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Initial

Document Control Officer
Information Management Division
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

100 MAR 17 11 08 AM '86

Dear Sir:

This letter is being submitted under Section 8(e) of the Toxic Substances Control Act. Based on previous data we had concluded that acrylic acid does not represent a significant risk to health or the environment, and at this point the attached information does not change our assessment. However, we have decided to forward this information for your review.

The work was conducted independently at New York University, and was presented by the study's principal author in a poster session at the Society of Toxicology meeting in New Orleans on March 6, 1986.

Sincerely yours,

George A. Rodenhauen
Director
Environmental, Health
and Safety Affairs

GAR:js
att.

Acrylic Acid: Skin Carcinogenesis in ICR/HA Mice

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2

INTRODUCTION

Acrylic acid (AA) is a substituted ethylene compound used in the production of plastics, coatings and acrylic fibers. Occupational exposure occurs primarily via inhalation and/or skin contact (1). Little previous work has been done in regard to the mutagenic or carcinogenic potential of this compound. The present study was initiated to investigate 1) the effects of chronic dermal application of AA, 2) its potential as a tumor promotor in two stage carcinogenesis, 3) the ability of AA to bind to DNA in vitro.

3

METHODS

Bioassay

Female ICR/HA mice were obtained at four weeks of age (Harlan Sprague Dawley Industries; Indianapolis, Indiana). The animals were divided into groups of 30 animals each, housed in polycarbonate cages (5/cage), given Purina Rodent Chow and water ad libitum. Animals were kept on 12 hour day/night lighting schedule.

Experiments began when animals were 6 weeks of age. Acrylic acid (4 mg) was applied to shaved dorsal skin by micropipette in 0.1 ml acetone, 3 times per week for 1.5 years. This dose of AA was determined to be near the maximum tolerated dose in a previous six week dose reponse testing. Mice in another group were first initiated with 20 ug DMBA, as previously described (2), then began receiving AA two weeks later (3 times per week for 1.5 years). Control animals received acetone alone or DMBA followed by acetone alone as described above. Animals were observed daily and weighed every other month. At the time of sacrifice (1.5 years) animals were necropsied. Tissue from skin, kidney, liver, spleen, brain, bladder, and colon as well as any grossly abnormal tissue were examined histologically.

Biochemistry

Acrylic acid was reacted with calf thymus DNA at 37° and pH 7.0. DNA was hydrolyzed using acid and enzyme hydrolysis (3,4,5,6,). Adduct formation was detected by analytical paper chromatography and high performance liquid chromatography (HPLC). Adducts were isolated by preparative paper chromatography and purity verified by HPLC and/or paper chromatography. Structures were elucidated by gas chromatography-mass spectrometry (GCMS) as previously described (3,4,5,6).

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RESULTS

Bioassay

Acrylic acid produced skin tumors in both the DMBA/AA group and the AA alone group. Four tumors were observed in the DMBA/AA group (Fig. 1). ($p < .05$; Fisher exact test). One of these tumors proved to be a squamous cell carcinoma. In the AA alone group two tumors were observed, both of which were squamous cell carcinomas (Fig. 2). This is marginally significant when compared to historical controls ($p < .07$; Fisher exact test). No skin tumors were observed in control groups. There was no statistical difference in the numbers of tumors or latency of tumor appearance between the DMBA initiated group and the AA alone group. This suggests that AA may act as a complete carcinogen and not as a promoter. If these two acrylic acid groups are combined, irrespective of DMBA, the incidence of carcinoma is 5%.

AA also produced an increase in leukemia which normally occurs in 20 to 40% of historical controls and occurred in 30% of the control groups in this study. Leukemia in AA treated animals was 86%. The tumor incidence for the sum of all other tumor types was also significantly different from control ($p < 0.05$; Fisher exact test). However, other than those discussed above, no single tumor type alone was significant.

Biochemistry

The adducts formed by the *in vitro* reaction of AA with calf thymus DNA were 1-(2-carboxyethyl)-adenine, 3-(carboxyethyl) cytosine, 7-(2 carboxyethyl) guanine and 3-(2 carboxyethyl) thymine. The reaction time of AA is slow with a $t_{1/2}$ in the order of days.

DISCUSSION

Our work with AA is part of a larger study on the carcinogenicity and biochemistry of substituted ethylene compounds. We chose to study AA based on what we felt was its potential to form adducts similar to the well known carcinogen beta-propiolactone (BPL). In the studies completed to date, there are similarities between AA and BPL, as well as the carcinogen acrylonitrile (3-6). In DNA treated with AA and these compounds, several major adducts are found in common including 1-(2-carboxyethyl) adenine, 3-(2-carboxyethyl) thymine, 3-(carboxyethyl) cytosine, and 7-(2-carboxyethyl) guanosine. The $t_{1/2}$ of BPL is more rapid ($t_{1/2}$ = hours) than that of AA and acrylonitrile. Acrylic acid reacts with a nucleophilic center in DNA by Michael addition while BPL reacts by aliphatic nucleophilic substitution at the beta carbon atom via an S_N2 mechanism. Using identical protocols, both BPL and AA are both only mildly carcinogenic in mouse skin painting bioassays. Acrylonitrile is not carcinogenic by dermal application.

In summary, AA appeared mildly tumorigenic *in vivo*. This is the first report of any tumorigenic activity of acrylic acid, as well as the first report of acrylic acid-DNA adduct formation and preliminary identification of those adducts.

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1. International Agency for Research on Cancer Monograph: Evaluation of Carcinogenic Risk (1979) 19:187-211.
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3. Mate, U., Solomon, J. J., Segal, A. (1977) Chem.-Biol. Interactions 18: 327-336.
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Chronic Skin Application in Mice

Thirty female ICR/He Swiss mice per group were randomly assigned to test and control groups. The experiment began when animals were 35 days of age and continued approximately 500 days.

<u>Primary^a treatment</u>	<u>Secondary^b treatment</u>	<u>Time to first tumor</u>	<u>Mice with papillomas/ total papillomas</u>
None	acrylic acid 4.000/0.1 ml acetone	361 days	2/2 ^c (2) ^e
DMBA 20 ug/.1 ml acetone	acrylic acid 4.000/0.1 ml acetone	357 days	4/5 ^d (1)
DMBA 20 ug/.1 ml acetone	acetone 0.1 ml	-	0/0
None	none	-	0/0

- ^a One application only.
- ^b Applications 3 times/week beginning 14 days after primary treatment for duration of test in 0.01 ml acetone/application.
- ^c Fisher Exact Test, $p = .07$ as compared to historical controls (1 tumor in 180 animals).
- ^d Fisher Exact Test, $p = .0035$ as compared to historical controls (1 tumor in 90 animals).
- ^e Numbers in parentheses, mice with squamous cell carcinomas.

00006

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