



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

1-31-92

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Response to the Peer Review of Amitraz
EPA Reg.No.45639-51

Project No.1-1729
Tox.Chem.No.374A

From: Ray Landolt *1/30/92*
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Thru: Mike Ioannou, Section Head
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and
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Registrant: Nor-Am Chemical Company, letter of June 18, 1991

Action Requested: 1. Review and comment on the registrant's
response to the EPA Peer Review document of
January 3, 1991 with a request:

a. for reconsideration of the carcinogenicity
classification from Group C to D and

b. to formally address the Peer Review
Committee.

2. Review and comment on a Tissue Residue Study
Following Dermal Spray Application of Amitraz
(Taktic to Beef Cattle in the USA (Vol.4
and 5).



Conclusion: 1. Nor-Am Chemical Company's response (MRID 419184-01) to the Peer Review of Amitraz acknowledges that this request "does not report any new experimental data". The existing data base has been cited by NOR-AM Chemical in support of their rebuttal to the Peer Review of Amitraz. "The Committee concluded that the data available for Amitraz provided evidence to classify the chemical as a Group C, possible human carcinogen", requiring a quantitative risk assessment.

In the absence of new data, a reevaluation of the existing data base does not merit a referral of this request to the Peer Review Committee for reconsideration of the Group C classification of amitraz.

2. The Tissue Residue Study Following Dermal Spray Application of Amitraz (Tactic) to Beef Cattle in the USA, Vol. 4 of 5 (MRID No. 419184-03) and Vol. 5 of 5 (MRID No. 419184-04) are duplicate copies of the same studies in both documents. These studies were previously reviewed by Residue Chemistry (John H. Onley) February 14, 1984 in support of Pesticide Petition 4F2968. Review and comment on these studies is not applicable to this review.

Background Information:

The carcinogenic potential of amitraz has been evaluated and reevaluated by the Cancer Assessment Group (CAG) and recently by the Health Effects Division (HED) Peer Review Committee. (DER 008336)

Initially, CAG's evaluation of a carcinogenic study in CFLP mice, December 21, 1978, concluded that there was a significant increase in the incidence of lymphoreticular tumors in the high dose (400 ppm) female mice when compared to the controls. The second carcinogenic study in B₆C₃F₁ mice, evaluated by CAG March 29, 1985, demonstrated a significant increase in hepatocellular adenomas and carcinomas in the high dose female mice accompanied by a significant increase in lung adenomas in the high dose males. CAG concluded that amitraz is a Group C, possible human carcinogen with an estimated carcinogenic potency of 0.05 (mg/kg/day)⁻¹.

The HED Peer Review document of January 3, 1991 evaluated the weight of evidence on the carcinogenic potential of amitraz and concluded that amitraz should be classified as a Group C, possible human carcinogen, with an estimated carcinogenic potency of 0.05 (mg/kg/day)⁻¹.

Consideration Given To The Information Presented by Nor-Am Chemical

In the first carcinogenic study, four groups of 50 CFLP mice per sex per dose were fed dietary levels of amitraz at 25, 100, and 400 ppm for 80 weeks. In this study there was a significant increase in the incidence of lymphoreticular tumors in the high dose (400 ppm) female CFLP mice.

Nor-Am Chemical has resubmitted their response presented to the Agency July 10, 1986 on the incidence of lymphoreticular tumors in the high dose (400 ppm) female CFLP mice accompanied by historical control data for the CFLP mouse.

CAG responded September 6, 1986, with the conclusion that "The original CFLP mouse study should be considered a valid study which is positive for lymphoreticular tumors in females at 400 ppm".

Nor-Am has resubmitted the issue of "the possible influence of viral factors on the incidence of lymphoreticular tumors in the original mouse study".

CAG Risk Assessment on Amitraz, December 21, 1978, addressed the clustering of lymphoreticular tumors in female mice and possible viral spread with the conclusion that "the hypothesis of viral etiology in the induction of tumors is not clearly established and can not be used to cast doubt on the fact that the occurrence of tumors is related to the presence of amitraz".

The Peer Review Committee has confirmed the conclusion of the Cancer Assessment Group (1978 and 1985) that there is a "statistically significant ($p < 0.03$) increase in the incidence of lymphoreticular tumors in the high dose (400ppm) female mice (49%) when compared to the controls (23%).

Nor-Am has cited the Agency Peer Review with the following quotation "there is 'weakly positive evidence' that amitraz is a possible human carcinogen based on the positive effects demonstrated in the mouse study although there were questions raised regarding the reliability of the study."

This citation presented by Nor-Am does not appear in the Peer Review document of January 3, 1991.

The second carcinogenicity study was conducted with B₆C₃F₁ mice since the CFLP strain used in the first study was not available. This second study was conducted with three groups of 75 B₆C₃F₁ mice per sex per group fed dietary levels of 25, 100, or 400 ppm with 100 mice per sex in the controls, for 104 weeks.

In this study there was a significant increase in the incidence of liver tumors (adenomas and carcinomas) in the high dose females and a significant increase in the incidence of lung tumors in the high dose male mice.

Nor-Am Chemical has acknowledged that there is a significant increase in the incidence of liver tumors in female mice at the high dose level (400ppm). However, Nor-Am is of the opinion in their response presented to the Agency July 10, 1986 and June 18, 1991 that the MTD was exceeded as evidenced by body weight decrement, reduced food efficiency, hormonal effects and increased mortality in female mice at the 400ppm level.

CAG (September 6, 1986), was of the opinion "Although it appears that the MTD was exceeded, CAG did not evaluate the mice as being severely compromised from the intended purpose of the biotest for carcinogenicity".

The Peer Review Committee determined that the highest level fed may be excessive for males, but not for the female mice "since it (dose) was not life-threatening and there were no significant differences in mortality compared to controls".

In females there was a significant (p<0.01) increase in the incidence of hepatocellular adenomas (18%), carcinomas (21%) and adenomas/carcinomas combined (38%) at the high dose (400ppm) when compared to controls (adenomas 4%, carcinomas 2% and adenomas/carcinomas combined 6%) with a significant (p<0.01) positive trend.

Also in the second mouse study, a statistically significant (p<0.01) increase in the incidence of lung adenomas (25%) in male B₆C₃F₁ mice at the high-dose when compared to controls (9%) was demonstrated with a significant (p<0.01) dose-related trend.

Nor-Am Chemical has contended that the incidence of lung adenomas presented in the Peer Review document is inconsistent with previous Agency reviews.

CAG (September 6, 1986) concluded that "male lung adenomas were significantly increased (21%) as compared to control (7%), whereas the females showed no lung carcinogenesis" does not support this contention of inconsistency.

Table 4 in the Peer Review document for the incidence of lung adenomas in male mice at the 100 ppm level is incorrect. The incidence of lung adenomas in male mice reported at the 100 ppm level of 8/89 should be corrected to read 8/69. Each test group was composed of 75 animals of each sex (B.Fisher memo of September 12, 1990).

Two mouse carcinogenic studies have been conducted in two different strains of mice, one in the CFLP strain and the other in the B₆C₃F₁ strain. The target organ site for carcinogenic activity is the lymphoreticular system in the CFLP female mice, the liver in B₆C₃F₁ female mice and the lung in the B₆C₃F₁ male mice. Amitraz has manifest its carcinogenic potential in two different strains of mice.

Dietary administration of amitraz to Wistar male and female rats at 15, 50, or 200 ppm for two years did not alter the spontaneous tumor profile for this strain of rat. The highest dose tested was considered adequate for carcinogenicity testing.

In addition to the evidence of carcinogenic potential of amitraz in two strains of mice, but not in the rat, the Peer Review Committee in the weight-of-evidence determination considered the structural similarity of amitraz to chlordimeform, the similarity of the substituted aniline metabolites of amitraz and chlordimeform and the mutagenic and carcinogenic activity of the 2,4-dimethylaniline metabolite of amitraz.

These factors are relevant, but were not critical to the consideration given by the Peer Review Committee to the classification of amitraz as a Group C, possible human carcinogen, requiring a quantitative risk assessment.

Nor-Am contends "While 4-chloro-o-toluidine is a major urinary metabolite of chlordimeform in the rat, that 2,4-dimethylaniline is not a metabolite of amitraz".

This is contrary to the Nor-Am response to the Agency July 10, 1986 in which 2, 4-dimethylaniline was identified as a minor metabolite recovered in the urine of mice(1.9%), rats(2.1%), baboon(2.2%) and human(1.4) from a single oral dose of ¹⁴C amitraz. HED Dietary Exposure Branch memo of July 30, 1990 identified "the residue to be regulated as the parent amitraz and its metabolites containing 2,4-dimethylaniline moiety" from the use on cotton. In addition, the technical product.

Nor-Am contends "while chloro-o-toluidine can be conclusively shown to be carcinogenic in animals, the evidence for carcinogenicity of 2,4-dimethylaniline is questionable. This conclusion is supported by the physicochemical properties of the two chemical structures."

The Peer Review document of December 20, 1985 concluded that there is sufficient evidence to classify chlordimeform a Group B₂, probable human carcinogen. Chlordimeform and its metabolite 4-chloro-o-toluidine (4-chloro-2-methylaniline) are clearly oncogenic in the mouse. Subsequently, the Agency concluded that 4-chloro-o-methylaniline a metabolite of chlordimeform is linked to bladder cancer in occupationally exposed workers (FR Vol.54 No.25, February 8, 1989, Decision and Order to Cancel Chlordimeform).

CAG (December 21, 1978 and September 6, 1986) concluded that 2,4-dimethylaniline, a metabolite of amitraz, induced a statistically significant increased incidence of lung tumors in HaM/ICR female mice at the high dose level in a National Cancer Institute mouse study.

The Peer Review document of January 3, 1991 concluded that "2,4-dimethylaniline, 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".

Ghali, G.Z., 1980 (Ph.D Thesis, Purdue University) observed that the aniline metabolites common to chlordimeform and amitraz "are the proximate carcinogens responsible for the oncogenicity of the parent formamidines in mice. This hypothesis is strengthened by the work of Weisburger, E.K. (1978), who concluded that 4-chloro-o-toluidine (a metabolite of chlordimeform) was carcinogenic in both sexes of HaM/ICR mice but not in Charles River CD rats, and that 2,4-dimethylaniline (a metabolite of amitraz) caused pulmonary tumors in female mice". Both of these aniline metabolites were found to be mutagenic in the Salmonella assay.

The carcinogenic and mutagenic potential of amitraz and chlordimeform have been demonstrated by the aniline metabolites common to these two formamidines. The question remains as to the degree or potency of the respective aniline metabolites to induce carcinogenic and mutagenic activity.

Nor-Am contends that "the structure-activity relationship between 4-chloro-o-toluidine (4-chloro-2-methylaniline) and 2,4-dimethylaniline is not valid."

The structure activity relationship of these two substituted aniline metabolites of chlordimeform and amitraz can be characterized by a methyl group in the ortho position to the NH₂ group which is common to both 4-chloro-2-methylaniline and 2,4-dimethylaniline metabolites. The para position of both metabolites is occupied by chlorine in the case of 4-chloro-2-methylaniline and a methyl group in the para position of the 2,4-dimethylaniline metabolite.

Although these two aniline metabolites differ in the substituted group in the para position this difference would have a quantitative rather than a qualitative effect on the mutagenic and carcinogenic potential of these two aniline metabolites. This similarity of these two aniline metabolites to induce carcinogenic and mutagenic activity was presented in the Peer Review document of January 3, 1991.

With the recommendation of several members of the Peer Review Committee, a reevaluation of the existing data base does not permit a referral of this request to the Peer Review Committee for reconsideration of the Group C classification of amitraz.