FILE COPY

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

MAY 29 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Acetamide

FROM: Esther Rinde, Ph.D. Esther Rinde 5/10/90
Science Analysis and
Coordination Branch
Health Effects Division (H7509c)

TO: Dennis Edwards
Product Manager #12
Registration Division (H7505c)

The Health Effects Division Peer Review Committee met on Feb.28, 1990 to discuss and evaluate the weight-of-the-evidence on Acetamide with particular reference to its carcinogenic potential.

A. Individuals in Attendance:

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

Penelope A. Fenner-Crisp	Penelope A Fenner-Crisp
William L. Burnam	
Reto Engler	
Marcia Van Gemert	
Karl Baetcke	
Robert Beliles	
Marion Copley	
Kerry Dearfield	
Julie Du	
Richard Hill	

A. 1. Peer Review Committee (contd.)

Richard Levy

John Quest

Esther Rinde

Yin-Tak Woo

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

Clark Swenzel

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

George Ghali

William Sette

4. Other Attendees: (Observers)

Victor Miller (HED)
Hugh Pettigrew (HED)

B. Material Reviewed:

The material available for review consisted of data summaries prepared by Clark Swenzel with the following attachments: 1) Memorandum, Paynter to Melone, 2/9/83; 2) Memorandum, Litt to Ellenberger, 5/24/83; (3 and 4: CBI documents); 5) Four published studies; 6) Examples of cancer risk calculations for Thiodicarb; 7) Memorandum, Katz to Edwards, 6/18/85. The material reviewed (except items 3 and 4) is attached to the file copy of this report.

C. Background Information:

Acetamide (not itself a pesticide) is a metabolite of the pesticides Methomyl and Thiodicarb , neither of which were reported to have carcinogenic activity in acceptable studies in the rat (1981, 1980 respectively) or mouse (1981, 1980 respectively) [C. Swenzel].

In 1985, the Toxicology Branch classified Acetamide as a Group C carcinogen with quantitation, based on the available data (a formal Peer Review was not held: Memorandum, Katz to Edwards, 6/18/85). Since a 409 tolerance was required at that time, and the only way to grant it was to do a Quantitative Risk Assessment, the rationale for quantitation was administrative rather than scientific. At this time the granting of any additional tolerances, will result in exceeding a carcinogenic risk of 10^{-6}.

Structure of Acetamide:

CH_3CONH_2

D. Evaluation of Carcinogenicity Evidence for Acetamide:

1. Study in Male Rockland Albino Rats

Reference: Dessau, F.I. and Jackson, B. (1961). Acetamide-Induced Liver Cell Alterations in Rats. Lab. Invest. 4:387-397.

Acetamide (40% aqueous solution) was administered by intubation to 2 groups of male rats at 0 (control) or 4000 mg/kg for 5 days a week. Group 1 rats, consisting of 5 control and 8 treated males, were sacrificed and autopsied after 117 days of treatment. Group 2 rats (5 control and 5 treated males) were sacrificed and autopsied after 205 days of treatment.

Cytologic irregularities were reported in the liver sections of 5 of 8 treated rats of group 1 and in 3 of 5 treated rats in group 2. One treated rat in group 1 was found to have a hepatocellular adenoma.

The focus of this study was on liver cell alterations, and it was not designed to investigate the carcinogenicity of acetamide.

2. Study in Male Wistar Rats

Reference: Jackson, B. and Dessau, F.I. (1961). Liver Tumors in Rats Fed Acetamide. Lab. Invest. 10:909-923.

Acetamide was administered in the diet (Wayne Lab Blox) in 3 experiments for 1 year at levels of 0, 1.25, 2.5 or 5.0%.
Experiment 1: 50 male rats were fed 5% acetamide in the diet (50 rats were on the control diet).
Experiment 2: 25 male rats were fed 0, 1.25%, 2.5% or 5% acetamide in the diet.
Experiment 3: 99 male rats were fed 5% acetamide in the diet at the start; each (succeeding) week, treatment of 2 rats was stopped and they were placed on control diet for the remainder of the year.

The incidences of gross liver lesions are given in Table 1. Histologic examination revealed some adenocarcinomatous and anaplastic patterns (neither the incidence of tumors examined microscopically nor the correlations of noted histological findings with dosage and/or experiment number were given). Pulmonary metastases and peritoneal implants were detected grossly in 6 rats which died spontaneously with liver tumors; the presence of secondary tumors in the lungs of 5 of these rats was confirmed microscopically.

Table 1

Incidence (%) of Gross Liver Lesions in Male Wistar Rats (Jackson & Dessau)

Dietary Level	0	1.25%	2.5%	5.0%
Experiment 1	0/43	----	----	4/48(8.3%)
Experiment 2	0/25	4/24(16.7%)	6/22(27.3%)**	1/18(5.6%)
Experiment 3	----	----	----	22/81(27.2%)

** $p < 0.01$, Fisher's exact

3. Carcinogenicity Study in Male Wistar Rats

Reference: Weisburger, J.H., Yamamoto, R.S., Glass, R.M. and Frankel, H.H. (1969). Prevention by Arginine Glutamate of the Carcinogenicity of Acetamide in Rats. Toxicol. and Appl. Pharm. 14:163-175.

Acetamide was administered in the diet (Wayne Lab Blox) to groups of 40 male Wistar rats at 2.5% acetamide or 2.5% acetamide + 5.6% arginine glutamate; control groups (15 rats each) included rats fed Wayne Lab Blox diet alone and rats fed diet plus 5.6% arginine glutamate. Treatment was stopped after 1 year and a "sample of each group was killed" and autopsied; the remaining rats were given control diet for another 3 months, then sacrificed.

The weight gain, compared to Wayne Lab Blox controls, in rats fed acetamide or acetamide plus arginine glutamate or arginine glutamate alone was depressed (less so in rats fed acetamide plus arginine glutamate). According to graphed data, the survival in Wayne Lab Blox control rats was approximately 12 out of 15 (80%) at 1 year; survival in rats fed acetamide supplemented with arginine glutamate was comparable: 32 rats out of 40 (80%) survived after 1 year. Rats fed acetamide had increased mortality (after 1 year only 25 out of 40 (63%) rats survived).

At the 15 month sacrifice, in male rats administered 2.5% acetamide in their diet, there was a statistically significant increase in "malignant liver tumors" (author's description, note: these were designated as "hepatomas" in his table); there was also a numerical increase at the 12 month sacrifice. In rats fed the same concentration of acetamide supplemented with 5.6% (equimolar) arginine glutamate, the incidence of hepatomas was considerably decreased when compared to the incidence in rats fed acetamide alone. (The incidence of neoplastic liver lesions in animals histologically examined is given in Table 2.)

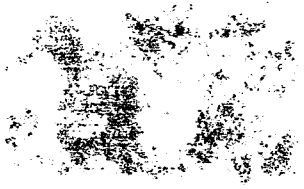

Table 2.

Incidence (%) of Neoplastic Liver lesions in Acetamide Treated Male Wistar Rats (Weisburger et al.)

Time of Sacrifice (months)		Hyperplastic Nodules	Hepatoma
Control Wayne Lab Blox Diet	12	0/4	0/4
	15	0/7	0/7
Control	12	0/3	0/3
5.6% Arg. glutamate	15	0/10	0/10
2.5% Acet-amide	12	0/8	2/8 (25%)
	15	3/16 (19%)	7/16 (44%)*
2.5% Acet-amide + 5.6% Arg.glutamate	12	0/11	1/11 (9%)
	15	1/19 (5%)	0/19

*$p<0.05$, Fisher's Exact Test

4. Carcinogenicity Study in Fisher 344 Rats and C57Bl/6 Mice

Reference: Fleischman, J.R.B., Baker, M.H., Wade, G.G., Hayden, D.W., Smith, E.R., Weisburger, J.H. and Weisburger, E.K. (1980). Carcinogenesis Bioassay of Acetamide, Hexanamide, Adipamide, Urea and P-Tolylurea in Mice and Rats. J.Envtl. Path. and Toxicol. 3: 149-170.

Acetamide was administered in the diet (Wayne Lab Blox) to groups of 50 male and 50 female Fisher 344 rats at 0 (control) or 2.36% (rats) for 12 months, followed by a 4-month recovery period during which control diet was administered to all groups. (Acetamide was also administered at 1.18% and 2.36% to C57Bl/6 mice, but the results were not considered valid due to mixed sources of animals and inability to characterize tumor types.)

Weight gains in male and female rats were essentially comparable to that in controls throughout the study. Male rats had an increased mortality: 44% compared to 13% in controls (suggesting that the dose used in this study may have been too high). The survival of female rats was not adversely affected by treatment. The proportion of rats that died with liver tumors was not reported.

In both sexes of rats administered acetamide in the diet there was a statistically significant increase in hepatocellular carcinomas (Table 3). There were also statistically significant increases in non-neoplastic liver lesions in both sexes of treated animals (Table 4).

Metastases occurred in 15/47 (32%) of male rats and in 5/48 (10%) of female rats; lungs were the most frequent metastatic site. In males, metastases occurred in the lung, kidney, peritoneal cavity, pancreas, diaphragm, heart and mediastinum (in descending order); in females metastases to the lung only were reported.

Table 3

Incidence (%) of Liver Neoplastic Lesions in Acetamide Treated F344 Rats (Fleischman et al.)

		Control	2.36% Acetamide
Hepatocellular Carcinoma	M	0/50	41/47 (87%)**
	F	0/49	33/48 (69%)**
Neoplastic Nodule	M	0/50	1/47 (2)
	F	0/49	3/48 (6)

Table 4

Incidence (%) of Non-Neoplastic Liver Lesions (Fleischman et al.)

		Control	2.36% Acetamide
Focal fatty changes	M	0/50	20/47 (43)**
	F	0/49	29/48 (69)**
Mixed cell foci	M	0/50	6/47 (13)*
	F	0/49	11/48 (23)**

*p,0.05, **p<0.01, Fisher exact test

5. Other Relevant Studies - Mechanism of Action

Dybing, E., Soderlung, E., Gordon, W., Holme, J., Christensen, T., Becher, G., Rivedal, E. and Thorgeirsson, S. (1987). Studies on the mechanism of acetamide hepatocarcinogenicity. Pharmacol. Toxicol., 60(1):9-16.

A modified Solt and Farber (Nature (1976) 263:701-703) procedure was used to study acetamide initiation activity. Acetamide administered IP to male Fischer 344 rats was followed after 2 weeks by injection with 2-acetylaminofluorene (AAF); after 1 week with a partial hepatectomy and continued AAF treatment, then phenobarbital in the feed for 2 months.

Acetamide induced gamma glutamyl transpeptidase foci (GGT-positive) at IP doses of 100 and 400 mg/kg. At the lower dose the GGT-positive livers displayed basophilia, lipidosis and periportal hypertrophy; the livers which did not stain for GGT had normal morphology. At the higher dose, all livers showed prominent basophilia, sometimes as discrete foci with large cells in the periportal region and lipidosis. The investigators concluded that acetamide was clearly positive for initiating activity, although precise quantitation was not deemed possible.

> The Peer Review Committee noted the lower doses used in this study (as compared to those used in the chronic study) and the confounding effect of AAF (itself an initiator) administration.

The authors also investigated the genotoxicity of acetamide and found that it was negative in the following assays:

Salmonella typhimurium (± activation)
DNA damage in rat hepatoma cells
Unscheduled DNA synthesis in isolated rat hepatocytes.
(See section E.2 for a more complete discussion.)

In contrast, N-hydroxyacetamide (a possible metabolite of acetamide) was positive in all 3 of these assays. The authors reported that neither N-hydroxyacetamide nor acetic acid was excreted in rat urine in significant amounts following IP injections of acetamide at 100 and 1000 mg/kg; however, it could not be determined whether the authors used isolation procedures which would optimize the detection of this metabolite. (see also discussion in Section E.1.).

E. Additional Toxicology Data on Acetamide:

1. Metabolism

Reference: Kennedy, Gerald, L. Jr. (1986). Biological Effects of Acetamide, Formamide, and their Monomethyl and Dimethyl Derivatives. CRC Critical Reviews in Toxicology 17:129-182.

In sheep, 28 to 34% of orally administered C14-acetamide was absorbed and degraded to CO2 within 7 to 12 hours after dosing. In the rat, 62% of a 1.5 to 5 g. dose was excreted unchanged in the first 24 hrs (rat liver cells in vitro have been shown to metabolize acetamide to acetate). In the dog and cat, a large proportion of an oral dose was excreted unchanged in the urine.

Dybing et al. reported that neither N-hydroxyacetamide nor acetic acid was excreted in rat urine in significant amounts following IP injections of acetamide at 100 and 1000 mg/kg; however, it could not be determined whether the authors used isolation procedures which would optimize the detection of the N-hydroxy metabolite. (The cytochrome P-450 containing monooxygenases found in liver endoplastic reticulum (ER) of almost every animal species so far examined catalyze N-hydroxylation of both aromatic and aliphatic amines. The N-hydroxy derivative may then be conjugated with glucuronic acid, catalyzed by glucuronyl transferase (also found in the liver ER) and excreted in the urine or bile (less soluble compounds are excreted in the bile) [Cassarett and Doull's Toxicology. The Basic Science of Poisons. 2nd Edition. Macmillan Publishing Co., Inc. pp. 57-60].

* * * * * * * * * * * * *

Thiodicarb (the parent compound) in mammalian systems is metabolized (via syn-methomyl) to carbon dioxide; conversion of syn-methomyl to anti-methomyl also can occur with subsequent metabolism to acetonitrile (which is either respired or excreted, or further metabolized to acetamide).

In rats, approximately 40-50% of a dose was recovered as respired CO2 and/or acetonitrile during a 72 hr. period; the cow similarly respired 66% of the dose. The question remains as to whether there may be a species-related difference in conversion of syn-methomyl to anti-methomyl and resultant excretion as acetonitrile or metabolic hydrolysis to acetamide. The registrant has submitted a protocol for a metabolism study in monkeys (recommended in Memo: Katz to Ellenberger, 4/19/85).

2. Mutagenicity

Studies published by Dybing, E., Gordon, W. Holme, J., Christensen, T., Becher, G., Rivedal, E. and Thorgeirsson, S. (1987). Studies on the mechanism of acetamide hepatocarcinogenicity. Pharmacol. Toxicol. 60:9-16.

a) S.typhimurium (TA 98 or TA 100); 50-10000 ug/plate; with and without activation: negative

b) DNA damage in Reuber rat hepatoma cells; 10-250 mM did not increase alkaline elution rates of cellular DNA: negative

c) UDS test; 2.5-25 mM did not induce unscheduled DNA synthesis in isolated rat hepatocytes: negative

d) Cell transformation, SHE cells: negative

GeneTox data file - EPA GeneTox Program
a) SHE clonal assay: positive

b) Cell transformation assay, mouse embryo: positive

c) Cell transformation assay, RLV F344 rat embryo: positive

d) Host-mediated assay, E. coli polA: negative

e) Salmonella assay: negative

f) Sperm morphology, mouse: negative

g) S. cerevisiae, homozygosis: negative

h) E. coli polA without S9: inconclusive

> The GeneTox's panel of experts for carcinogenicity concluded, on the basis of the 1980 Fleischman et al. study, that acetamide was a "sufficient positive" based on: "malignant lymphoma (site not specified) and stomach squamous papilloma in male C5751/6 mice and hepatocellular carcinoma in male and female F344 rats".

The overall conclusion of the Committee was that there was not a large concern for mutagenicity for acetamide (or methomyl); however, there were cell transformation studies that were positive.

3. Developmental Toxicity

No data were located for acetamide. Studies with Thiodicarb in rats, and with Methomyl in rats and rabbits have not shown evidence of developmental toxicity.

acetamide

E. 4. Structure-Activity Correlations

No appropriate structurally related analog for which there was carcinogenicity data was located.

Hepatocarcinogenesis has been demonstrated with thioacetamide in rats; however, it was not considered to be a good analog, because thioacetamide can be activated by a metabolic pathway that is not applicable to acetamide.

F. Weight of Evidence Considerations:

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

The data on the carcinogenic potential of Acetamide are derived from the following 4 published studies (described in Section D):

1) Dessau and Jackson
2) Jackson and Dessau
3) Weisburger et al.
4) Fleischman et al.

None of these studies meet current test protocol standards (the first 2 were not even designed to investigate carcinogenicity). The Committee concluded, however, that these studies collectively demonstrated an association of acetamide with liver tumors in the rat, albeit at excessive doses.

The tumors in these studies were early appearing, and in the Fleischmann study occurred in both sexes and metastasized by direct invasion outside the liver.

The parent compounds, Thiodicarb and Methomyl, were reported to be negative in bioassay and methomyl for genotoxicity.

Acetamide did not demonstrate genotoxicity, but was positive in cell transformation assays. In addition, a probable intermediate metabolite of acetamide, N-hydroxy acetamide, was positive in S. typhimurium, for DNA damage in rat hepatoma cells and for unscheduled DNA synthesis in isolated hepatocytes.

G. Classification of Carcinogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Committee reached a consensus on the classification of Acetamide as a Group C carcinogen. This was based on the collective data base (from the published studies) which showed an association of liver tumors with administration of Acetamide in male Wistar and male and female F344 rats.

The Committee also considered whether or not to quantify the risk for Acetamide, and concluded that the data were not suitable for quantitative risk assessment because of the deficiencies in the individual studies.

It was also agreed to recommend that the Registrant (s) of the parent chemicals not be granted any additional tolerances, until satisfactory completion of the following studies:

1) Metabolism study (with the parent chemicals) in an appropriate species (primate) and information on whether there is a species-specific metabolic conversion of thiodicarb/methomyl to acetamide.

2) Substantiation of the isomeric form of the registered product(s).

3) Initiation and promotion mechanistic studies with acetamide in vivo at appropriate doses, in an appropriate system, including histochemistry.

4) S-Phase analysis with acetamide to provide information concerning its cell proliferation potential.

5) Studies designed to identify and measure (as the glucuronide, or other conjugate) the N-hydroxy acetamide metabolite.