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

JAN 3 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Amitraz (BAAM®)

FROM: Ray Landolt 12/20/90
Toxicology Branch II - Herbicide, Fungicide, and
Antimicrobial Support
Health Effects Division (H7509C)

and

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Science Analysis and Coordination Branch
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TO: Dennis H. Edwards, Jr., PM 12
Insecticide-Rodenticide Branch
Registration Division (H7505C)

The Health Effects Division (HED) Peer Review Committee met on October 31, 1990 to discuss and evaluate the weight-of-the-evidence on amitraz with particular reference to its carcinogenic potential. The Committee concluded that amitraz should be classified as a Group C, possible human carcinogen. Quantification of potential human cancer risk, using a low-dose extrapolation model (Q^*_1), was recommended. The Q^*_1 will be calculated based upon the combined hepatocellular adenomas and carcinomas in female mice.

A. Individuals in Attendance

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

Penelope Fenner-Crisp
William Burnam
Karl Baetcke
Marcia van Gemert
for John Quest
Kerry Dearfield
Esther Rinde
Hugh Pettigrew
Yin-Tak Woo
Marion Copley
George Ghali

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

Reto Engler
Richard Hill —
Robert Beliles
William Sette
Julie Du
Jean Parker

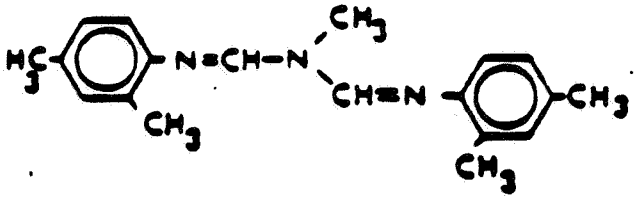
3. Reviewers: (Non-Committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Ray Landolt
Mike Ioannou
Bernice Fisher

4. Others: (Observers) Flora Chow, HED/OPP.

B. Background Information and Regulatory History

Amitraz (N'-[2,4-dimethylphenyl]-N-[[(2,4-dimethylphenyl) imino] methyl]]-N-methylmethanimidamide) is a formamidine insecticide/acaricide, which was first synthesized by the Boots Pure Drug Company in England in 1969, and subsequently underwent extensive field testing in the United States by the Upjohn Company. It is marketed in the United States by the Upjohn Company and NorAm Chemical Company under the trade name of BAAM. Tolerances have been established under 40 CFR 180.287 for amitraz residues in/on raw agricultural commodities.



Amitraz
(BAAM)

On April 6, 1977, the Agency published a notice of rebuttable presumption against registration (RPAR) of pesticide products containing amitraz (42 FR 18299) pursuant to 40 CFR 162.11(a)(5). This notice of determination constituted a preliminary notice of intent to deny registration of amitraz for use on apples and to grant conditional registration of amitraz for use on pears.

"Briefly, the three major findings made in support of the RPAR are: (1) amitraz induced lymphoreticular (LR) tumors in the highest-dose female mice in the Boots Mouse Study; (2) amitraz induced lung tumors in the highest dose female mice in the Boots Mouse Study [this tumor type was later discounted by CAG (final report Dec. 1978) as not statistically significant] ; and (3) 2,4-dimethylaniline, a metabolite of amitraz, induced sarcomas in female mice and malignant tumors in male rats in a National Cancer Institute study" (FR Vol. 44, No. 9, Jan. 12, 1979).

For more details of the regulatory history, see FR Vol. 44, No. 9, January 12, 1979, pages 2678 to 2683, and the Reregistration Guidance, EPA Case No. 234, CAS No. 33089-61-1 dated October 1987.

Although the Agency's Cancer Assessment Group (CAG) concluded that the lymphoreticular tumors in female CFLP mice were treatment related, the FIFRA Scientific Advisory Panel (SAP), in their meeting in 1979, did not concur with this conclusion. The panel indicated that a statistically significant increase in lymphoreticular tumors in amitraz-treated mice had not been shown. In the panel's opinion, the carcinogenicity of amitraz was sufficiently questionable that the restriction on its use was not warranted.

Thereupon, the Agency registered amitraz on pears in January 1980 on the condition that the mouse carcinogenicity study be repeated in order to resolve the controversial interpretation of the mouse study.

A second mouse study was initiated using $B_6C_3F_1$ mice since the CFLP strain used in the first study was no longer available. The same dose regimen was used in the second study. Although the 90-day range-finding study indicated that a lower dosing regimen should be followed because of significant body weight gain decrease, the Agency suggested that the same dose levels fed in the original 80-week study be used in the repeat study.

A significant increase in hepatocellular tumors (carcinoma and adenoma) was reported in females of the high-dose group. CAG (1986) classified amitraz in Group C, possible human carcinogen. However, SAP (1986) recommended that amitraz be classified as a Group D because the weight-of-the-evidence was inadequate to clearly categorize the carcinogenicity of amitraz.

Although the toxicology issues concerning the classification of amitraz were addressed by the Toxicology Branch of the Hazard Evaluation Division (HED) with the conclusion that amitraz should be classified as Group C carcinogen not requiring a quantitative risk assessment and for which additional uses should be compared to the RfD (R.B. Jaeger, April 2, 1987), a comprehensive determination of the-weight-of-the-evidence regarding the carcinogenicity of amitraz has never been performed by the HED Peer Review Committee. It was reasonable, therefore, to submit the chemical to the HED Peer Review Committee for further evaluation of the weight-of-the-evidence and classification of amitraz with respect to its carcinogenic potential.

C. Evaluation of the Carcinogenicity Evidence

1. Carcinogenicity Study in CFLP Mice - Boots Company, Ltd., Report Nos. 76039 and 76059 dated May 21, 1976, EPA Accession Nos. 11186, 30491, and 44484.

   a. Experimental Design - In this study, four groups of 50 CFLP mice/sex/dose were administered amitraz in the diet at concentrations of 0, 25, 100, or 400 ppm for 80 weeks. [redacted] a stabilizing agent, was present in the treated diet at concentrations of 0.6, 2.5, and 10 ppm, respectively, during the final 14 weeks of the study.

   b. Consideration of Adequacy of Dose Selection - The high-dose tested was considered adequate for carcinogenicity testing in females based upon a significant ($p < 0.01$) decrease in body weight gain of 18 percent. In males, the maximum tolerated dose might have been exceeded based upon decreased body weight gain of 37 percent in the high-dose group. The body weight gain decrease was accompanied by a statistically significant ($p < 0.01$) increase in food consumption of about 42 and 12 percent, respectively, in males and females of the high-dose group. The treatment had no effect on mortality.

   c. Neoplastic Lesions - There was a significant ($p < 0.03$) increase in the incidence of lymphoreticular tumors in females of the high-dose group (49%) when compared to concurrent controls (23%) (DER 001116, August 30, 1976 and J. Holder evaluation, September 6, 1986).

2. Two-Year Carcinogenicity Study in $B_6C_3F_1$ Mice - Report No. 456-49 dated December 1983, prepared by Huntingdon Research Center, EPA Accession Nos. 252098 and 252102.

   a. Experimental Design - Three groups of 75 $B_6C_3F_1$ mice/sex/group were fed dietary levels of 25, 100, or 400 ppm with 100 mice/sex in the control group. Animals were housed with five mice/sex/cage. This strain of mice was used in this repeat study since the CFLP strain used in the 80-week study was no longer available.

b. General Observations - Hyperactivity and aggressive behavior was observed for males fed the 400 ppm and to a lesser degree in males fed the 100 ppm level during the first 12 weeks of the study. Cutaneous lesions, as evidence of fighting, accompanied by inflammation of the perigenital and perianal areas were also observed in male mice fed the 100 and 400 ppm levels. These gross effects were not observed in females fed dietary levels of amitraz.

A decrease in food consumption was reported during weeks 13 to 19 for males and females fed the 100 and 400 ppm levels, followed by an increased food intake for both sexes (9-13%) at the 400 ppm level for the duration of the study. Group mean body weight gains were reduced in males (25%) and females (14%) of the high dose group by week 13. Group mean body weight changes were significantly ($p < 0.001$) reduced at the 400 ppm level by week 18 for males (30%) and for females (31%), and by week 52 body weight gains were significantly ($p < 0.01$) reduced for males (29%) and females (55%) as compared to control values. A significant decrease ($p < 0.01$) in group mean body weight gain was reported at the 100 ppm level for males (29%) and for females (32%) by week 52.

Statistical evaluation of survival (Table 1) indicated that males had a significant positive trend in mortality with incremental doses of amitraz. Female mice had no differential mortality with dose increments of amitraz.

The bone marrow myeloid/erythroid ratio was significantly ($p < 0.001$) reduced, as compared to controls, for males (24%) at the 400 ppm level and for females at the 100 ppm (22%) and 400 ppm levels (26%).

The incidence of focal hyperkeratosis of the forestomach and spleen hematopoiesis was increased in males fed the 25, 100, and 400 ppm levels as compared to the controls. Females fed all three dietary levels and males fed the 400 ppm level exhibited an increased incidence of hyperplastic nodules, telangiectatic and basophilic foci of the liver (DER 004252, May 23, 1984).

Table 1. Amitraz $B_6C_3F_1$ Mouse Study - Mortality Rates[+] and Cox or Generalized K/W Significant Test Results (Bernice Fisher, September 12, 1990)

Males	Week				
Dose (ppm)	1-26	27-52	53-78	79-104[a]	Total
0	0/100	0/100	2/100	18/98	20/100 (20)*
25	0/75	2/72	1/73	7/72	10/75 (13)
100	1/75	3/74	2/71	9/69	15/75 (20)
400	1/75	1/74	7/73	11/66	20/75 (27)

Females	Week				
Dose (ppm)	1-26	27-52	53-78	79-107[a]	Total
0	2/100	0/98	4/92	14/88	20/100 (20)
25	0/75	2/75	2/73	10/71	14/75 (19)
100	0/75	0/75	3/75	10/72	13/75 (17)
400	1/75	1/74	1/73	16/72	19/75 (25)

[+]Number of animals that died during interval/ number of animals alive at the beginning of the interval.
( ) Percent.
[a]Final sacrifices at weeks 105-106 for males and 105-107 for females.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
*$p < 0.05$, **$p < 0.01$.

c. Consideration of Adequacy of Dose Selection - Two 90-day mouse studies were undertaken to identify the dose levels and most appropriate strain of mouse to be used in the repeat study since the CFLP strain was no longer available. The $B_6C_3F_1$ strain was selected.

The results of the two 90-day studies indicated that a lower dosing regimen should be followed because of significant decrease in body weight gain at the 400 ppm level. The Agency suggested that the same dose levels fed in the original 80-week CFLP study be used in the repeat study with $B_6C_3F_1$ mice

In the repeat study, body weight gain reduction and a significant positive trend in mortality in male mice suggested that the HDT (400 ppm) was excessive. In females, however, the Peer Review Committee agreed that the HDT was high but not excessive, since it was not life-threatening (there were no significant differences in mortality compared to controls). Group mean body weight gains were reduced in males (25%) and females (14%) of the high dose group by week 13. Group mean body weight changes were significantly ($p < 0.001$) reduced at the 400 ppm level by week 18 for males (30%) and for females (31%), and by week 52 body weight gains were significantly ($p < 0.01$) reduced for males (29%) and females (55%) as compared to control values. A significant decrease ($p < 0.01$) in group mean body weight gain was reported at the 100 ppm level for males (29%) and for females (32%) by week 52.

Neoplastic Lesions - Table 2 shows the incidence of hepatocellular tumors in males and females, respectively. The incidence of hepatocellular adenomas and carcinomas was not increased in males.

In females there were significant dose-related positive trends in hepatocellular adenomas, carcinomas, and in the combined group of adenomas and/or carcinomas. Female mice also had a significant difference in the pair-wise comparison of controls and the highest dose group in hepatocellular adenomas, carcinomas and in the combined group of adenomas and/or carcinomas.

Table 2. Hepatocellular Tumor Rates in Males and Females (Bernice Fisher, September 12, 1990)

	Dose (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Adenomas	6/99	3/72	4/70	6[a]/71	4/98	1/73	3/75	13[c]/73
Percent	6	4	6	8	4	1	4	18
p =	0.23	0.72[n]	0.51	0.28	0.00**	0.29	0.65	0.00**
Carcinomas	14/99	8/72	6/70	8[b]/73	2/98	0/73	1/75	15[d]/73
Percent	14	11	9	11	2	0	1	21
p =	0.77[n]	0.44[n]	0.72[n]	0.78[n]	0.00**	0.33	0.60	0.00**
Combined	20/99	11/72	10/70	14/73	6/98	1/73	4/75	28[a]/73
Percent	20	15	14	19	6	1	5	38
p =	0.58[n]	0.67[n]	0.83[n]	0.65[n]	0.00**	0.12	0.55	0.00**

[+]Number of tumor-bearing animals/number of animals at risk (males were evaluated by Peto's Prevalence tests, females were evaluated by Cochran-Armitage Trend and Fisher Exact test).
examined excluding those that died before first tumor was observed.
n-Negative change
[a]First liver adenoma observed at week 70, dose 400 ppm.
[b]First liver carcinoma observed at week 68, dose 400 ppm.
[c]First liver adenoma observed at week 84, dose 400 ppm.
[d]First liver carcinoma observed at week 94, dose 400 ppm.
Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
*$p < 0.05$, **$p < 0.01$.

Table 3 shows the background incidence of hepatocellular tumors in two other studies conducted concurrently on the same strain of mice in the same testing facilities (Huntingdon Research Center, 1983).

Table 3. Incidence of Hepatocellular Adenomas and Carcinomas in Concurrent Controls

	Study A		Study B	
	Males (%)	Females (%)	Males (%)	Females (%)
Adenomas	17	5	30	13
Carcinomas	14	5	18	6
Combined	31	10	48	19

The incidence of hepatocellular adenomas, carcinomas and adenomas/carcinomas combined in the high-dose female $B_6C_3F_1$ mice were higher than the incidence of these types of lesions in other concurrent controls.

Table 4 shows the incidence of lung tumors in males and females. In males there was a significant dose-related positive trend in lung adenomas. Also, in the pair-wise comparison of the controls and the highest dose group, there was a significant difference in this tumor type in the high-dose males when compared to controls.

Table 4. Lung Tumor Rates[+] and Peto's Prevalence Test Results for Males, and Cochran-Armitage Trend Test and Fisher's Exact Test Results for Females (Bernice Fisher, September 12, 1990)

Males	Dose (ppm)			
	0	25	100	400
Adenomas	9/95	12/71	8[a]/89	16/64
Percent	9	17	12	25
p =	0.01**	0.11	0.35	0.01**
Females	**Dose (ppm)**			
	0	25	100	400
Adenomas	7/98	8/73	4/75	10[b]/73
Percent	7	11	5	14
p =	0.08	0.27	0.44	0.12

[+]Number of tumor-bearing animals/number of animals examined.
[a]First male lung tumor observed at week 87, dose 100 ppm.
[b]First female lung tumor observed at week 98, dose 400 ppm.
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
*p < .05, **p < .01.

Table 5 shows the background incidence of lung tumors in two other experiments conducted in the same time period in the same testing facilities using the same strain of mice.

Table 5. Incidence of Lung Tumors in Concurrent Control $B_6C_3F_1$ Mice (Huntingdon Research Center, 1983)

	Study A (%)	Study B (%)
Male	13	7
Female	5	7

The incidence of lung tumors in the high dose male and female $B_6C_3F_1$ mice were higher than those for other concurrent controls.

3. Two-Year Chronic Toxicity/Carcinogenicity Study in Rats (Report No. TX73043 dated November 1973 - EPA Accession Nos. 44585, 41197, and 41197.)

   a. Experimental Design - Four groups of 40 Ash Wistar pathogen-free rats/sex/group were fed dietary levels of 15, 50, or 200 ppm for 2 years.

   b. General Observations - Animals fed the 200 ppm level exhibited aggressive and excitable behavior. A temporary decrease in food consumption was reported during weeks 1 to 4 for males (7-28%) and females (9-14%). During the first 12 weeks of the study a significant ($p < 0.001$) decrease in body weight gain was reported for males and females by 14 and 20 percent, respectively. At the termination of the study a significant ($p < 0.001$) decrease in body weight gain was reported for males (13%) fed the 200 ppm level. Survival rates among the three test levels and the controls were comparable for both sexes.

      No hematological or biochemical changes between the control and test levels were reported. An increase in male relative liver and thyroid weights by 8 and 24 percent, respectively, was observed at the high-dose level. No dose-related histopathological changes were reported. There were no significant changes in the incidence, type, or time of appearance of tumors in the treated groups as compared to controls (DER 001124, February 19, 1975).

   c. Consideration of Adequacy of Dose Selection - The high dose was considered adequate for carcinogenicity testing based upon a reduced body weight gain of 14 and 20 percent in males and females, respectively.

   d. Neoplastic Lesions - The treatment did not alter the spontaneous tumor profile in this strain of rats.

D. Other Relevant Toxicology Data

1. Subchronic Toxicity in Rats - Daily oral doses of 3, 12, 50, or 200 mg/kg body weight

were administered by gavage to male and female rats for 90 days.

Animals at the 50 and 200 mg/kg levels exhibited excitable and aggressive behavior, a reduced body weight gain, and were terminated after 7 days. A decrease in liver weights and congestion of the major organs were also observed at the 50 and 200 mg/kg levels.

Animals dosed at the 12 mg/kg level were excitable and exhibited a significantly ($p < 0.05$) reduced body weight gain (8%) as compared to control values. No hematological, biochemical, or histopathological changes were observed at the 12 mg/kg level. Male relative liver weights were significantly reduced at the 3 mg/kg level ($p < 0.05$) by 5 percent and at the 12 mg/kg level ($p < 0.01$) by 6 percent (DER 001124, February 19, 1975).

2. Chronic Toxicity in Nonrodents - Four groups of four beagle dogs/sex/group received daily oral doses at 0, 0.1, 0.25, or 1.0 mg/kg body weight in gelatin capsules. Central nervous system depression and hypothermia accompanied by increased blood sugar levels were reported at the 1.0 mg/kg level (DER 001124, February 19, 1975).

   The present RfD of 0.0025 mg/kg body weight is based on the 2-year dog feeding study NOEL of 0.25 mg/kg with a hundred fold uncertainty factor.

3. Reproductive Toxicity - Dietary levels of 15, 50, or 200 ppm were fed to Boots-Wistar strain rats over three generations.

   A decrease in food consumption (12% during the first week) and significant ($p < 0.05$) decrease in body weight gain (14%) were reported for the P1 females fed the 200 ppm (20 mg/kg/day) level. There was a decrease in litter size (6.7 vs. 7.9 in control), and a decrease in viability (48%) and lactation (9%) indices at the high-dose level. With 5/114 pups alive on day 21, the F1 generation was terminated.

   A significant ($p < 0.05$) decrease in the mean number (5.5 vs. 7.5 in control) of pups alive on day 21 was reported for the 50 ppm (5.0 mg/kg)

level of the F3 generation (DER 001124, February 19, 1975).

The pregnancy index for the three dose levels was comparable to the control values.

Systemic NOEL = 15 ppm (1.5 mg/kg/day).
Systemic LEL = 50 ppm (5.0 mg/kg/day) with increased pup mortality during the F3 generation.

Reproductive NOEL = 50 ppm with no adverse effects on reproductive indices or mating performance.

4. Developmental Toxicity - Dosage levels of 1, 3, or 12 mg/kg were administered orally to Wistar rats during days 8 through 20 of gestation (DER 001124, February 19, 1975).

Maternal NOEL = 3 mg/kg, and
Maternal LEL = 12 mg/kg with a decrease in body weight.

Developmental NOEL = 3 mg/kg, and
Developmental LEL = 12 mg/kg with decreased body weight and litter size were reported at this level.

Dosage levels of 1, 5, and 25 mg/kg were admininistered orally to rabbits during days 6 through 18 of gestation (DER 001124, February 19, 1975 and DER 001122, June 19, 1975).

Maternal NOEL = 5 mg/kg, and
Maternal LEL = 25 mg/kg with a decrease in body weight and an increase in the number of abortions on days 17 to 20.

Developmental NOEL = 5 mg/kg, and
Developmental LEL = 25 mg/kg with a decrease in litter size and weight and a decrease in implantation and viability indices reported at this level.

5. Special Studies on Hormone Levels - Alterations in the estrus cycle and hormone levels of $B_6C_3F_1$ female mice were reported from feeding dietary levels of 25, 100, or 400 ppm for 28 weeks. Prolongation of proestrus and decreased duration

of the diestrus phase without an effect on the length of the estrus cycle was reported at the 400 ppm level. A dose-related decrease in blood levels of progesterone and prolactin and an increase in dehydroepiandrosterone sulfate levels were reported at the 100 and 400 ppm levels. At the 400 ppm level, blood glucose and urea levels were reduced as well as a decrease in body weight gain. An increase in liver weight was observed at the 100 and 400 ppm levels. The 25 ppm level (3.8 mg/kg/day) is the NOEL (DER 004175, January 2, 1985).

Effects on the estrus cycle of female rats was demonstrated from feeding amitraz at 200 ppm (20 mg/kg/day) for 18 weeks. The average cycle length of the control rats was 4.3 days as compared to 6.1 days for the treated group. The cycle length was significantly altered by treatment, either estrus or diestrus being prolonged (DER 001116, August 30, 1976).

6. Metabolism - Studies conducted on both sexes of mouse, rat, dog, baboon, and male human volunteers (DER 004175, January 2, 1985) indicated no remarkable differences between species or sexes for total urinary excretion with peak level recoveries in the urine within 24 hours and to a much lesser extent in the feces.

The following table summarizes the metabolic fate of ^{14}C-amitraz (BTS 27419) in mouse, rat, dog, baboon, and humans (DER 004175, January 2, 1985).

Percent of the Dose Recovered within 48 Hours were as follows:

Urine

Human		Baboon		Dog		Rat		Mouse	
M	F	M	F	M	F	M	F	M	F
78	-	62	80	74	82	82	80	70	64

Feces

-	-	24	9	13	12	17	17	31	39

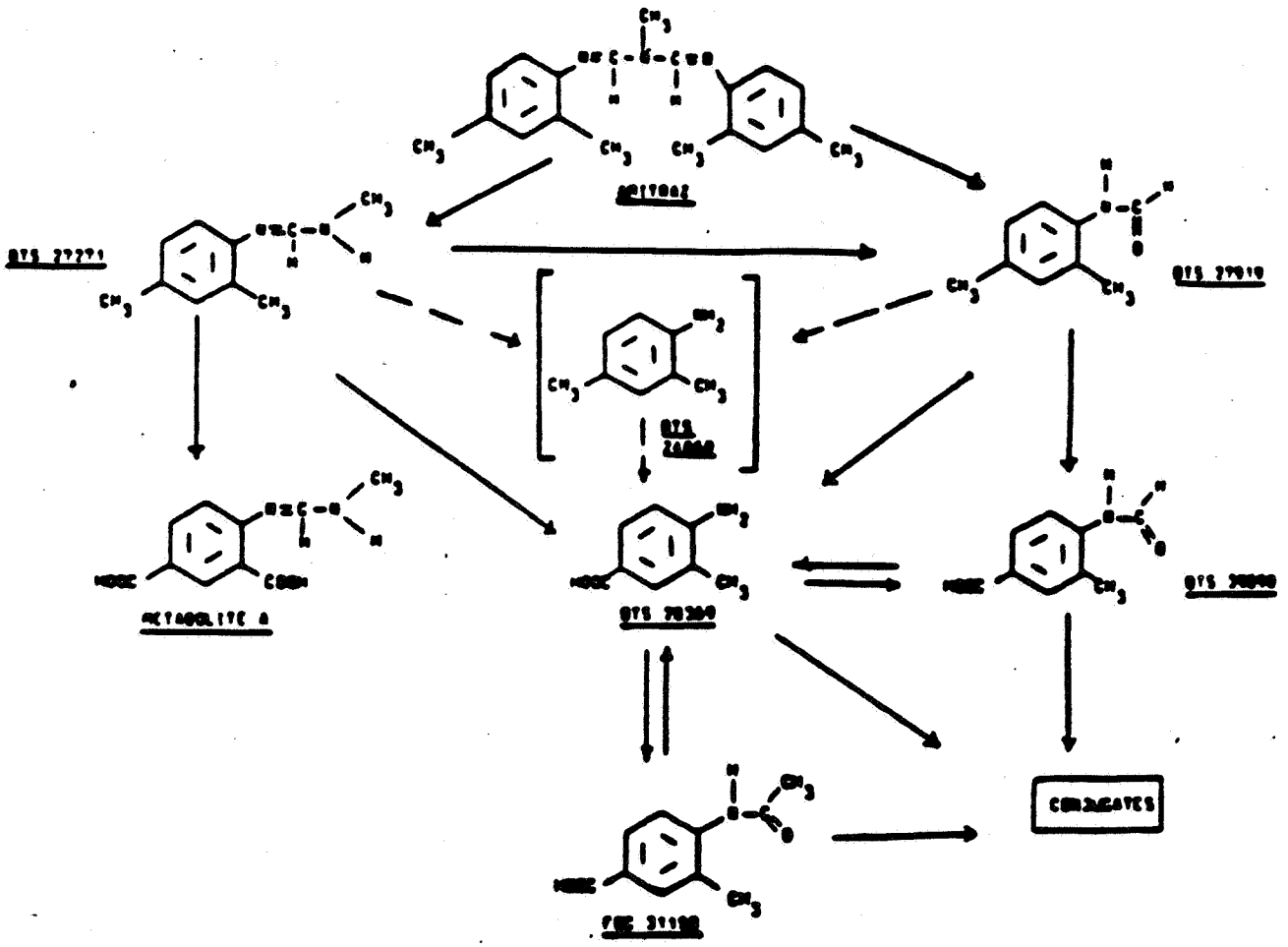
M = Male
F = Female

The highest levels of ^{14}C tissue residues were reported in the liver-bile, pigmented area of the eye, adrenals, and kidney within 72 to 96 hours of oral doses to mice, rats, dogs, and baboons.

Dietary levels of 400 ppm were fed to male and female $B_6C_3F_1$ mice for 3 weeks followed by a single oral dose of 10 mg/kg body weight. ^{14}C-Labeled amitraz resulted in 72.3 and 75.7 percent of the total dose eliminated in the urine within 48 hours by males and females, respectively, with 21 percent eliminated in the feces of both sexes.

Metabolism studies conducted with two adult male human volunteers showed clinical effects as the result of ingestion of 0.25 mg/kg body weight of ^{14}C-labeled amitraz. The clinical effects which were reported within 90 to 160 minutes after ingestion included sedation, dry mouth, disorientation, bradycardia, hypertension, and hypothermia persisting up to 12 hours after dosing.

In all of the species studied, both sexes rapidly hydrolyzed amitraz in the stomach to (BTS 27271) N-(2,4dimethylphenyl)-N-methyl formamidine and (BTS 27919) 2,4-dimethylformanilide with conjugates of free (BTS 28369) 4-amino-3-methylbenzoic acid reported upon hydrolysis in the urine.



BTS 24 868: 2,4-dimethylaniline
BTS 27 919: 2,4-dimethylformanilide
BTS 28 369: 4-amino-3-methylbenzoic acid
BTS 39 098: 4-formamido-3-methyl benzoic acid
BTS 31 158: 4-acetamido-3-methyl benzoic acid
BTS 27 271: N-(2,4-dimethylphenyl)-N- methyl formamidine

Figure 1. Proposed Metabolic Pathway for Amitraz in Animals

Proportion of various metabolites recovered in the urine of rats, mice, baboons, and humans given a single oral dose of ^{14}C-amitraz are similar. The percentages of the radioactivity in urine, are indicated in the following table.

Metabolites	Human 0.25 mg/kg	Rat 10 mg/kg		Mouse 10 mg/kg		Baboon 10 mg/kg	
	M	M	F	M	F	M	F
BTS 24 868	1.4	1.7	2.1	1.9	1.8	1.9	2.2
BTS 27 919	3.6	1.5	1.6	1.4	1.7	2.0	1.8
BTS 28 369	3.8	1.9	2.0	2.5	2.8	2.8	2.7
BTS 39 098 + > BTS 31 158	27.1	26.6	6.4	15.4	19.0	26.0	19.3
BTS 27 271	5.8	3.1	4.6	5.1	5.7	4.1	6.5
Polar material	59.6	55.6	53.6	64.4	59.1	51.8	55.1
Remainder	1.5	9.6	9.7	9.4	10.1	11.4	12.4

The concentration of metabolite BTS 27271 excreted in the urine was dose dependent with its degradation products in the urine similar to amitraz (DER 004175, January 2, 1985).

7. Mutagenicity - Amitraz was negative in several mutagenicity tests, including the Salmonella assay with and without metabolic activation, host mediated assay in male and female mice, mouse lymphoma assay with and without metabolic activation, transformation assay in mice C3H/10T 1/2 cells, and unscheduled DNA synthesis (UDS) assay in human embryonic cells.

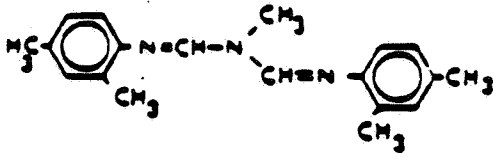
The metabolites N-(2,4-dimethylphenyl)-N-methyl formamidine and 2,4-dimethylformanilide were reported to be negative for gene mutation in the Salmonella assay with and without metabolic activation.

On the other hand, 2,4-dimethylaniline was positive in the mouse lymphoma mutation assay with metabolic activation and negative in the absence of metabolic activation (DER 001112, June 16, 1980 and DER 004174, January 2, 1985).

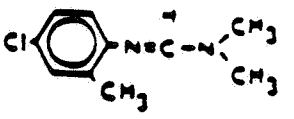
The NTP showed 2,4-dimethylaniline to be positive in the Salmonella assay with metabolic activation. In many published studies, it is usually positive with tester strain TA 100 with activation. It also induces UDS in primary rat hepatocytes (Mut. Res. 206: 183-191, 1988).

The behavior of amitraz and its metabolites including the aniline metabolites, has been investigated in the Ames Salmonella assay using four tester strains in the presence and absence of metabolic activation from livers of rats and mice, different concentrations of the test chemicals and the activating systems (G. Ghali, 1980, PhD Dissertation, Purdue University). No positive response was obtained with or without metabolic activation with the parent compound or any of its metabolites, except for 2,4-dimethylaniline. This aniline was positive only with the tester strains TA97 and TA100 with metabolic activation from either rats or mice, but not with TA1535 or TA1537 with or without metabolic activation.

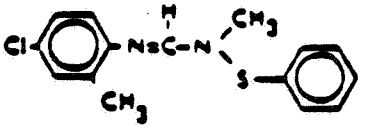
8. Structure-Activity Relationship - Amitraz is structurally similar to other formamidine pesticides such as chlordimeform, chlormethiuron, U-42558, and U-42662. As formamidines, naturally, they all possess the formamidine moiety in their structure and are expected to behave biologically in a somewhat similar, though not identical, manner. Most important is the fact that they all contain a substituted aniline moiety in their structure that can be released biologically. Other than amitraz, chlordimeform was the only formamidine pesticide registered for use in the United States, and was cancelled as of February 8, 1989 (FR Vol. 54, No. 25). The other formamidines



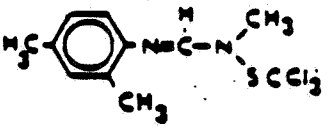
Amitraz



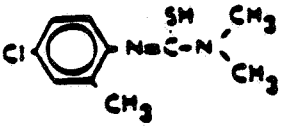
Chlordimeform



U-42558



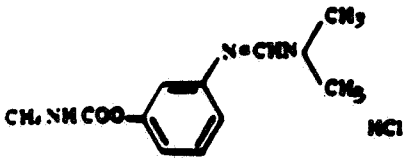
U-42662



Chlormethiuron

listed above are either used in other countries or are still in the experimental stage, and therefore information may not be available for SAR purposes.

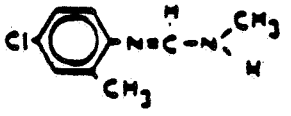
Another structural analogue to amitraz is formatenate, a formamidine/carbamate pesticide. However, the fact that it has a carbamate moiety and lacks the appropriate aniline substitution (as will be discussed later in this section), may disqualify this chemical as an amitraz analogue and may result in different biological behavior uncommon to other formamidines.



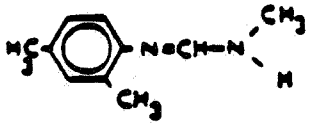
Formatenate Hydrochloride

Chlordimeform is considered an appropriate structural analogue with a wealth of information available. Chlordimeform was reported to be carcinogenic in mice and was classified as a Group B2, probable human carcinogen, based upon a significant, dose-related increase in the incidence of malignant hemangioendotheliomas in both sexes of Tif:Mag:SPF mice. Furthermore, a metabolite of chlordimeform 4-chloro-2-methyl-aniline, was also carcinogenic in rats and/or mice. In addition, this metabolite was linked to bladder cancer in occupationally exposed workers (R. Kimbrough, memo to J. Moore, Nov. 27, 1987; G. Burin, memo to J. Auerbach dated May 27, 1987; J. Blondell, memo to G. Burin dated Dec. 7, 1987, for copies of these memos, see chlordimeform peer review file).

Chlordimeform is metabolized by hepatic microsomal enzymes to N-demethyl chlordimeform, a metabolite structurally similar to the hydrolysis product of amitraz. Acid hydrolysis of amitraz is the first biotransformation step catalyzed by the acidic



(1)



(2)

1) N-demethyl chlordimeform
2) hydrolysis product of amitraz (BTS 27 271)

media in the stomach upon ingestion. After the initial hydrolysis of amitraz, it is expected to proceed biologically in a manner similar to chlordimeform. Both compounds release substituted anilines as metabolites, i.e., 4-chloro-2-methyl aniline in the case of chlordimeform and 2,4-dimethyl aniline in the case of amitraz. The mutagenic and carcinogenic potential of these anilines is well documented in the open literature.

The behavior of chlordimeform and its metabolites in the Ames Salmonella assay in the presence and absence of metabolic activation from livers of rats and mice was qualitatively similar to amitraz, i.e. no positive response was obtained with or without metabolic activation with the parent compound or any of their metabolites, except for the substituted aniline (4-chloro-2-methylaniline). This aniline metabolite was positive only in the tester strains TA97 and TA100 with metabolic activation from either rats or mice, but not with TA1535 or TA1537 with or without metabolic activation (G. Ghali, PhD Dissertation, 1980, Purdue University).

When other mono and disubstituted anilines were tested with the tester strain TA100 in the presence of an activating system from rat liver, only those 2,4-disubstituted anilines with a methyl group in the ortho position were positive (G. Ghali, 1980, PhD Dissertation, Purdue University). It is likely that the mutagenic/ carcinogenic potential of amitraz and chlordimeform is the result of the release of the 2,4-disubstituted anilines as metabolites, and subsequent activation of these substituted anilines to the ultimate mutagens/carcinogens. This is consistent with reports by other investigators (IARC Monograph Vol. 27, April 1982). The evidence of aniline carcinogenicity is limited, but for other substituted anilines, particularly those with a methyl group in the ortho position, there is sufficient evidence that they are carcinogenic in rats or mice or both.

Weisburger et al. (J. Environ. Pathol. Toxicol. 1978, 2:325-356) concluded that 4-chloro-2-methylaniline was carcinogenic in both sexes of

HaM/ICR mice but not in Charles River CD rats, and that 2,4-dimethylaniline caused pulmonary tumors in female mice, but not in male mice or rats.

According to Miller and Miller (Ann. NY. Acad. Sci., 1969, 163:731-750), aromatic amines are not active as such but they require metabolic activation. N-hydroxylation is now recognized as the first step in the activation of aromatic amines (McCann et al., 1975, Proc. Nat. Acad. Sci.[USA], 72:5135-5139; McMahon et al., 1979, Cancer Res. 39:682-693; Hollstein et al., 1979, Mut. Res. 65:153-226) The resulting hydroxylamines themselves have shown very little reactivity with tissue nucleophiles under physiological conditions, but subsequent esterification converts them to strong electrophiles which arylate, arylamidate, or arylaminate nucleophiles such as DNA (Miller and Miller, Ann. Ny. Acad. Sci., 1969, 163:731-750).

E. Weight-of-the-Evidence Considerations

The Committee considered the following facts to be of importance in the weight-of-the-evidence determination of the carcinogenic potential of amitraz.

1. Dietary administration of amitraz to male and female CFLP mice for 80 weeks was associated with a statistically significant ($p < 0.03$) increase in the incidence of lymphoreticular tumors in females of the high-dose group (49%) when compared to controls (23%). In this study, the high dose tested was considered adequate for carcinogenicity testing in females based upon a statistically significant ($p < 0.01$) decrease in body weight gain (18%). The maximum tolerated dose might have been exceeded in males of the high dose with a reduction of body weight gain of 37 percent.

2. Dietary administration of amitraz to female $B_6C_3F_1$ mice was associated with a significant ($p < 0.01$) increase in the incidence of hepatocellular adenomas (18%), carcinomas (21%), and adenomas/ carcinomas combined (38%) in high-dose females when compared to controls (adenomas, 4%; carcinomas, 2%; and adenomas/ carcinomas combined, 6%). This type of tumor is relatively uncommon in females.

3. Dietary administration of amitraz to male $B_6C_3F_1$ mice was associated also with a statistically significant ($p < 0.01$) increase in lung adenomas in high-dose males (25%) when compared to controls (9%). There was also a significant ($p < 0.01$) positive dose-related trend for this lesion. Although body weight gain decrease in the high dose group of $B_6C_3F_1$ was progressive, it was not a remarkably life-threatening effect.

4. The Committee discounted any possible contribution of the [redacted] to the overall carcinogenic effect in the CFLP mice study, given the low concentration of [redacted] in the diets and the short duration of exposure. [redacted] was present as a stabilizing agent in the treated diets at cocentration of 0.6, 2.5, and 10 ppm, respectively, during the final 14 weeks of the study.

5. Dietary administration of amitraz to Wistar male and female rats for two years did not alter the spontaneous tumor profile for this strain of rats. The high dose tested was considered adequate for carcinogenicity testing.

6. Amitraz is structurally similar to chlordimeform, another formamidine pesticide, which has been reported to be carcinogenic in mice and was classified by the HED Peer Review Committee as a Group B2, probable human carcinogen (December 20, 1985). Furthermore, both amitraz and chlordimeform produce several similar metabolites including substituted anilines (2,4-dimethylaniline in the case of amitraz, and 4-chloro-2-methylaniline in the case of chlordimeform). These two aniline derivatives were reported to be mutagenic/carcinogenic. Furthermore, 4-chloro-2-methylaniline was linked to bladder cancer in occupationally exposed workers.

7. Amitraz, and all its animal metabolites except 2,4-dimethyl aniline, were negative in several mutagenicity testing regimens.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

F. Classification

Considering criteria contained in EPA Guidelines [FR 51:33992-34003, 1986] for classifying a carcinogen, the Committee concluded that the data available for amitraz provided evidence to classify the chemical as a Group C, possible human carcinogen. This classification was based upon:

1. Statistically significant ($p < 0.03$) increase in the incidence of lymphoreticular tumors in the high-dose (400 ppm) female CFLP mice (49%) when compared to controls (23%).

2. Statistically significant ($p < 0.01$) increases in the incidence of hepatocellular adenomas (18%), carcinomas (21%), and adenomas/carcinomas combined (38%) in females of the high-dose (400 ppm) $B_6C_3F_1$ mice when compared to controls (adenomas, 4%; carcinomas, 2%; and adenomas/carcinomas combined, 6%) with a significant ($p < 0.01$) positive dose-related trend.

3. Statistically significant ($p < 0.01$) increase in the incidence of lung adenomas in males (25%) of the high-dose $B_6C_3F_1$ mice when compared to controls (9%), with a significant ($p < 0.01$) dose-related trend.

4. Structural similarity of amitraz to chlordimeform, another formamidine, which has been reported to be carcinogenic in mice and classified by the HED Peer Review Committee as a Group B2, probable human carcinogen (HED report dated Dec. 20, 1985).

   Furthermore, both amitraz and chlordimeform produce several similar metabolites including substituted anilines (2,4-dimethylaniline in the case of amitraz, and 4-chloro-2-methylaniline in the case of chlordimeform). These two aniline derivatives were reported to be mutagenic/carcinogenic.

   2,4-dimethyl aniline, a metabolite of amitraz, was reported to be mutagenic in several mutagenicity tests, and induced sarcomas in female mice and malignant tumors in male rats in a National Cancer Institute Study.

   In addition, 4-chloro-2-methylaniline, a metabolite of chlordimeform, was linked to bladder cancer in occupationally exposed workers.

Quantification of potential human cancer risk, using a low-dose extrapolation model (Q^*_1), was recommended. This decision was based on the fact that amitraz was associated with the induction of multisite benign and malignant tumors in different strains of male or female mice. Some of these tumors, i.e., hepatocellular tumors, are considered relatively uncommon in female $B_6C_3F_1$ mice. Furthermore, 2,4-dimethylaniline, a metabolite of amitraz, is a positive mutagen and was reported to be carcinogenic in mice. Amitraz and chlordimeform are structurally similar and produce 2,4-disubstituted anilines as metabolites. 4-Chloro-2-methylaniline, a metabolite of chlordimeform was linked to bladder cancer in occupationally exposed workers.

The calculation of Q^*_1 will be based on the incidence of hepatocellular tumors in female $B_6C_3F_1$ mice.