incidences reported for groups 1 and 2 are for dams, their offspring, or some combination of both. Third, the total number of dams and/or offspring treated with amitrole was not reported in the study. Mortality was also not reported for either the treated dams or their offspring.

According to Vesselinovitch (1983; Table 4 of the paper), Group 3 animals (offspring) were treated when adults. However, in a footnote to the same paper, it is stated that Group 3 animals were treated from weaning through 90 weeks. Given that the age of mice at maturity is 7-9 weeks (PND 49-63), it is unclear whether offspring were really treated beginning on PND 21 (weaning) or beginning somewhere from PND 49-63, i.e.,

as adults. The IARC Monograph for Amitrole (IARC, vol. 79, 2001) and the USEPA Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (2005) interpreted the study authors to mean that animals in Groups 1 and 2 were exposed to amitrole *in utero* and perinatally (through milk), respectively, and then allowed to consume untreated feed until 90 weeks (age at death). However, Vesselinovitch (1983) nowhere states that this was the experimental procedure utilized.

Even if one were to accept the validity of the results presented in Vesselinovitch (1983) presented in Table 12 (above), the highest incidences of adenomas and carcinomas were observed in Group 3 males and females, i.e, offspring treated (beginning either at weaning or at attainment of maturity) for 90 weeks. However, each of these incidences falls within current historical control ranges reported for B6C3F$_1$ mice by Charles River Laboratories (CRL) and the National Toxicology Program (NTP). Historical control data from these sources are reported in Table 13. It should be noted that the concurrent control incidences reported in Vesselinovitch (1983) (Table 12 above) are much lower than those reported by CRL and NTP. However, given 1) the insufficiencies in study reporting by Vesselinovitch (1983), 2) the fact that the values are based on apparently 1 study only, 3) the lack of clarity as to whether they are concurrent or historical control values, and 4) the apparent fact that 100 mice in both sexes lived to 142 weeks (2.7 years), the control incidences reported in Table 12 (above) are unreliable.

Table 13. Historical Control Ranges for Liver Tumors in B6C3F$_1$ Mice (New Analysis)

Source	Adenomas		Carcinomas	
	Males	Females	Males	Females
CRL (1989; Purina Rodent chow)[8]	1-41.3%	1.3-17.1%	4.2-24.6%	1.4-6.3%
NTP (1999; NIH-07 diet)[9]	18-60%	12-50%	10-40%	4-20%
NTP (2003; NTP-2000 diet)[10]	10-30%	6-12%	8-20%	2-6%

[8] Based on 24-month oral feeding studies; http://www.criver.com/flex_content_area/documents/rm_rm_r_lesions_b6c3f1_crlbr_mouse.pdf

[9] Based on 19 feeding studies/sex; http://ntp-server.niehs.nih.gov/ntp/research/database_searches/historical_controls/path/m_orlfd.txt

[10] Based on 6 chronic feeding studies/sex; http://ntp-server.niehs.nih.gov/index.cfm?objectid=0362203A-C968-D248-D8158B9D6838C6B4

4. Unacceptable studies

In the first of two lifetime experiments (Hodge et al. 1966[11]; MRID 00072280), 50 C3H/Anf mice/sex were treated once by subcutaneous injection (midscapular region) with either vehicle (trioctanoin) or 10 mg amitrole in vehicle. According to the published paper, "there were only 5 cages in which 2 or more deaths did not occur on a single day" (p. 587). This may have been explained by the fact that the mice were housed in groups of 12 or 13 per cage (cage dimensions not reported). In addition, mortality in females was relatively high, with 50% of female controls having died after 11 months of treatment. Although subcutaneous cysts were observed occasionally at the site of injection (incidence not reported), no tumors were reported for either group. It should be noted, however, that histological analysis was performed on only 15% (controls) or 33% (treated) animals.

In the second lifetime study, 50 C3H/Anf mice/sex were treated weekly with 0 (methanol/acetone vehicle), 0.1, or 10 mg amitrole in vehicle by the dermal route (scapular region). As in the first study, multiple deaths were observed in single cages on single days. Median survival times for male and female mice treated with 0.1 mg amitrole were approximately 12 and 11 months, respectively. Median survival times for male and female control animals for the high dose group were approximately13 and 10 months, respectively. No tumors were observed either locally or systemically after either low- or high-dose treatment with amitrole; however, not all animals were observed histologically. Both the injection and dermal application studies are considered unacceptable due to inappropriate housing conditions, elevated mortality in control and treated animals, and missing histopathology data.

5. Other studies

Two studies were included in the evaluation of amitrole by IARC (2001), but not in the "3rd and 4th Peer Review of Amitrole". Feinstein et al. (1978) treated 87 C3H mice (both sexes combined) with 1% amitrole in the diet for 70 weeks. Only 17% of mice survived to week 40 (10 months). Because of the apparent reduced survival of treated animals (no concurrent control data were reported), the study design was changed, such that mice received a pulse diet of 1% amitrole for 4 weeks (beginning at weaning) followed by a normal diet for 1 week. Pulse feeding was continued for 1 year. The incidence of mice with "liver tumors" was 100%; however, the types of liver tumor were not reported. Twelve (out of 45) mice (27%) died during the year. An untreated control group was not included in the study.

In two separate experiments, Mori et al. (1985) treated 20 (or 20), 5 (or 20), and 5 (or 20) female mice of the NOD, DS, and ICR strains, respectively, for 3 (or 6) months with 1% amitrole in the drinking water. A hepatocellular carcinoma was observed in 1/20 NOD

[11] Hodge HC, Maynard EA, Downs WL, Ashton JK, Salerno LL. (1966). Tests in mice for evaluating carcinogenicity. Toxicol Appl Pharmacol 9(3): 583-596.

mice at 6 months. Untreated control groups for each strain were not reported for either experiment.

C. **Hamster (Oral Carcinogenicity Study; MRID 00061340)**
Author: Steinhoff D, Boehme K, Lorke D, et al. (1978)[12]
Strain: Golden Hamster
Testing Lab: Bayer AG Inst. of Toxicology for Union Carbide Agricultural Products Co.
Total Rats In Study: 75/sex/dose
Classification: Supplementary

The following text and table are taken directly from the HED CPRC "3rd and 4th Peer Review of Amitrole":

"Hamsters were fed 0, 1, 10 or 100 ppm (equivalent to 0, 0.1, 1.0 or 10.0 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."**

IV. MODE OF ACTION ANALYSES

A. Thyroid Follicular Cell Carcinogenesis

As a group, the submitted carcinogenicity studies support the published fact that amitrole is a thyroid carcinogen, specifically of follicular cells, in rats. The mode of action of amitrole-induced thyroid tumorigenesis has been published previously by Hurley et al. (1998), and involves perturbation of thyroid-pituitary functioning. Amitrole is considered to be nonmutagenic and does not mediate thyroid carcinogenesis through a mutagenic mode of action (IARC 2001). The purpose of this section is to provide a summary of the currently accepted mode of action for thyroid tumors caused by amitrole in rats (thyroid tumors have not been observed in hamsters). Evidence for the mode of action, using the framework published in Assessment of Thyroid Follicular Cell Tumors (USEPA 1998; so-called "Purple book") will also be provided here. **It is important to note that this framework was used eight years ago by Hurley et al. (1998) in their evaluation of the mode of action of 12 pesticides, including amitrole.** Evidence from two new studies is also presented that addresses the differences in amitrole-induced thyroid-pituitary disruption between rats and monkeys.

[12] Published in the open literature as Steinhoff D, Weber H, Mohr U, Boehme K. (1983). Evaluation of amitrole (aminotriazole) for potential carcinogenicity in orally dosed rats, mice, and golden hamsters. Toxicol Appl Pharmacol 69(2):161-9.

There are several key steps in the mode of action of thyroid follicular cell carcinogenesis induced by amitrole. First, amitrole irreversibly binds to and inhibits thyroid peroxidase (Strum and Karnovsky 1970; Hard 1998) and possibly the iodide pump (Hurley et al. 1998), thereby leading to decreased iodide uptake by the thyroid gland in rodents (Figure 1). In addition to its intrathyroidal site of action, there is some evidence that amitrole acts extrathroidally by enhancing the conversion of active (T_4) to inactive (reverse T_3; rT_3) thyroid hormone levels in the peripheral tissues (e.g., skeletal muscle, kidneys, liver). This is accomplished by the 3'-deiodination of T_4 by 5'-monodeiodinase (IARC 2001).

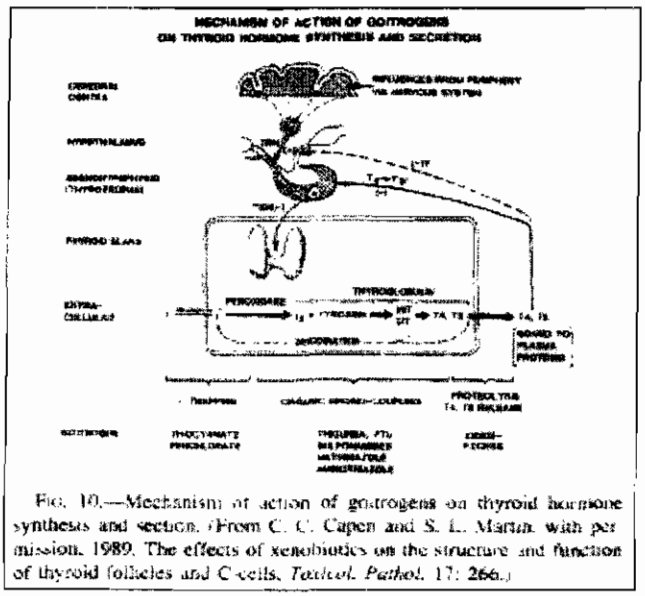

FIG. 10.—Mechanism of action of goitrogens on thyroid hormone synthesis and secretion. (From C. C. Capen and S. L. Martin, with permission, 1989. The effects of xenobiotics on the structure and function of thyroid follicles and C-cells. *Toxicol. Pathol.* 17: 266.)

Figure 1. Sites of action of intrathyroidal xenobiotics (Capen 1997; Reproduced with permission from author).

Second, due to the decreased uptake of iodide by the thyroid, there is a consequent reduction in thyroid hormone (T_4/T_3) production and therefore release into the blood of rodents. The third key event is a compensatory increase in serum thyroid stimulating hormone (TSH) that is secreted from the pituitary. Following these two hormonal changes, the fourth key step is cellular hypertrophy and hyperplasia in the thyroid, followed by an increase in thyroid weight, but without an increase in thyroid hormone blood levels. Over time, hyperplasia leads to the fifth key step, which is the formation of thyroid tumors following persistent stimulation of the thyroid by TSH. It is important to note that rodent thyroid follicular cell cancer is a *secondary* effect (i.e., indirect) of thyroid-pituitary disruption. For chemicals that act through this pathway, TSH is the actual thyroid mitogen. Unlike chemicals that act through a nongentoxic mode of action, mutagens induce neoplasia by acting directly on the thyroid. Figure 2 depicts the ways by which xenobiotic-induced chronic stimulation of the rodent thyroid by TSH may disrupt the thyroid-pituitary axis leading to neoplasia.

The reversibility of the effects of amitrole on the thyroid was demonstrated over 40 years ago (Jukes and Shaffer 1960). Using the five categories of evidence required by the USEPA (1998), Hurley et al. (1998) also noted that decreases in thyroid hormone

production have been shown to be reversible upon cessation of treatment with amitrole, thereby strengthening the accepted mode of action. Additional strength for the published mode of action is provided by an initiation-promotion study, in which amitrole was demonstrated to promote thyroid follicular cell tumorigenesis initiated by *N*-nitrosobis(2-hydroxypropyl)amine (NBHPA) (Hiasa et al. 1982). **Amitrole alone initiated no tumors over the 12-week study period.** Please refer to the IARC Monograph (2001) for additional details of the study. The sedative, phenobarbital, has also been shown to be a promoter of rodent thyroid carcinogenesis and to act by way of nongenotoxic mode of action. There is no evidence that phenobarbital produces thyroid cancer in humans (IARC 2001).

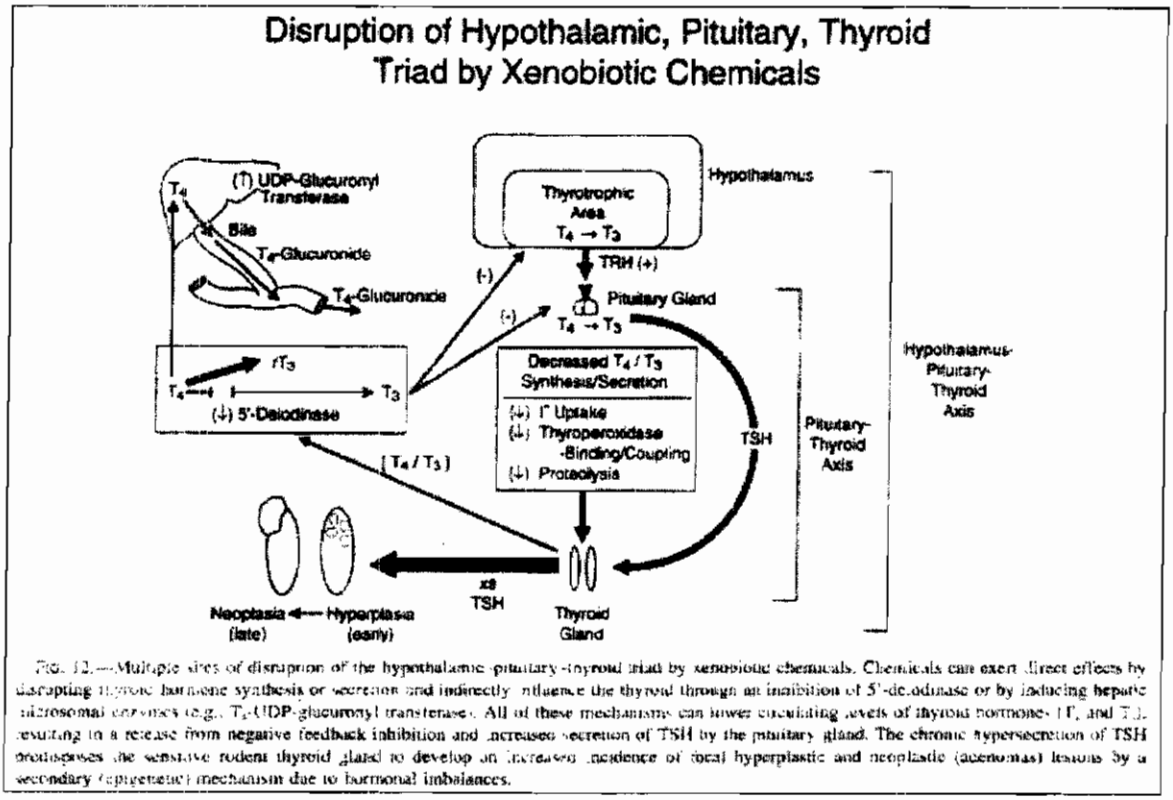

FIG. 12.—Multiple sites of disruption of the hypothalamic-pituitary-thyroid triad by xenobiotic chemicals. Chemicals can exert direct effects by disrupting thyroid hormone synthesis or secretion and indirectly influence the thyroid through an inhibition of 5'-deiodinase or by inducing hepatic microsomal enzymes (e.g., T_4-UDP-glucuronyl transferase). All of these mechanisms can lower circulating levels of thyroid hormones (T_4 and T_3), resulting in a release from negative feedback inhibition and increased secretion of TSH by the pituitary gland. The chronic hypersecretion of TSH predisposes the sensitive rodent thyroid gland to develop an increased incidence of focal hyperplastic and neoplastic (adenomas) lesions by a secondary (epigenetic) mechanism due to hormonal imbalances.

Figure 2. A schematic of the manner in which xenobiotic-induced chronic stimulation of the rodent thyroid by TSH disrupts thyroid homeostasis (Capen 1997; Reproduced with permission from author).

IARC Consensus Report: Publication 147 (1999)

The primary focus of the International Agency for Research on Cancer (IARC) Consensus Report of Scientific Publication 147 (1999) was to point out the differences in response between species to thyroid carcinogens. Specifically, IARC noted that nongenotoxic thyroid follicular cell carcinogens (e.g., amitrole) operate through a common pathway that involves perturbation of the thyroid-pituitary axis, specifically of TSH. In addition, the body concluded that the overall weight of the evidence suggests

that humans are less sensitive than rodents to thyroid tumorigenesis caused by elevated TSH.

IARC Monograph for Amitrole (2001)

The IARC Consensus Report (1999) was followed up in 2000 by a meeting of international experts in France to specifically evaluate the carcinogenic potential of 19 chemicals, including amitrole, to the human thyroid. The panel concluded that although evidence in humans was inadequate to support a causal association between exposure to amitrole and carcinogenicity – this was the case for all 19 chemicals assessed – sufficient evidence did exist in experimental animals to support such a relationship. The experts concluded that amitrole induces thyroid tumors in rodents by a nongenotoxic mode of action, specifically by inhibiting the activity of thyroid peroxidase leading to decreased thyroid hormone levels and increased TSH in the circulation. However, the expected magnitude of this effect is species-dependent. Consequently, amitrole would not be expected to cause thyroid tumors in humans at concentrations that do not perturb thyroid hormone homeostasis (IARC 2001). Please refer to the full IARC Monograph for Amitrole (2001).

Assessment of Thyroid Follicular Cell Tumors (USEPA 1998)

According to the USEPA "Purple book" (1998), five categories of evidence are required to evaluate a carcinogenic mode of action centered upon disruption of the thyroid-pituitary axis. Hurley et al. (1998) actually used each of EPA's five categories in its analysis of the mode of action of thyroid follicular cell carcinogenesis by amitrole. Understandably, the evidence presented in the "3rd and 4th Peer Review of Amitrole" (1991) was not arranged according to the EPA framework for evaluating thyroid carcinogens (published in 1998). For the purpose of consistency, that evidence has been re-categorized below according to the existing EPA "Purple book". Two newly submitted studies that measured the effect of amitrole on several thyroid endpoints in rats and monkeys are also presented. According to EPA "Purple book", the most important lines of evidence are considered to be 1) increases in thyroid growth and 2) changes in thyroid and pituitary hormones (USEPA 1998).

1. Increases in thyroid growth

Steinhoff, Boehme, and Lorke (1979): "In the published article (Steinhoff, 1983), the authors reported that 'the percentage accumulation of radioiodine in the thyroid of 100-ppm [high dose] 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' [p.166]." The explanation provided by the authors for the increase in iodine accumulation is plausible, given that the thyroid concentrates iodine under normal conditions and increases iodine accumulation during heightened activity (Hill et al. 1989). Depending on sex, thyroid

weights were 2-8X greater ($P<0.05$) than controls at 100 ppm over the course of the study.

Steinhoff and Boehme (1979): "Increased *[mouse]* 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." In fact, in males thyroid weights were increased by a factor of 2, depending on the time of collection. Iodine concentrations were also increased in both sexes.

Steinhoff, Boehme, and Lorke (1978): "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." Increases in iodine accumulation and thyroid weight observed in rats and mice, respectively, were not observed in hamsters, thereby suggesting rodent species differences in the effects of amitrole on the thyroid.

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."

Fregley (1968): "Male rats (10/dose; Blue Spruce Farms strain) were given 0.25 and 0.50 ppm amitrole (equivalent to 0.01, 0.02 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 0.02 mg/kg/day doses."

Bagdon et.al. (1956): "Male rats (10/dose; "albino rats") were given 0, 50, 250 and 1250 ppm amitrole (equivalent to 0, 5.0, 25.0 and 125.0 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 14 below]".

Table 14. Microscopic Examination

	Thyroid[a]
Concentration (ppm)	
0	-normal
50	-enlarged; follicles contain colloid
250	-hyperplasia; most follicles lack colloid
1250	-hypertrophy; hyperplasia; no follicles with stored colloid

	Liver
Concentration (ppm)	
0	-scattered vacuolization (1 animal)
50	-"no liver lipid" (1 animal)
250	-slight fatty infiltration (1 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 in the thyroid.

Based on the adverse effects in the liver at 1250 ppm (125 mg/kg/day), dosing appeared to be adequate.

Vidone et.al. (1958): "Male 'albino' rats (10/dose) were given amitrole in the diet 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.'"

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."

Alexander (1959): "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."

2. Changes in thyroid and pituitary hormones

Rat (New Data)

Fabreguettes (1998): In an acceptable/non-guideline study (MRID 46366601; 5 Sprague-Dawley rats/sex/dose were administered amitrole by gavage at doses of 0, 1.5/330, 12, or 100 mg/kg/day for 10 weeks. An additional 5 rats/sex/dose were administered the known thyroid inhibitor, 6-propyl-2-thiouracil (PTU), at a dose of 30/60 mg/kg/day as a reference group. For weeks 9 and 10, animals that had received 1.5 mg/kg/day (low dose group) during weeks 1-8 were administered 330 mg/kg/day, while the dose administered to the animals in the PTU group was also increased from 30 to 60 mg/kg/day. The reason for this increase was unreported. In a concurrent toxicokinetic study, in which a satellite group of 9 rats/sex were dosed once with 100 mg/kg/day amitrole, absorption of amitrole was observed at all measured time points (15 and 30 minutes and 1, 2, 4, 8 and 24 hours post-dosing) and was comparable between sex. The C_{max} was identified about 2 hours post-dosing.

Decreases (>10%) in body weight and body weight gain were observed in both male and female rats treated with 100 mg/kg/day only. Inhibition of thyroid function was noted by moderate to marked decreases in T3 and T4 and increased TSH levels starting as early as day 7 in both sexes administered 100 mg/kg/day. Male rats also had a 2.6-fold increase in TSH levels at the 1.5 mg/kg/day dose and males and females at the 12 mg/kg/day dose by the end of week 6 (day 42). PTU also caused thyroid inhibition, which was more significant than that for amitrole. Values for rT3 were for the most part unmeasurable (reason unspecified). The results of the thyroid function tests are reported below in Tables 15 and 16.

TABLE 15. Results of thyroid function tests in male rats (mean ±SEM) [1]

	Dose (mg/kg/day)				
	0	**1.5/330**	**12**	**100**	**PTU (30/60)**
T3 (fmol/L)					
Day 7	432 ± 17	546 ± 40 (+26) [2]	410 ± 27 (-5)	327 ± 24 (-24)	13 ± 6 (-97)
Day 21	331 ± 17	369 ± 22 (+11)	519 ± 35 (−57)	58 ± 16 (-82)	0
Day 42	361 ± 9	424 ± 46 (+17)	432 ± 33 (+20)	56 ± 25 (-84)	0
T4 (pmol/L)					
Day 7	86.4 ± 3.1	120.3 ± 8.9 (+39)	103.3 ± 5.1 (+20)	111 ± 14.3 (+28)	45.8 ± 5.3 (-47)
Day 21	96.6 ± 2.6	93.7 ± 2.5 (-3)	163.1 ± 14.4 (+69)	41.4 ± 5.6 (-57)	9.8 ± 3 (-90)
Day 42	99.2 ± 3.5	93 ± 4 (-6)	89.2 ± 4.8 (-10)	9.4 ± 3.5 (-90)	2 ± 0.1(-98)
TSH (ng/mL)					
Day 7	10.4 ± 1.9	15.7 ± 1.4 (+51)	9.5 ± 1 (-9)	39.5 ± 7.1 (x 3.8)	81 ± 9 (x 7.8)
Day 21	12.7 ± 1.2	21.1 ± 1.7 (+66)	16.6 ± 2.3 (+31)	114 ± 11 (x 9)	184 ± 14 (x 15)
Day 42	10.6 ± 0.3	27.8 ± 2.9 (x 2.6)	27.1 ± 6.8 (x 2.6)	179 ± 10 (x 16.9)	217 ± 8 (x 20.5)

[1] Data obtained from p. 54. MRID 46366601
[2] % change from control in parentheses

TABLE 16. Results of thyroid function tests in female rats (mean ±SEM) [1]

	Dose (mg/kg/day)				
	0	**1.5**	**12**	**100**	**PTU (30/60)**
T3 (fmol/L)					
Day 7	579 ± 40	457 ± 31 (-21) [2]	450 ± 8 (-22)	450 ± 16 (-22)	142 ± 4 (-75)
Day 21	365 ± 20	495 ± 18 (+36)	447 ± 24 (−22)	303 ± 36 (-17)	0
Day 42	534 ± 16	457 ± 19 (-14)	436 ± 21 (- 18)	105 ± 30 (-80)	0
T4 (pmol/L)					
Day 7	59.7 ± 4.5	53.6 ± 5.5 (-10)	48 ± 5.3 (-20)	78.5 ± 9.7 (+31)	19.3 ± 2.4 (-68)
Day 21	66.7 ± 3.1	79.3 ± 7.7 (-19)	85.4 ± 13.4 (+28)	30.7 ± 3.7 (-54)	16.7 ± 3.2 (-75)
Day 42	40.3 ± 4.1	37.4 ± 2.7 (-7)	40 ± 2.4 (-1)	4.6 ± 1.4 (-89)	1.8 ± 0.2 (-96)
TSH (ng/mL)					
Day 7	2.6 ± 0.5	3.1 ± 0.4 (+19)	5.2 ± 0.5 (x2)	15.5 ± 0.8 (x 6)	82.2 ± 4.9 (x 31.6)
Day 21	4.8 ± 0.8	3.4 ± 0.5 (-29)	3.2 ± 0.3 (-33)	44.9 ± 9.1 (x 9.1)	194 ± 6 (x 40.4)
Day 42	2.8	2.9 ± 0.2 (+4)	7.2 ± 1.5 (x 2.6)	137 ± 14 (x 14)	267 ± 15 (x 95.4)

[1] Data obtained from p. 55, MRID 46366601
[2] % change from control in parentheses

Mean absolute and relative thyroid weights were 4-8X greater in high-dose males and females, compared to controls. An increase was also noted in males and females in the 1.5/330 mg/kg/day group likely due to the increase in dose (1.5 to 330 mg/kg/day), while

values were comparable to controls in the 12 mg/kg/day group. Organ weight data are provided in Table 17.

TABLE 17. Summary of mean body and organ weights in rats[1]

	Dose (mg/kg/day)				
	0	**1.5/330**	**12**	**100**	**PTU (30/60)**
Males					
Mean final body weight (g)	430.7 ± 36.57[2]	427.1 ± 40.06	463.8 ± 18.70	316.6 ± 59.16	234.1 ± 6.32#
Absolute pituitary weight (g)	0.012 ± 0.001	0.012 ± 0.002	0.014 ± 0.003	0.013 ± 0.002	0.012 ± 0.003
Relative pituitary weight (g)	0.003 ± 0.0	0.003 ± 0.00	0.003 ± 0.001	0.004 ± 0.001*	0.005 ± 0.001**
Absolute thyroid weight (g)	0.023 ± 0.002	0.072 ± 0.018	0.029 ± 0.006	0.129 ± 0.043**	0.088 ± 0.018*
Relative thyroid weight (g)	0.005 ± 0.001	0.017 ± 0.003	0.006 ± 0.001	0.040 ± 0.008**	0.038 ± 0.009**
Females					
Mean final body weight (g)	277.5 ± 17.00	290.1 ± 33.84	276.7 ± 25.48	250.4 ± 45.65	183.0 ± 13.06
Absolute pituitary weight (g)	0.018 ± 0.001	0.017 ± 0.004	0.017 ± 0.001	0.016 ± 0.003	0.013 ± 0.001**
Relative pituitary weight (g)	0.007 ± 0.00	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.007 ± 0.001
Absolute thyroid weight (g)	0.019 ± 0.003	0.072 ± 0.02	0.027 ± 0.007	0.088 ± 0.046*	0.093 ± 0.028**
Relative thyroid weight (g)	0.007 ± 0.001	0.025 ± 0.004	0.009 ± 0.002	0.038 ± 0.024*	0.051 ± 0.015**

[1] Data from pp. 56-7, MRID 46366601
[2] Weight (g) ± SD
* P<0.05
** P<0.01

Enlarged thyroid glands were noted in all male (5/5) and female (5/5) rats dosed with either 1.5/330 or 100 mg/kg/day of amitrole or PTU. Microscopic lesions suggestive of thyroid hyperactivity were observed in all treated groups. These changes included follicular cell hypertrophy, small follicles, and increased vascularity. The severity of lesions identified were dose-related with the lesions being Grade 2 or less in the 12 mg/kg/day group and Grade 3 or 4 in the 1.5/330 or 100 mg/kg/day group and PTU group. Most animals in the 1.5/330 and 100 mg/kg/day groups showed vacuolated cells in the pituitary gland. In males, pituitary lesions were observed in 0/5, 4/5, 1/5, 5/5 and 5/5 in the control, 1.5/330, 12, 100 or 30/60 mg/kg/day groups, respectively. In females, pituitary lesions were seen in 0/5, 5/5, 0/5, 3/5, and 5/5 in the control, 1.5/330, 12, 100 and 30/60 mg/kg/day groups. These lesions increased in severity as the dose increased in the males but were similar in the degree of severity at all doses in the females.

Rat (Old Data)

Johnson et al (1981): **"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. Contrary 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 18 below]. The reported results are not unexpected. Given the lack of consistent dose administration in this study, they are uninterpretable.

Table 18. Summary of Thyroid Hormone Results (Old Data)

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 (Control)	122	7.0	119	6.9	85	7.8	67	3.6
B (3.4)**	173*	7.8	217	6.3	64	3.7	251*	6.9
C (0.35)	124	5.8	128	6.4	78	1.9	101	5.5
D (1.04)	143	6.2	187	7.8	132	3.1	59	4.7
E (3.5)	298*	10.0*	320*	7.7	148	7.7	62	5.2
Females								
A (Control)	125	3.9	66	5.8	37	3.2	65	3.6
B (3.4)**	122	3.6	120	5.8	72	1.4	190	4.7
C (0.35)	104	2.9	95	5.5	109	2.6	62	3.2
D (1.04)	131	2.7	75	5.7	121	2.5	69	3.4
E (3.5)	212*	4.9	275*	7.5	214	6.4	70	4.7

[b] 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, $p < 0.05$."

** Average dose over entire study (mg/kg/day)

Monkey (New Data)

Fabreguettes (1998): In an acceptable/non-guideline study (MRID 46366602), 1 Cynomolgus monkey/sex/dose was administered amitrole by gavage at doses of 0, 30/1000, 100, or 330 mg/kg/day for 14 weeks. An additional 1 monkey/sex/dose was administered the known thyroid inhibitor, 6-propyl-2-thiouracil (PTU), at a dose of 30 mg/kg/day as a reference group. From weeks 8-14, animals in the low dose group (30 mg/kg/day) were treated with 1000 mg/kg/day, and the animals originally dosed with 30

mg/kg/day PTU were treated with 60 mg/kg/day. At the end of week 10, the PTU dose was again increased to 100 mg/kg/day until week 14. Due to the lack of any adverse clinical signs in any group, an additional group of 1 monkey/sex was added to the study at the beginning of week 12 and administered amitrole at increasing dose levels of 1500-4000 mg/kg/day over six periods of three or four days each. After three weeks of treatment (week 14), the male was found dead (cause undetermined) and the female was kept for a reversibility period of 2 weeks. Absorption of the test material was observed in all treated animals. An increase in dose-level resulted in a proportional absorption up to 330 mg/kg/day in both males and females. Absorption was slightly higher in males than in females on days 42 and 98.

Decreases (≥10%) in body weight were observed in males in all treatment groups during the course of the study, whereas no significant differences in body weight were observed in the females. Thyroid function was also evaluated and included T3, T4, TSH, rT3 and TBG (thyroxine binding globulin). Only the results for T3, T4, and TSH were provided, and they are presented below in Tables 19-21. Reported serum rT3 values were unreliable, because the lower limit of the assay did not account for the values. In addition, serum TBG values slightly increased in the 100 and 330 mg/kg/day male and female (no further information).

Males at ≥100 mg/kg/day demonstrated effects on thyroid function, while no effects were observed in females <330 mg/kg/day. No significant changes in the thyroid tests were observed in the male or female PTU-treated animals. The male exhibited slightly more thyroid inhibition but the effects were minor. This suggested a possible error in or an insufficient dose of the PTU in the primates.

TABLE 19. Results of thyroid function tests in male monkeys (n=1/dose) [1]

	Dose (mg/kg/day)				
	0	**30/1000**	**100**	**330**	**PTU (30/60/100)**
T3 (nmol/L)					
day -3	2.4	1.8	2.0	2.2	2.4
day 7	2.6	2.3	2.2	2.7	2.2
day 21	2.2	1.8	1.5	3.2	2.1
day 42	2.2	1.6	0.2	2.6	1.7
day 97	2.3	<0.5	0.5	0.5	1.9
T4 (nmol/L)					
day -3	23	25	42	41	41
day 7	24	22	36	59	43
day 21	25	29	25	73	39
day 42	25	14	<7.0	67	35
day 97	31	<7.0	<7.0	<7.0	22
TSH (mUL/L)					
day -3	1.42	0.1	1.22	0.46	0.48
day 7	1.88	0.14	0.4	0.10	0.67
day 21	0.67	0.1	4.31	0.04	0.44
day 42	1.12	1.76	77.7	0.25	0.47
day 97	1.37	>75	>75	60	1.16

[1] Data obtained from p.34, MRID 46366602

TABLE 20. Results of thyroid function tests in female monkeys (n=1/dose) [1]

	Dose (mg/kg/day)				
	0	**30/1000**	**100**	**330**	**PTU (30/60/100)**
T3 (nmol/L)					
day -3	2.0	2.5	2.0	2.0	2.1
day 7	2.5	2.4	2.3	2.1	2.3
day 21	2.4	2.3	2.4	2.1	2.2
day 42	2.1	1.8	2.3	1.6	1.8
day 97	2.7	1.5	2.5	<0.5	2.3
T4 (nmol/L)					
day -3	26	47	23	21	14
day 7	25	39	22	16	27
day 21	32	30	33	30	49
day 42	21	40	27	16	19
day 97	27	19	37	<7.0	31
TSH (mUL/L)					
day -3	0.35	0.20	0.5	0.23	0.63
day 7	0.43	0.49	0.22	0.43	0.67
day 21	0.67	0.40	0.43	0.60	0.87
day 42	0.27	0.32	0.65	2.79	1.51
day 97	0.17	11	0.42	>75	0.47

[1] Data obtained from p. 35, MRID 46366602

TABLE 21. Results of thyroid function tests in 1 female monkey treated with 1500-4000 mg/kg/day (stepwise dose increase every 3-4 days for 3 weeks)

T3 (nmol/L)	
day -3	2.2
day 20	1.6
day 35	3.3
T4 (nmol/L)	
day -3	30
day 20	50
day 35	27
TSH (mUL/L)	
day -3	0.85
day 20	0.11
day 35	0.19

[1] Data obtained from p. 35, MRID 46366602

All males in the treated groups had increased absolute and relative thyroid weights on necropsy compared to controls (Table 22). Absolute and relative thyroid weights were increased in females at the high dose only.

TABLE 22. Summary of mean body and organ weights in monkeys (n=1/sex/dose)[1]

	Dose (mg/kg/day)					
	0	**30/1000**	**100**	**330**	**PTU (30/60/100)**	**1500-4000**
Males						
Terminal body wt. (kg)	6.3	4.40	5.56	4.70	6.54	NR[2]
Thyroid Absolute (g)	0.4060	1.104 (172)[3]	0.4870 (20)	0.539 (32)	0.8440 (108)	
Relative (% BW)	0.0065	0.025 (285)	0.0087 (34)	0.011 (69)	0.0129 (98)	NR
Adrenal Absolute (g)	0.4890	0.4950	0.5110	0.3770 (-23)	0.5620 (15)	
Relative (% BW)	0.0078	0.0112	0.0092	0.0080	0.0086 (10)	NR
Females						
Terminal body wt.(kg)	3.98	3.76	3.34	3.86	4.12	3.62
Thyroid Absolute (g)	0.3870	0.2960 (-24)	0.3250 (-16)	1.1410 (195)	0.4090	0.2310 (-40)
Relative (% BW)	0.0097	0.0079 (-19)	0.0097	0.0295 (204)	0.0099	0.0063 (-35)
Adrenal Absolute (g)	0.5750	0.4150 (-28)	0.337 (-41)	0.7000 (22)	0.4130 (-28)	0.5520
Relative (% BW)	0.0144	0.0110 (-24)	0.010 (-31)	0.0181 (26)	0.0100 (-31)	0.0153

[1] Data obtained from pp. 141-154, MRID 46366602
[2] NR = not recorded
[3] % change from control in parentheses

In all animals receiving the test substance, dark thyroid glands were observed. Each male in the 100, 330, or 30/1000 mg/kg/day group and the female in the 330 or 30/1000 mg/kg/day group was observed to have microscopic lesions indicative of thyroid hyperactivity. These included follicular cell hypertrophy, increased vascularity, and follicular cell hyperplasia. Severity was rated as Grade 1-4. Lesions in the males showed increased severity with dosing but this trend was not observed in the females. No microscopic changes in the thyroid were noted in the PTU dose group or in either the male or female treated with 1500-4000 mg/kg/day amitrole.

3. Site(s) of antithyroid action

Decreased iodine uptake by the thyroid in several studies (above) suggests that the action of amitrole is intrathyroidal. The following data confirm this conclusion.

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 monitored in perfused thyroid tissue of treated and control rats and was reported to show, in treated rats, a decrease in activity at one week which continued to decrease over the six month study."

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.'"

Alexander (1959): "Male Sprague-Dawley rats were injected with 1 mg of amitrole followed 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.'"

4. Reversibility of effects

Wynford-Thomas et al. (1982c): "Rats were treated with 0.1% amitrole in the drinking water for a period of 80 days. Amitrole was then withdrawn for 25 days and subsequently re-introduced for a further 35 days. Groups of animals were killed at various intervals and the following were measured: serum TSH, T_3, T_4, thyroid weight, follicular cell number and mitotic activity. The authors stated that 'the initial period of treatment led to a 5-fold increase in serum TSH, a 10-fold increase in thyroid weight and a 9-fold increase in follicular cell number. Mitotic activity stabilized at a few times [to] control levels. Following withdrawal of amitrole, TSH and mitotic activity fell to below normal. Thyroid weight fell by 66% but there was no significant fall in follicular cell number. Re-introduction of amitrole simply led to a return of all variables to their previous 'stimulated' levels. There was no second burst of mitotic activity and no renewed thyroid growth.' After the 80 days of treatment, serum T_3 and T_4 dropped to below detectable levels. Withdrawal of amitrole led to a return to normal levels of T_3 and to a slightly supranormal level of T_4 by 25 days. Subsequent re-introduction of amitrole produced a rapid fall to reach undetectable levels again by 14 days."

Babish et al. (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."

5. Dose-response concordance

In most of the rat carcinogenicity studies, antithyroid effects were not measured alongside thyroid tumor response. However, dose-response concordance was observed between hyperplasia and thyroid tumors in the lifetime pulse feeding study in the rat (MRID 00132445), despite the unique study design.

The best evidence available to perform a dose-response concordance analysis for non-tumor, antithyroid endpoints is provided by the newly submitted data in the rat and monkey (summarized above). Although the administered doses to the animals were changed in the rat study, clear changes in thyroid hormone levels, serum TSH, thyroid weight, and histopathology were observed with increasing dose. These changes are what would be expected to be produced by the established mode of action in rodents. In the monkey study, changes in antithyroid parameters were observed; however, the limited number of subjects tested (1/sex/dose) makes study interpretation difficult.

6. Other (concordance across antithyroid effects)

Several well-done studies were published by Wynford-Thomas (1982) that demonstrate excellent concordance among several antithyroid (non-tumor) endpoints observed after treatment of rats with amitrole in the drinking water. Although only one dose was tested, changes in thyroid hormone levels, thyroid weight, and/or hyplerplasia were observed that are consistent with the established mode of action. These changes were not observed in control animals. The studies were summarized in the "3rd and 4th Peer Review of Amitrole" and are reported here below:

Wynford-Thomas et al. (1982a): "Groups of male Wistar rats were administered amitrole at a concentration of 0.1% in the drinking water. Appropriate controls were provided. The treated and control groups were killed at various times up to 153 or 154 days. On each sacrifice day, the thyroid was removed for microscopic examination and TSH levels

were measured. The authors stated that the level of serum TSH rose rapidly from 979 to 4507 ng/ml by day 24. This was followed by a slower rise to reach a sustained maximum of over 5000 ng/ml by day 116. Controls rose as well, but only to a level of 1407 ng/ml by day 154. The thyroid weights increased in phases: an initial lag of a few days, a period of rapid growth for 46 days, and a final period of declining growth rate, reaching a maximum 12-fold plateau (266.6 $\pm$ 44.3 mg) by day 25. Control weights rose from approximately 22 to 28 mg by day 252, following which no further growth occurred. **There was a close correlation between the thyroid weight and the level of serum TSH.** The authors stated that 'there was a rapid change during the first week in the proportional volumes of component tissues; a rise in the epithelial cell and blood vessel components being accompanied by a corresponding fall in follicular lumen and non-vascular stroma. **Total epithelial cell volume rose in parallel with thyroid weight to reach a 16-fold maximum.** There was a more marked increase in vascular volume and there were smaller increases in the other two components...The morphological changes persist, but growth eventually ceases, despite a sustained rise in the level of serum TSH, thus pointing to the existence in the thyroid of a growth-limiting control mechanism.'"

Wynford-Thomas et al. (1982b): "Animals [rats] maintained on 0.1% amitrole in the drinking water were sacrificed together with controls at frequent intervals over a period of 5 months. The following were measured: serum TSH, T_3, T_4, thyroid/serum iodide levels, thyroid weight, follicular cell number and follicular cell mitotic activity. The authors stated that 'serum T_3 and T_4 rapidly fell to undetectable levels within 2 weeks. **The level of serum TSH rose to a stable 5-fold maximum after 4 weeks. The *thyroid/serum ratio followed a closely similar pattern rising to a sustained* 7-fold maximum.** Thyroid weight and follicular cell number increased rapidly for the first few weeks but the growth rate declined progressively, falling almost to zero after 80 days, consistent with the observed change in cell number.'"

Wynford-Thomas et al. (1982c): "Rats were treated with 0.1% amitrole in the drinking water for a period of 80 days. Amitrole was then withdrawn for 25 days and subsequently re-introduced for a further 35 days. Groups of animals were killed at various intervals and the following were measured: serum TSH, T_3, T_4, thyroid weight, follicular cell number and mitotic activity. The authors stated that 'the initial period of treatment led to a ***5-fold increase in serum TSH, a 10-fold increase in thyroid weight* and a 9-fold increase in follicular cell number.**"

Species Differences in Thyroid Carcinogenesis

No chemical, including amitrole, has been shown to be carcinogenic to the human thyroid, and the only known thyroid carcinogen in humans is ionizing irradiation, specifically from either therapeutic X-irradiation (acute exposure) or nuclear fallout (radioiodine) (Hard 1998). More importantly, there are several species differences that account for the reduced relevance to humans of rodent thyroid follicular cell carcinogenesis induced by amitrole. Most are summarized below in Table 23. Although the architecture of the pituitary feedback mechanism that regulates thyroid function is the same in rats and humans, constitutive thyroid hormone and TSH levels are approximately

10X and 25X higher, respectively, in rats than in humans (Meek et al. 2003). These observations, along with the fact that the major thyroid hormone carrier, thyroxine binding globulin (TBG), is present in humans, but not in the rat, explain the shorter half-lives of T_4 and T_3 in rats than humans. In addition, McClain (1995) has reported that the mean background incidence of thyroid follicular cell tumors is 500X higher in Fischer male rats (2%) than in humans (0.004%). This may occur because thyroid tumors in humans tend to be papillary, rather than follicular, as in rats (McClain 1995). It has also been noted that rats treated with iodine-deficient diets have a high incidence of thyroid tumors, whereas epidemiological data linking the presence of goiter (due to iodine deficiency) and thyroid cancer in humans are weak and inconclusive (McClain 1995). Therefore, while the established mode of action of amitrole for follicular thyroid carcinogenesis in animals is qualitatively plausible in humans, it is quantitatively implausible based on differences in thyroid physiology (also reflected in differences in incidence data) between rats and humans. "*Quantitatively*, if humans develop cancer through thyroid-pituitary disruption, it appears that humans are less sensitive to the carcinogenic effects than are rodents. Rodents show significant increases in cancer with thyroid-pituitary disruption; humans show little, if any" (USEPA 1998).

Table 23. Metabolic and histopathological differences between rats and humans in basal thyroid-pituitary axis endpoints (USEPA 1998).

Parameter	Human	Rat
Thyroxine-binding globulin	present	essentially absent
T_4 Half-life	5-9 days	0.5-1 day
T_3 Half-life	1 day	0.25 day
T_4 Production rate/kg b.w.	1	10 × that in humans
TSH	1 ×	6-60 × that in humans
Follicular cell morphology	low cuboidal	cuboidal
Sex differences		
Serum TSH	sexes equal	M ≥ 2 × F
Cancer sensitivity	F ≥ 2.5 × M	M > F

Other Modes of Action (Mutagenicity)

According to several published papers, amitrole is considered to be nonmutagenic (IARC 2001; Dybing and Sanner 1999, Hill et al. 1989). Amitrole been shown to be negative in prokaryotes in all *in vitro* mutagenicity assays, either with or without exogenous metabolic activation. Amitrole is also negative in *in vivo* clastogenicity assays, including the mouse micronucleus (bone marrow) assay, and did not induce unscheduled DNA synthesis in mouse hepatocytes. Amitrole was also demonstrated to be negative in 4/5 *in vitro* mammalian gene mutation assays. In the only positive study, Tsutsui et al. (1984)

reported increased mutation rates at the hypoxanthine phosporibosyl transferase (HPRT) and Na^+: NK^+ ATPase loci in Syrian hamster embryo cells. However, only one replicate was performed per concentration in the study and the result for the appropriate concurrent control was not reported. In addition, amitrole was negative for gene mutation using Syrian hamster BP6T cells, as well as in 3 separate gene mutation assays using mouse lymphoma cells. Therefore, the weight of the evidence supports the conclusion that amitrole is nonmutagenic in mammalian cells. For a complete listing of the genotoxicity database, please refer to the attached IARC Monograph for Amitrole (2001). Many assays are also listed in Hill et al. (1989).

No new genotoxicity studies have been submitted to the Agency since the "3rd and 4th Peer Review of Amitrole" (1991). In 1991 the 3rd and 4th Peer Review of Amitrole cited Hill et al. (1989), who concluded that amitrole is not generally genotoxic and does not likely mediate its carcinogenic effects by way of a mutagenic mode of action. In addition, the Peer Review itself concluded that the database suggests that amitrole is a nongenotoxic carcinogen. However, there was residual uncertainty concerning the results of two *in vivo* micronucleus tests and a cell transformation assay submitted to the Agency.

Cell transformation assays are *in vitro* assays used to predict potential rodent carcinogenicity and that, in contrast to standard genotoxicity assays, can detect both genotoxic and nongenotoxic carcinogens (Mauthe et al. 2001). However, the assay itself cannot discriminate between carcinogens that operate through a nongenotoxic mode of action and those that do not. The assay in question was performed in BALB/3T3 cells (a mouse embryonic fibroblast cell line). In the 3rd and 4th Peer Review, amitrole was reported to be positive in a BALB/3T3 assay conducted by Brusick (1976; MRID 00052648). However, reanalysis of the study report indicated that amitrole was positive at 2 concentrations in only 1/3 trials and that the effect was not dose-dependent.

Similarly, review of the two *in vivo* micronucleus tests indicated that amitrole was indeed negative in both assays. Re-analysis also indicated that sampling times were adequate in Salamone et al. (1981). In Tsuchimoto and Matter (1981), 2 animals/sex were injected twice i.p. with appropriate doses of amitrole. Although the number of animals tested was not ideal, the number of polychromatic erythrocytes (1500/animal) was considered to be adequate and the negative results supported those reported by Salamone et al. (1981).

Last, the "3rd and 4th Peer Review of Amitrole" (1991) hypothesized that amitrole might generate genotoxic products after being activated by to the enzyme, prostaglandin H synthase (PHS), in the thyroid. In the presence of PHS, amitrole failed to induce gene mutations in the Ames test using the TA98 strain of Salmonella typhimurium (Croker et al. 1992). The authors concluded that these negative results were consistent with the accepted nongenotoxic mode of action of amitrole.

Based on the overall weight of the evidence found in the genotoxicity database (published by Hill et al. 1989 and IARC 2001), amitrole is not considered to be a mutagen or to cause thyroid follicular cell carcinogenesis through a mutagenic mode of

action. This conclusion is the same as that reached by Hurley et al. (1998), IARC (2001), and USEPA (2005).

Structure-Activity Relationships (SAR)

Examination of structure-activity relationships is most helpful when there is limited or inconclusive data on the carcinogenic potential or mode of action of a chemical. For amitrole, however, the carcinogenic potential in the rodent thyroid (not hamster), as well as the nongenotoxic mode of action of follicular cell carcinogenesis are both well established.

As the "3rd and 4th Peer Review of Amitrole" itself pointed out, the SAR discussion in that 1991 document was quite speculative. Much of the discussion was centered on attempting to link the (at that time) supposed genotoxicity of amitrole with what were viewed to be structurally related chemicals that were mutagenic and carcinogenic, specifically bridged double ring aromatic amines. Later, in 1998, however, Hill et al. pointed out that amitrole was not a structural analogue of members of the thionamide, aromatic amine, or halogenated hydrocarbon class of chemicals. Hill et al. (1998) contended that amitrole shared a *functional* similarity only, to certain thionamides and aromatic amines that inhibited thyroid peroxidase. This supports the established mode of action for amitrole. In addition, the ability to inhibit thyroid peroxidase was not correlated with genotoxicity in the analysis by Hill et al. (1998). Moreover, the evidence for the genotoxicity of amitrole was considered by Hill et al. (1998) to be limited.

B. Pituitary Neoplasia

Statistically significantly increased incidences of "pituitary tumors" were observed at the highest dose tested (100 ppm) in rats in a lifetime feeding study conducted by Steinhoff et al. 1979 (MRID 00061351). Although a differentiation between adenomas, carcinomas, and cysts was not made in the original study report, the increased incidences in pituitary tumors were also evident when the data were published in the open literature (Steinhoff et al. 1983). Pituitary hyperplasia progressing to neoplasia is observed in some cases when there is a demand for increased TSH secretion (Paynter et al.; 1988 USEPA 1998). In addition, Hill et al. (1989) concluded, "pituitary hyperplasia and neoplasia appear to result from the same treatments causing thyroid neoplasia – conditions leading to prolonged circulating thyroid hormone decrease and excessive secretion of TSH by the pituitary gland".

C. Hepatocarcinogenesis

Without any evidence of a treatment-related tumor response in the liver of animals in any submitted study, a mode of action analysis is unnecessary.

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V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

Rat

Thyroid Follicular Cell Tumors

-As a group, the submitted carcinogenicity studies provide sufficient information to support the published fact that amitrole is a thyroid carcinogen, specifically of follicular cells, in rats. This was determined by HED's 3rd and 4th Peer Review of Amitrole and re-confirmed by the present CARC. Although adequacy of dosing for each study was not explicitly assessed by the 3rd and 4th Peer Review of Amitrole, the carcinogenic potential of amitrole in the rat thyroid is so well accepted that this dimension of the assessment is not emphasized in the current CARC review. The treatment-related increases in thyroid follicular cell tumors were seen in the following studies as excerpted from the 3rd and 4th Peer Review of Amitrole:

a) Keller 1959 (MRID 00082176): Amitrole administered in the diet to Charworth Farms rats at 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 was associated with numerical increases, in both sexes at the terminal sacrifice, in the incidence of thyroid adenoma at the mid (50 ppm) and high dose (100 ppm) and in thyroid adenoma/carcinoma combined at the high dose. There were statistically significant dose-related positive trends in the incidence of thyroid gland tumors. However, there were no significant pairwise comparisons between any dose group and control. Although serious conduct problems were identified in this study, the data did show increased incidences of thyroid neoplasia.

b) Johnson et al. 1981 (MRID 00132445): Amitrole administered in the diet (0-0 (A), 1-20 (C), 3-60 ((D), 5-100 (B), or 10-200 (E) ppm to Fischer 344 rats was associated (in treatment groups B, D and E) with statistically significant increases in the incidences of thyroid follicular cell adenoma and combined adenoma/carcinoma in both sexes. "Treatment groups B, D and E show statistically significant increases in the incidences of thyroid follicular cell adenoma and combined adenoma/carcinoma in the pairwise comparison for both sexes. There are also statistically significant positive trends for adenoma and carcinoma, individually and combined, in both sexes"

c) Steinhoff et al. 1979 (MRID 00061351), Steinhoff et al. 1983: Amitrole administered in the diet (0,1,10 or 100 ppm equivalent to 0, 0.05, 0.5, and 5.0 mg/kg/day) to Wistar rats was associated with statistically significant increases in the incidence of "thyroid tumors" for the high dose (100 ppm) [5 mg/kg/day] males and females when compared to controls, as well as a statistically significant positive trend in both sexes

d) Tsuda et al. (1976) (MRID 00052654): A fourth study was performed in rats (Wistar) exposed for 70 weeks to 0 or 2500 ppm [250 mg/kg/day] amitrole in drinking water. In the 3rd and 4th Peer Review of Amitrole, this study was reported as a subchronic study. The study did measure carcinogenic activity, however, and tumors and tissue invasion were observed in treated animals by the end of the study. "The author indicated that although there was evidence of follicular tissue invasion in all treatment groups exposed

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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.'

Pituitary Tumors

c) Steinhoff et al. 1979 (MRID 00061351); Steinhoff et al. 1983: Amitrole administered in the diet (0, 1, 10 or 100 ppm equivalent to 0, 0.05, 0.5, and 5.0 mg/kg/day) to Wistar rats was associated with statistically significant increases in the incidence of "pituitary tumors" in both sexes at the high dose level, with a statistically significant positive trend for females.

Mouse

a. Innes 1969 (MRID 00043595): 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. 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 dose selected exceeded the Maximum Tolerated Dose (MTD) for this strain of mice. Therefore, this study did not contribute to the WOE for carcinogenicity and was not used for regulatory purposes.**

b Steinhoff and Boehme (1979) (MRIDs 00061348; 41317901;41462501)-**UPDATED ANALYSES:** Amitrole was administered in the diet (0,1,10 or 100 ppm equivalent to 0, 0.15, 1.5, or 15 mg/kg/day) to NMRI mice for 18 months. The incidences of liver tumors in NMRI mice were not correctly reported in the "3rd and 4th Peer Review of Amitrole". Therefore, a **new analysis** of the incidences of benign, malignant, and combined benign/malignant primary liver tumors was performed based on a review of the individual animal data contained in the original study report (MRID 00061348). **A dose-dependent increase in benign (adenomas), malignant (carcinomas), or combined benign/malignant liver tumors was not evident in either sex.** Statistical analysis was not warranted since tumor incidences in each treatment group were less than or at control levels. In addition, the recalculated liver tumor incidences for both hepatocellular adenomas and carcinomas were below the historical control values for NMRI mice from 1983-1993 reported by Carmichael et al. (1997) for mouse studies conducted for 18 months (n=2 studies). **Therefore, the CARC did not consider the liver tumors to be treatment-related. This conclusion reverses the 1990 CPRC assessment of the liver tumors.**

c.Vesselinovitch 1983 (**Updated interpretation**): 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. This study is unreliable and could not be evaluated due to major insufficiencies in study reporting. **Therefore, the CARC concluded that this study did not contribute to the weight-of-the-evidence.**

Hamster

Steinhoff et al. 1978 (MRID 00061340): Amitrole administered in the diet (0, 1, 10, or 100 ppm equivalent to 0, 0.1, 1 or 10 mg/kg/day) to Golden hamsters for 2 years was negative for carcinogenicity. **There was no evidence of treatment related induction of tumors of any type including the thyroid, liver and pituitary.**

2. Mutagenicity

Based on the overall weight of the evidence found in the genotoxicity database (published by Hill et al. 1989 and IARC 2001), amitrole is not considered to be a mutagen or to cause thyroid follicular cell carcinogenesis through a mutagenic mode of action. This conclusion is the same as that reached by Hurley et al. (1998), IARC (2001), and USEPA (2005).

3. Structure Activity Relationship

Hill et al. (1998) contended that amitrole shared a *functional* similarity only, to certain thionamides and aromatic amines that inhibited thyroid peroxidase. This supports the established mode of action for amitrole.

4. Mode of Action

The mode of action of amitrole-induced thyroid tumorigenesis has been published previously by Hurley et al. (1998), and involves perturbation of thyroid-pituitary function. The CARC concluded that evidence for an antithyroidal mode of action in rodents, using the framework published in Assessment of Thyroid Follicular Cell Tumors (USEPA 1998; so-called "Purple book") was established. Amitrole is considered to be nonmutagenic and does not mediate thyroid carcinogenesis through a mutagenic mode of action (IARC 2001).

Amitrole induces thyroid tumors in rodents by inhibiting the activity of thyroid peroxidase leading to decreased thyroid hormone levels and increased TSH. In addition, the increases in cell growth *in vivo* (e.g. increases in thyroid weights and thyroid hypertrophy and hyperplasia) progressing to thyroid follicular cell tumors were seen in the presence of thyroid/pituitary hormone changes. Decreased iodine uptake by the thyroid points to an intrathyroidal site of action. Decreases in thyroid hormone production have been shown to be reversible upon cessation of treatment with amitrole, which strengthens the accepted mode of action.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March 2005), the CARC classified amitrole as **"Not Likely To Be Carcinogenic To Humans At Doses That Do Not Alter Rat Thyroid Hormone Homeostasis"**. This decision was based on the following:

(i) Treatment-related thyroid follicular cell tumors were seen in male and female rats (multiple strains) based on evidence provided collectively from several studies (Keller 1959; Johnson et al. 1981; Steinhoff et al. 1979; and Tsuda et al. 1976); pituitary tumors were also seen in male and female rats in one study by Steinhoff et al. 1979;

(ii) No treatment-related tumors were seen in male or female mice (Steinhoff and Boehme 1979);

(iii) Amitrole is considered to be nonmutagenic and does not mediate thyroid carcinogenesis through a mutagenic mode of action

(iv) The overall weight-of-the-evidence was considered sufficient to indicate that amitrole induced thyroid follicular tumors through an antithyroidal mode of action;

(v) Rats are substantially more sensitive than humans to the development of thyroid follicular cell tumors in response to thyroid hormone imbalance.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The quantification of carcinogenic potential is not applicable.

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