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Re: BEHQ-0386-0592

Dear Sir:

By letter dated March 14, 1986, Celanese Corporation submitted, for your review, results of a study conducted at New York University (NYU) on acrylic acid. This letter supplies additional follow-up information.

First, I would like to clarify that the study reported in our previous letter was not sponsored by Celanese Corporation. It was independently conducted at NYU.

Subsequent to submitting our first letter to you, Celanese hired a consultant to review the NYU study. We are submitting to you, at this time, a copy of the consultant's report. This report concludes that the skin tumors observed are the result of treatment with acrylic acid, but many questions regarding the conduct of the study remain unanswered. The consultant's report also suggests that the observations of leukemia and DNA adduct formation are of questionable relevance.

In addition, we would like to inform you of the results of two tests sponsored by Celanese Corporation regarding possible cytogenetic response to acrylic acid. An in vitro test for cytogenetic response showed activity with and

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without metabolic activation; however, an in vivo test showed no activity at doses toxic in animals. We believe this information is useful in evaluating potential responses from exposure to acrylic acid. These test results indicate to us that there is no genetic hazard in live animals from this material even though in vitro activity has been detected.

Sincerely yours,



George A. Rodenhause  
Director  
Environmental, Health  
and Safety Affairs

CAR:js  
att.

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**EVALUATION OF ACRYLIC ACID MOUSE  
SKIN TUMOR BIOASSAY AND DNA ADDUCT  
STUDY PERFORMED AT NEW YORK UNIVERSITY  
MEDICAL CENTER, NEW YORK, NEW YORK**

**A REPORT TO**

**CELANESE CORPORATION**

**JULY 11, 1986**

**ADL Ref: 55846**

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## BACKGROUND

At a poster session at the 1986 annual meeting of the Society of Toxicology (Abstract #945), a report was presented describing the induction of skin tumors in ICR/Ha mice following topical administration of an acetone solution of acrylic acid. The report was authored by Ila L. Cote, Anne E. Hochwalt, Irving Seidman, Greb N. Budzilovich, Jerome J. Soloman and Alvin Segal of the Department of Environmental Medicine, New York University Medical Center, New York, NY. The senior author, Ila L. Cote, is now with the Environmental Protection Agency in North Carolina.

In order to gain more detailed information to evaluate the experiments performed with acrylic acid, the Celanese Corporation retained Arthur D. Little, Inc. with Dr. Andrew Sivak as the principal task leader.

This evaluation of the bioassay began in mid-April, 1986, and the report was submitted at the end of June, 1986. During this interval two visits were made to the Sterling Forest, N.Y. laboratories of the Institute of Environmental Medicine to discuss details of the study with Susan Melchionne, Alvin Segal, Jerome Soloman and Anne Hochwalt and to review the histological slides. Additional review of the slides was carried out by Alice Liberson, a board certified veterinary pathologist, whose experience is summarized in Appendix A. In addition two personal conversations were held with Ila Cote in North Carolina in May and June, 1986.

Written material that was supplied included:

- (1) copies of the individual animal cards (Ila Cote)
- (2) a summary of histopathology data (Ila Cote)
- (3) a table showing the data base on which the statistics were calculated (Susan Melchionne)

- (4) a letter from Alvin Segal dated May 12, 1986 describing the DNA adduct studies and the subcutaneous bioassay
- (5) a copy of a progress report (undated) obtained from Alvin Segal on May 21, 1986 (Appendix E).
- (6) a copy of the material presented in the Society of Toxicology meeting poster (J. Clary) and,
- (7) a copy of the 8e notice filed by the Celanese Corporation to the Environmental Protection Agency (J. Clary).

#### Laboratory Study Management

This study was carried out in the Laboratory of Organic Chemistry and Carcinogenesis whose director is Benjamin L. Van Duuren. The experiments were directly supported by a research grant to Alvin Segal from the National Cancer Institute. The actual design and execution of the skin painting bioassay, was done by Ila Cote, a postdoctoral fellow, with the technical assistance of Anne Hochwalt, a graduate student. Chemical analysis support was provided by Jerome Solomon. The histopathological diagnoses were done by Irving Siedman of the Department of Pathology and Greb Budzilovich of the Department of Neuropathology. Ila Cote was responsible for collection, recording and summarizing the data associated with the skin painting bioassay and she acted independently in these tasks.

From observations during the visits and discussions with Susan Melchionne, it is apparent that the animal housing and treatment facilities are clean and well cared for and are operated on a department wide basis by an animal care committee that is organized in keeping with the present U.S. Department of Health and Human Services regulations. Although there are no formal animal care committee records for the time interval of the acrylic acid study (November, 1981 to March, 1983), there is every expectation that the animal husbandry was carried out in a fully

acceptable manner. The tissue blocks and histology slides for this study were organized and clearly labeled in the histology laboratory of the Institute. Written data that were not discovered included

- (1) in life animal records
- (2) detailed histopathology reports and
- (3) the laboratory notebooks for the skin painting bioassay.

## METHODS

### Housing and Animal Care

There was no formal written protocol prepared for this study and no formal quality assurance audit. Conversations with Ila Cote, Susan Melchione and Anne Hochwalt confirmed that the skin exposure bioassay was carried out essentially as described in the poster reported at the 1986 Society of Toxicology meeting with 30 mice per group treated 3 times weekly for 300 days. The mice used in the study were female ICR/Na (Marion Syngene Dowley Industries, Indianapolis, Indiana) and were housed in polycarbonate boxes 5 animals per box. "Bettachip" heat-treated hardwood chips were used as bedding with the cages being changed weekly. Based on conversations with Ila Cote, the mice were shaved as needed, approximately monthly, but never on the day of treatment.

The acrylic acid study was carried out in a room that also contained two sets of mice being treated with acrylonitrile. A common set of solvent (acetone) treated controls was used.

### Dose Selection and Preparation

According to conversations with Ila Cote and Alvin Segal, the selection of the dose for the bioassay was based on the results of a 6 week study at 1, 2, 3, 4 and 5% acrylic acid in acetone. At 5%, severe skin damage was observed, and at 4%, transient gross inflammation was observed, and 4% was selected as the dose for the bioassay. The written documentation and histological slides for the range finding study are no longer available. The acrylic acid used in the study was obtained from Aldrich Chemical Company, and contained 200 ppm hydroquinone monomethyl ether as a stabilizer. It is unlikely that the stabilizer had an impact on the results since it showed no promoting or tumor inducing activity in an experiment reported by Boutwell (*Cancer Res.*, 19:413, 1959). The solution of acrylic acid was prepared monthly in acetone and stored in a refrigerator in a brown bottle between treatments. The 0.1 ml dose was delivered with a plunger pipette that accurately delivers the required

volume. Alvin Segal indicated in conversations that no degradation was likely during the one month storage interval.

From the several documents reviewed originally, there was considerable uncertainty with respect to the dose of acrylic acid used in the chronic skin painting study.

The abstract states that, "Female mice were exposed to 25  $\mu$ l AA (4% v/v in acetone)." In subsequent conversations with Ila Cote, she indicated that this 25  $\mu$ l was made up to 100  $\mu$ l dose, yielding a 1% actual dosage solution. The poster states "Acrylic acid (4mg) was applied to shaved dorsal skin by micropipette in 0.1 ml acetone ...." A page of a progress report supplied by Alvin Segal on May 21, 1986 states, "Doses are 20 $\mu$ g of AN and 10 $\mu$ g of AA." In an undated letter from Ila Cote received on June 27, 1986, which had as an enclosure a xerox of what was identified as a laboratory notebook page, a dose of 72  $\mu$ g per application of acrylic acid was indicated (Appendix D). This amount of acrylic acid in 0.1 ml acetone would be an 0.072% solution.

#### Necropsy and Histological Analysis

There are no formal standard operating procedures for necropsy or tissue preparation.

For those mice dying during the study and not autolyzed or cannibalized and for those killed at the end of the study, a complete necropsy was performed by Ila Cote and tissue samples of skin, liver, kidney, stomach, colon, bladder and brain were taken. This selection of tissues is standard practice for skin exposure experiments done in the NYU laboratories. Organs were not weighed, nor was any hematology or clinical chemistry done. In addition, any tissues with unusual appearance and masses were also taken. All tissues were prepared for histopathological diagnosis by standard embedding procedures and with standard hematoxylin/eosin staining. These operations were carried out

by Susan Melchione who has many years experience in the procedures in the laboratory of the study. The histopathological diagnosis were done by Irving Seiden of the Department of Pathology of the New York University School of Medicine who has been associated with the Laboratory of Organic Chemistry and Carcinogenesis for nearly 20 years as a pathologist in experimental carcinogenesis studies.

Histopathology Analysis by Arthur D. Little, Inc.

The slides were made available for reexamination by Alice Liberson of Arthur D. Little, Inc., (Appendix A) and the results of this analysis are presented below in the results discussion.

Gross pathology reports were not available. Histological sections stained with hematoxylin and eosin, obtained from the Department of Environmental Medicine of New York University at Sterling Forest, containing samples from 21 acetone treated mice (Control, C), 29 acetone-acrylic acid treated mice (A-AA), and 26 7,12-dimethylbenz (a)-anthracene initiated: acetone-acrylic acid treated mice (D-AA) were available for review. Tissues were examined principally to determine the presence of neoplasia. Inflammatory and parasitic lesions are not generally reported. All sets of tissues were not complete. The findings of this review are presented in Table 3-6.

~~Lymphocytic aggregates were a frequent finding in many organs.~~ The following criteria were used in differentiating lymphocytic hyperplasia from neoplasia: number of lymphocytic aggregates in the section, size of the aggregates, cell type or types comprising the infiltrate, invasion of surrounding tissue, and cellular morphology. The great majority of the infiltrates were composed of mature appearing lymphocytes. It should be noted that only rarely were features of immaturity or atypia present. The term lymphoma will be used to describe abnormally proliferative, probably neoplastic diseases of lymphocytes. We did not obtain a written description of the criteria used by New York University to diagnose the leukemia/lymphomas.

### Other Methods

The procedures for the subcutaneous carcinogenesis studies and the DNA adduct formation are described in the letter from Alvin Segal to Andrew Sivak dated May 12, 1986.

### RESULTS

#### Mouse Skin Bioassay

The distribution of animal weights at the termination of the study was calculated from data shown on the copies of the animal cards provided (Table 1). While differences in group animal weights exist, there appears to be no pattern that would relate them to treatment or leukemia/lymphoma tumor burden, and the weights are in the range expected for female mice of about 1 1/2 years of age.

The survival of the 3 groups of mice in the study is shown on Figure 1. These data were also taken from the animal cards. No differences are apparent in survival among the groups.

Based on the data presented in the poster from Ila Cote, 2 skin tumor-bearing mice were observed in the acrylic acid-treated group, with each animal bearing a single squamous cell carcinoma. A later summary table obtained from Ila Cote reported one papilloma and one squamous cell carcinoma in this group (Table 2). The pathology analysis done by Arthur D. Little, Inc., revealed two squamous cell carcinomas in the acrylic acid treated group. In the portion of the study designed to test the tumor promoting ability of acrylic acid with a single 20  $\mu$ g treatment of the skin with 7,12-dimethylbenz(a)anthracene (DMBA) prior to repeated acrylic acid treatment, 4 skin tumor bearing mice were observed with 1 squamous cell carcinoma, 1 keratoacanthoma and 3 papillomas. One animal bore 2 papillomas. The first skin lesion appeared in each group at one year after the start of acrylic acid exposure (Table 2). A copy of the

data used for the statistical evaluation was supplied by Susan Melchione and is enclosed as Appendix B. These data show that skin tumor production in acetone-treated controls is an extremely rare event.

The occurrence of leukemia/lymphomas according to the NYU diagnosis is shown in summary in Table 1 and in detail in Table 2. In the absence of the detailed histopathology report by individual animals, it is not possible to confirm the patterns of tumor distribution reported in Table 2. A preliminary examination by Susan Melchione of historical data in control mice indicated a range of leukemia incidence of 20 to 40 percent.

Pathology Evaluation by Arthur D. Little, Inc.

Tumor incidence in the vehicle control group in this evaluation can not be readily compared to findings in the original study as only 21 cases were available for review. The other 9 sets were at the Department of Neuropathology, and we were not able to gain access to these slides. Overall, occurrence of tumor in this review was lower than that in the original report in the A-AA treated mice (23 vs. 28) and the D-AA treated mice (14 vs. 20). The total number of tissues examined was not noted in the original report. A greater discrepancy exists between individual organ involvement noted in the original report and this review. The following comments may explain some of these discrepancies.

**Liver:** Mild peripheral or perivascular cellular infiltrates were often present in all groups. Lymphoma was diagnosed only in those cases where infiltrates were extensive or invasive, and contained a relatively pure population of lymphocytes. The majority of small foci usually contained a mixed cellular infiltrate and were more consistent with inflammation than neoplasia.

**Spleen:** Marked extramedullary hematopoiesis and lymphocytic hyperplasia were frequent findings in all groups. However, unless invasion of normal structures was present, these changes were considered reactive and not neoplastic.

Lung: Proliferation of peribronchial lymphoid tissue was a frequent finding in all groups. This change can be difficult to interpret.

Kidneys: Most kidneys in all groups contained perivascular and subpelvic cellular infiltrates, some of which contained a mixed population of inflammatory cells. In those foci composed principally of lymphocytes, the cells were usually small and relatively mature. However, if the infiltrates were pure and extensive, they were categorized as lymphoma.

Urinary Bladder: Prominent submucosal lymphoid aggregates composed primarily of mature lymphocytes were often present. However, unless there was invasion of the epithelium or the tunica muscularis, these foci were considered hyperplastic.

Brain: No animals were observed to have neoplastic lesions involving the brain. Two animals (C-241, C-155) had mixed meningeal infiltrates characteristic of meningitis, and one animal (D-AA 127) had very mild, mononuclear perivascular cuffs.

Skin: Seven animals had proliferative skin lesions. Four mice had squamous cell carcinoma (A-AA, 153 and 179; D-AA 126 and 139). When more than one slide was present, they appeared to be from the same site. D-AA 146 had a squamous papilloma. The skin section from D-AA 144 contained only a mass and lacked underlying tissue to aid in orientation and diagnosis. However, a squamous papilloma was diagnosed due to the characteristic cellular proliferation. One section of skin from D-AA 145 contained a proliferative lesion, but severe artifact due to autolysis or processing precluded a diagnosis in this case. Skin treated with acrylic acid for the entire interval exhibited no pathological response, and acetone - treated and acrylic acid treated skins were indistinguishable at the end of the bioassay.

#### Conclusions of Arthur D. Little Histopathology Evaluation

Although the overall incidence of tumor diagnosed in the original report and in this review was similar, there were marked discrepancies in the evaluation of individual organ involvement. Part of this difference may

simply be due to the inherent difficulty in differentiating neoplastic from hyperplastic response of lymphocytes, or the use of varying criteria for the diagnosis of a malignancy in different mouse strains. In addition, the overall health status of the animal has a profound effect on the appearance of lymphoid tissue. This can be of particular importance in the spleen. A more thorough knowledge of the health status, including titers to viral diseases, and aging changes in untreated controls would be required for a more meaningful evaluation of changes seen.

It is apparent that mice in the A-AA (23/29, 72%) and B-AA (14/25, 56%) treated groups had a greater incidence of lymphoid proliferation than those in the control group (9/21, 43%). However, there were marked inconsistencies from organ to organ in the incidence of lymphoid lesions among the treated groups. For example, no elevation of lymphomas was found in the lungs of mice treated with DNBA followed by acrylic acid, although in the group treated with acrylic acid alone, lymphoma incidence in this organ was elevated. The inverse situation is apparent with lymphomas in the colon and liver, where the group treated with DNBA and acrylic acid exhibited the highest lymphoma yield. Moreover, in spleen, stomach, and urinary bladder, no increase in lymphomas in the treated groups was found.

As previously stated, most of these infiltrates were composed of mature lymphocytes. The possibility that this may represent marked hyperplasia, which potentially could be reversible, instead of frank neoplasia can not be ruled out with certainty in most of the cases.

#### Subcutaneous Assay Studies in Mice

In addition to the mouse skin exposure studies, Segal has found 2 sarcomas at the site of weekly subcutaneous injection with acrylic acid with the first tumor palpable at 327 days. The pathological evaluation of this study is not complete, and no data for leukemia/lymphomas were available. (See Appendix C).

### DNA Adducts

The experimental conditions and outcomes of the studies carried out by Dr. Alvin Segal are shown in a letter from Alvin Segal to Andrew Sivak dated May 21, 1986 (Appendix C). These studies were carried out by Alvin Segal and Jerome Solomon using procedures previously reported in the published literature and cited in the letter from Segal to Sivak.

Based on the laboratory visit, it is clear that adequate facilities were available to conduct the studies, including sophisticated mass spectrometry equipment for structure determination.

Initially, a set of reactions was carried out by dissolving 2'-deoxyadenosine, deoxycytosine, deoxyguanosine and deoxythymidine at a concentration 1-3  $\mu$ mole in a 50 ml solution of acrylic acid neutralized to pH 7.0 with sodium hydroxide and incubating the mixture at 37°C for 40 days in a shaker. Similarly, 150 mg of calf thymus DNA was incubated under the same conditions with neutralized acrylic acid.

Under both conditions, adducts were isolated and identified for all four nucleosides from both sets of studies. The specific details of this portion of the work are included in Appendix C.

### Other Considerations

In a verbal communication, Ila Cote indicated that the chemical evaluation of the acrylic acid used in the mouse skin bioassay may not have been able to find impurities and/or degradation products with HPLC column retention times similar to acrylic acid because of the broadness of the acrylic acid peak under the separation conditions and solvents employed. Alvin Segal indicated verbally that there is no reason to suspect substantial alterations of the acrylic acid in the acetone solutions used for the mouse skin applications.

### Conclusions of this Evaluation

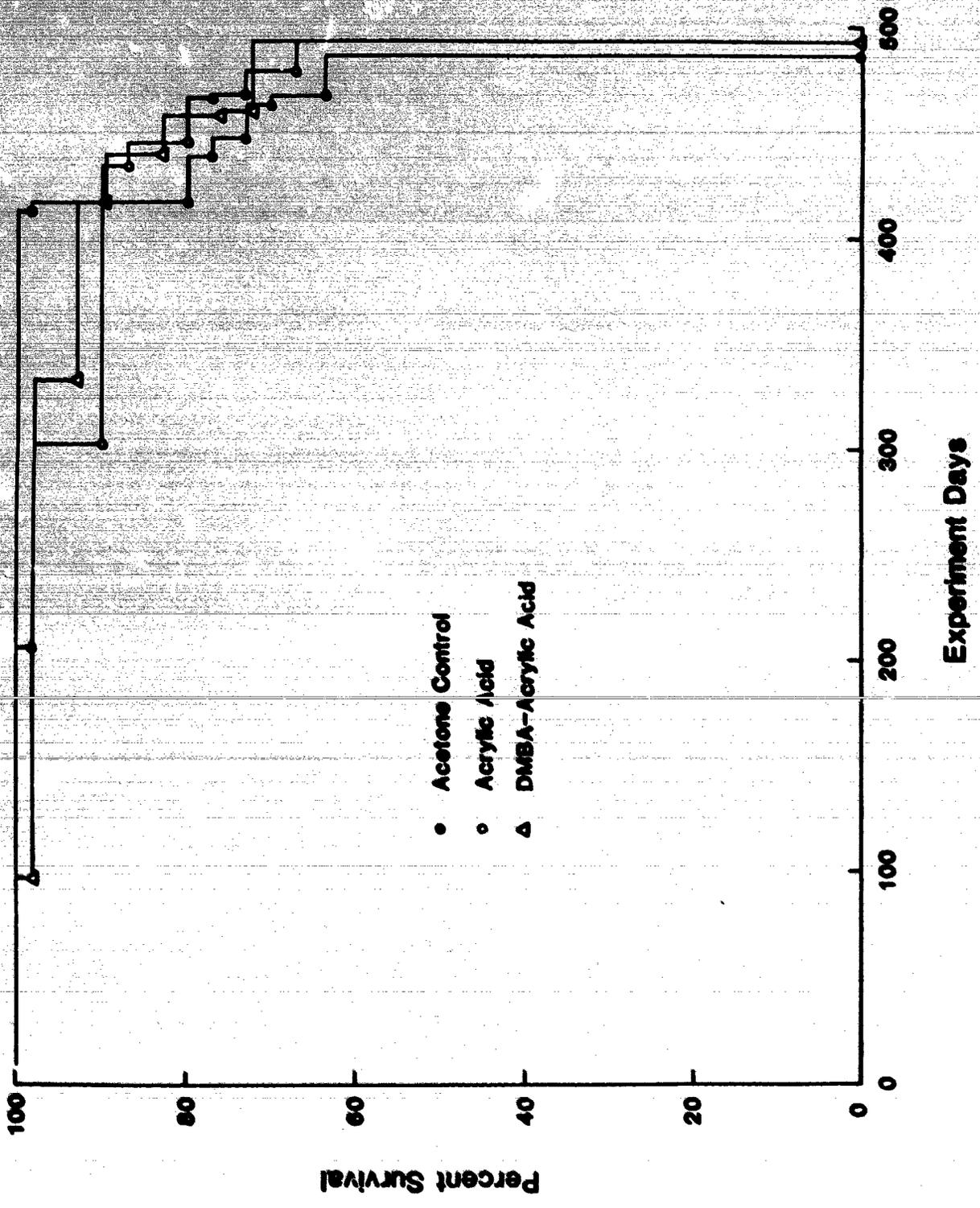
Although the dose cannot be established, acrylic acid delivered to the dorsal skin of ICR/Na female mice in most likely 1% solution in acetone 3 times weekly for 1 1/2 years resulted in the formation of both benign and malignant skin tumors. An independent histological evaluation confirmed these findings, and the consistency of necropsy record animal numbers and animal numbers on the slides add assurance to this conclusion. The absence of a written protocol and of definitive documentation of the dose of acrylic acid actually used in the bioassay make interpretation of these results a problem.

The reported incidence of leukemias/lymphomas in acrylic acid treated mice was not confirmed by an independent histopathological evaluation. Although the numbers of lymphomas were elevated in one of the treatment groups, inconsistent patterns of tumor occurrence from organ to organ would strongly suggest that the lymphomas were not treatment related. Similar inconsistencies were observed in the original histopathological analysis.

Subcutaneous injection of acrylic acid in trioctanoin (20  $\mu$ M per injection) into ICR/Na female mice resulted in fibrosarcomas at the site of injection in 2 of 28 mice.

Acrylic acid in a solution neutralized pH to 7.0 with sodium hydroxide and incubated at 37°C for 40 days formed adducts with all four single deoxynucleosides in solution. Adducts with these four primary nucleosides of DNA were also found with the same incubation conditions using calf thymus DNA. The relevance of this finding to the potential carcinogenic activity of acrylic acid is questionable given the unusually long incubation interval and the absence of a control incubation of DNA with a non-carcinogenic organic acid of similar structure and molecular weight.

FIGURE 1 - MOUSE SURVIVAL DATA



**Table 1**

**SUMMARY OF BODY WEIGHTS AT END OF STUDY**

	<b>Number of Mice</b>	<b>Mean Body Weight (Gm)</b>	<b>Standard Deviation</b>
<b>Control (Acetone)</b>	21	32.0	7.1
<b>Acrylic Acid</b>	20	36.5	3.9
<b>DMBA + Acrylic Acid</b>	20	38.9	5.2

Table 2

**Tumor Bearing Mice**

<b>Neoplastic Lesion</b>	<b>Control</b>	<b>Acrylic Acid</b>	<b>DMBA + Acrylic Acid</b>
Leukemia	6	28	20
Hemangioma	1	1	4
Skin (Total)	0	2	5 tumors/4 mice
Squamous cell carcinoma		1	1
Keratoacanthoma			1
Papilloma		1	2
Other	0	1	1

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From written data supplied by Ila Cote

Table 3

Number of Mice Bearing Leukemias

<u>Organ Site</u>	<u>Control</u>	<u>Acrylic Acid</u>	<u>DMBA + Acrylic Acid</u>
Lung	5	21	20
Liver	4	17	18
Spleen	6	25	19
Kidney	4	22	26
Stomach	1	0	0
Brain	0	0	10
Bladder	0	11	0
Colon	0	1	4
Miscellaneous	0	8	0
Total Mice Bearing Leukemias	6	28	20

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From written data supplied by Ila Cote

TABLE 4  
TUMOR INCIDENCE IN ACUTE TREATED MICE (CONTINUED)

Necropsy Number	Skin	Liver	Spleen	Kidney	Stomach	Primary Bladder	Colon	Prostate	Lung	Other	All Sites
241	NA	+	-	+	+	+	-	-	-	Subcutis: adenoma	+
242	-	-	-	+	+	+	-	-	-	-	+
243	-	-	-	-	-	NA	-	-	-	-	+
244	-	-	-	-	-	-	-	-	-	-	+
245	-	-	-	-	-	-	-	-	-	-	+
246	-	-	-	-	-	-	NA	-	-	Lymph node - Small Intestine - Small Intestine -	+
247	-	-	-	-	-	-	NA	-	-	-	+
248	-	-	-	+	+	+	NA	-	-	-	+
249	-	-	-	+	+	+	-	-	-	-	+
250	-	-	-	+	+	-	-	-	-	-	+
251	-	-	-	-	NA	-	-	-	-	-	+
252	-	-	-	-	-	NA	-	-	-	Uterus - Uterus -	+
253	-	-	-	-	-	-	-	-	-	-	+
254	-	-	-	-	-	-	-	-	-	-	+
255	-	-	-	-	-	-	-	-	-	-	+
256	NA	-	-	-	-	-	-	-	-	-	+
257	-	-	-	+	-	NA	+	-	NA	-	+
258	-	-	-	-	-	-	-	-	-	-	+
259	-	-	-	-	-	-	-	-	-	-	+
260	-	-	-	-	-	-	-	-	-	-	+
261	-	-	-	NA	-	-	-	-	-	Lymph node +	+
Total Evaluated	19	21	21	20	20	19	19	21	20		21
Total Lymphomas	0	1	3	6	3	3	1	0	4		9

Legend:

- No lymphoma
- + Lymphoma
- o Poor orientation of specimen may interfere with interpretation.
- NA Not available
- FN Proliferative nodule

TABLE 5  
INCIDENCE OF MYOPLASMA IN ACETONE ACRYLIC ACID TREATED MICE (PAGE 1 OF 2)

Mouse Number	Skin	Liver	Spleen	Kidney	Stomach	Urinary Bladder	Colon	Brain	Lungs	Other	All Sites
151	NA	NA	NA	+	NA	NA	NA	-	-	Pancreas/pancreatic lymph node +	+
152	-	+	+	+	-	-	-	-	+	Lymph node +	+
153	-/SQC/o	+	-	+	-	-	-	-	+		+
154	-	-	-	-	-	-	-	-	-		-
155	-	-	-	+	-	+	-	-	+	Small intestine	+
156	-	-	-	-	-	-	-	-	+		+
157	-	-	-	+	-	-	-	-	-	Lymph nodes carcinoma, site of origin unknown Subcutis -	+
158	-	-	-	+	-	-	-	NA	+	Lymph node + Thymus +	+
159	-	-	-	+	-	-	-	-	+		+
160	-	-	+	+	-	-	-	-	+ / PN	Lymph node -	+
161	-	- / H	-	+	-	NA	-	-	+		+
162	-	I	-	+	-	- / o	-	-	-		+
163	-	-	-	+	-	NA	-	-	- / PN		+
164	-	-	-	+	-	+	-	-	+		+
165	-	-	-	+	-	+	-	-	+		+
166	-	-	+	+	-	+	-	-	+	Thymus + Tongue -	+

Legend:

- No lymphoma
- + Lymphoma
- o Poor orientation of specimen may interfere with interpretation.
- NA Not available
- SQC Squamous cell carcinoma
- PN Proliferative nodule
- H Hemangioma
- I Specimen necrotic

TABLE 5 (continued)  
INCIDENCE OF NEURILS IN ACETONE AZOBLIC ACID TREATED WICE (PAGE 2 OF 2)

Mouse Number	Skin	Liver	Spleen	Salivary Glands	Stomach	Primary Biliary	Colon	Prostate	Uterus	Ovary	All Sites
167	-	-	-	-	-	-	-	-	-/PH	Neurois/Jejuna -	-
168	-	-	-	+	-	-	-	-	+/PH	Small Intestine +	-
169	-	-	-	+	-	-	-	-	+/PH	Small Intestine -	-
170	-	-	-	+	-	NA	-	-	-/PH	Small Intestine - Thymus	-
171	-	-	-	+	-	-	-	-	-	-	-
172	-	-	-	-	-	-	-	-	-	-	-
173	-	-	-	-	-	NA	-	-	-	-	-
174	-	-	-	-	-	-	-	-	-	-	-
175 <sup>a</sup>	-	-	-	-	-	-	-	-	-	-	-
176	NA	-	-	+	-	NA	NA	NA	-	-	-
177	-	-	-	+	-	NA	NA	NA	-	-	-
178	-	-	-	-	-	NA	NA	NA	-	-	-
179	-/SCC	-	-	+	-	-	-	-	-	-	-
Total Evaluated	27	27	28	29	28	20	24	29	28	-	28
Total Lymphomas	0	2	3	21	0	4	0	0	15	-	23

Legend:

- No lymphoma
- + Lymphoma
- o Poor orientation of specimen may interfere with identification.
- NA Not available
- SCC Squamous cell carcinoma
- PH Proliferative nodule
- A Autolysis

TABLE 6  
INCIDENCE OF NEOPLASMS IN DMBA - ACRYLIC ACID TREATED NICE (PAGE 1 OF 2)

Mouse Number	Skin	Liver	Spleen	Kidneys	Stomach	Urinary Bladder	Colon	Brain	Lungs	Other	All Sites
121	-	-	-	+	-	+	+	-	-	Pancreas +	+
122	-	-	-	+	-/o	-	-	-	-		+
123	-	-	-	+	-	-	NA	-	-	Subcutis -	+
124	-	-	-	+	-	-/o	-	-	-	Small intestine -	+
125	-	-	-	+	+SP	-	NA	-	+	Lymph node -	+
126	-/SCC	-	-	+	-	-	-	-	-	Thymus + Uterus/cervix -	+
127	-	+	-	+	-	-	-	-	+		+
128	-	+	+	+	-	-	NA	-	-	Small intestine -	-
129	-	-	-	-	-	-	NA	-	-/PN	Small intestine + Lymph node +	+
130	NA	-	-	+	-	NA	NA	-	+PN	Lymph node + Lymph node +	+
131	-	-	-	-	-	+	-	-	-	Lymph node + Thymus +	+
132	-	-	-	-	-	NA	-	-	-		-
133	-	-	-	-	-	-	-	-	PN		-
134	-	+	-	+	-	-	-	-	+PN		+
135	-	-	-	-	-	-	NA	-	-	Small intestine -	-
136	-	-/H	-	-	-	-	-	-	-		-

Legend:

- No lymphoma
- + Lymphoma
- o Poor orientation of specimen may interfere with interpretation.
- NA Not available
- SP Squamous papilloma
- SCC Squamous cell carcinoma
- PN Proliferative nodule
- H Hemangioma



TABLE 7

## NUMBER AND PERCENT OF LYMPHOMA BEARING MICE

ORGAN	Control		Acetone- Acrylic Acid		DMBA + Acrylic Acid				
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	(Total)
Lung	4	20 (20)	15	52 (20)	5	22 (23)			
Liver	1	5 (21)	2	7 (27)	5	17 (22)			
Spleen	3	14 (21)	3	11 (23)	1	5 (22)			
Kidneys	6	30 (20)	21	72 (26)	12	50 (24)			
Stomach	3	15 (20)	0	0 (20)	1	5 (22)			
Brain	0	0 (21)	0	0 (20)	0	0 (25)			
Urinary Bladder	3	16 (19)	4	20 (20)	5	22 (23)			
Colon	1	5 (19)	0	0 (24)	2	15 (13)			
-----									
Total Mice Bearing Lymphomas	9	43 (21)	23	79 (20)	14	56 (25)			

Number in parenthesis indicate number of cases examined.

## APPENDIX A

### **ALICE LIBERSON**

Dr. Alice Liberson joined Arthur D. Little, Inc., in January of 1986. She is a board certified veterinary pathologist who will be working in the Life Sciences Division.

Dr. Liberson has published in the Journal of the American Veterinary Medical Association (AVMA), and Veterinary Pathology. She is a member of the AVMA, The New England Comparative Pathology Colloquy, the Phi Zeta Veterinary Honor Society, and is a Diplomate of the American College of Veterinary Pathologists.

After receiving a B.A. in economics from the University of Michigan in Ann Arbor, she attended the College of Veterinary Medicine at Michigan State University where she graduated, with honors, in 1979. She accepted an internship at Angell Memorial Animal Hospital in small animal medicine and surgery. After completion of her internship, Dr. Liberson was an associate in a companion animal practice in Brookline, MA.

In 1982, Dr. Liberson entered a post-doctoral program in the Division of Comparative Medicine at the Massachusetts Institute of Technology where she trained in laboratory animal pathology and medicine. She continued her training in pathology as a resident at Angell Memorial. During this time, she also had the opportunity to train at the Large Animal Hospital at Tufts University, and the New England Regional Primate Center at Harvard University.

After completing her training, Dr. Liberson worked as a consultant and participated in an inhalation study at ADL. Since joining the company, she has set up the necropsy and histopathology laboratories, and is a member of the Animal Policy Care Committee. She has conducted subchronic studies in several species in support of EPA pesticide registrations.

APPENDIX B

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S. Melchionne

CONTROL SKIN TUMOR DATA (470-600 days)

No. Ex	Effective No. Animals	Bibliography* Reference	Number Mice Y/YaP	Number Mice Y/Ca	1st Tumor Days
Total: 705 animals	100	(269)	0	0	-
	100	(239)	0	0	-
	85	(181)	0	0	-
	60	(181)	0	0	-
<hr/>					
DMBA (1x) + Acetone (3 x per wk)	30	(181)	0	0	-
Total: 90 animals	20	(134)	0	0	-
	20	(140)	0	0	-
1 pap at 590 days	20	(150)	1	0	590
<hr/>					
Acetone (3 x per wk)	30	(269)	0	0	-
Total: 160 animals	30	(239)	0	0	-
1 pap at 417 days	50	(181)	1	0	417
	20	(176)	0	0	-
	20	(134)	0	0	-
	20	(140)	0	0	-

\* References in B.L. Van Duuren bibliography 1971-1983.



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ANTHONY J. LANZA RESEARCH LABORATORIES AT UNIVERSITY VALLEY

LONG MEADOW ROAD, STERLING FOREST, TUXEDO, N.Y.

MAIL AND TELEPHONE ADDRESS: 550 FIRST AVENUE, NEW YORK, N.Y. 10016

May 12, 1986

Dr. Andrew Sivak  
 Arthur D. Little, Inc.  
 20 Acorn Park  
 Cambridge, Massachusetts 02140

Dear Dr. Sivak:

In response to your recent inquiries concerning my work with acrylic acid (AA), I will summarize the progress made thus far in this letter. The research involving AA is related to my previous studies concerning the major adducts formed following in vitro reactions between 8-propiolactone (BPL), acrylonitrile (AN) and acrylamide (AM) with calf thymus DNA at pH 7.0 and 37°C. Those studies have been published.

1. BPL - Chem.-Biol. Interactions, 35:349-361 (1981) (and references cited therein).
2. AN - Chem.-Biol. Interactions, 51:167-190 (1984).
3. AM - Cancer Res., 45:3465-3470 (1985).

The results of my work with AA (except for the topical application studies in female ICR/Ha mice) have not been published thus far. However, with the exception of the studies on the in vitro reactions of AA with calf thymus DNA, the results are available to the public in the 1984 Environmental Medicine Core Report and in my progress reports to NIEHS (Grant ES 03043). I, of course, as Principle Investigator, take full responsibility for the results presented in this letter and the results dealing with the topical application of AA in mice which appeared in The Toxicologist, 6:235 (1986).

Reactions of AA with 2'-deoxynucleosides (dAdo, dCyd, dGuo and dThd). A stock solution (SS) of acrylic acid in water was prepared by titrating AA in water with aqueous NaOH to pH 7.0 so that the final solution contained 70 mmoles of mostly sodium acrylate in 50 ml SS. The 2'-deoxynucleosides (1.3 mmole) were

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each dissolved in 50 ml SS and shaken at 37°C for 40 days in a constant temperature water bath. The reaction of AA with dAdo gave 1-(2-carboxyethyl)-dAdo (5%) and N<sup>6</sup>-(2-carboxyethyl)-dAdo [11%, probably via Dimroth rearrangement of 1-(2-carboxyethyl)-dAdo, see references 1-3]. Reaction of AA with dCyd yielded 3-(2-carboxyethyl)-dCyd (7.5%). Reaction of AA with dGuo resulted in the isolation of 7-(2-carboxyethyl)guanine (4%) and 7,9-bis-(2-carboxyethyl)guanine (0.9%). Reaction of AA with dThd yielded 3-(2-carboxyethyl)-dThd (0.5%). Thus AA, like AN and AM, alkylates nucleophilic centers in 2'-deoxynucleosides by Michael addition (see references 2 and 3). In addition, the adducts formed by reactions of AA with the 2'-deoxynucleosides are identical to those found following reactions of the carcinogen BPL with the same 2'-deoxynucleosides, namely 2-carboxyethyl (-CH<sub>2</sub>-CH<sub>2</sub>-COOH) adducts, although the mechanisms of formation differ.

In vitro reactions of AA with calf thymus DNA. A solution of 150 mg of DNA in 50 ml SS was shaken at 37°C for 40 days. The reaction conditions are similar to those reported for the in vitro reactions of AN and AM with DNA (references 2 and 3 respectively). The adducts isolated and quantitated were 7-(2-carboxyethyl)guanine (1 mole/99 moles guanine); 1-(2-carboxyethyl)adenine (1 mole/91 moles adenine); N<sup>6</sup>-(2-carboxyethyl)adenine (1 mole/110 moles adenine) and 3-(2-carboxyethyl)thymine (1 mole/479 moles thymine). The adduct 3-(2-carboxyethyl)cytosine was not detected. Again the adducts are identical to those isolated following in vitro reaction of BPL with calf thymus DNA. The adducts formed following in vivo reactions of direct-acting alkylating agents are usually identical to those observed in in vitro reactions (see Discussion sections in references 1-3).

Bioassays - Topical Applications. The results for AN and AM are presented in Table 1. No tumors at the site of application were observed. The experiments reported in The Toxicologist, 6: 235 (1986) are as follows. AA [4 mg (55  $\mu$ mole/100  $\mu$ l acetone)] was applied to the dorsal skins of 30 female ICR/Ha mice, 3X/week for 1.5 years. Two squamous cell carcinomas were observed in 2 mice (1 per mouse) and confirmed by our pathologist, Dr. Irving Seidman. No malignancies were observed in acetone controls. In a second group of 30 mice, a dose of 20  $\mu$ g of DMBA in 100  $\mu$ l acetone was applied to the dorsal skins 1X and was followed by 3X/week applications of AA for 1.5 years. Tumors were observed in 4 mice in the DMBA/AA group (1 squamous cell carcinoma and 3 papillomas). The diagnosis of the slides was made by Dr. Seidman. No tumors were observed in mice treated with DMBA alone.

Dr. Sivak

-3-

May 12, 1986

Bioassays-Subcutaneous Injections. The results for AA, AN and AM are summarized in Table 1. No malignancies were observed at the time of application for AN and AM. Application of AA gave 2 sarcomas in 2 mice which were diagnosed by Dr. Seidman.

Thus of 60 mice exposed to AA alone, 4 mice have exhibited malignancies (2 sarcomas, 2 squamous cell carcinomas).

You have my permission to make the results which appear in this letter available to those who have requested you to undertake a study of our experiments with acrylic acid.

Sincerely yours,



Alvin Segal, Ph.D.  
Research Professor of  
Environmental Medicine

AS/dg

cc: Dr. I.L. Cote  
Dr. A.C. Upton

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Table 1

Bioassays in Female Hsd:(ICR)Br Mice (30 mice/group)<sup>1</sup>

<u>Compound</u>	<u>Dose/Route</u>	<u># Survivors</u>	<u>Mice w/Tumors Total # Tumors</u>	<u>Days to 1st Tumor</u>	<u># Mice with Malignant Tumors</u>
AN	300 $\mu$ mole/Topical <sup>3</sup>	15	0	-	0
AN	60 $\mu$ mole/Topical	22	0	-	0
Acetone	0.1 ml/Topical	28	0	-	0
AN	10 $\mu$ mole/SC <sup>4</sup>	27	0	-	0
AN	40 $\mu$ mole/SC	19	0	-	0
AA	20 $\mu$ mole/SC	28	2	323	2 <sup>5</sup>
Trioctanoin	0.05 ml/SC	28	0	-	0
Saline	0.05ml/SC	28	0	-	0
No treatment		94 of 100	0	-	0

<sup>1</sup> Topical applic., 450 days; SC applic., 365 days; duration of test, 540-626 days.

<sup>2</sup> Abbreviations: AN, acrylonitrile; AM, acrylamide; AA, acrylic acid.

<sup>3</sup> Topical applications, 3X/week in 0.1 ml acetone.

<sup>4</sup> SC applications, 1X/week in 0.05 ml trioctanoin (AN, AA) or 0.05 ml saline (AM).

<sup>5</sup> Saromas

*Alvin Legal*  
4/9/86

APPENDIX D

Dr. Andrew Sivak  
Arthur D. Little, Inc.  
20 Acorn Park  
Cambridge, Massachusetts 02140

Dear Dr. Sivak,

I wish to clarify several issues in regard to the acrylic acid study, which was reported in The Toxicologist, 6:235 (1986). There has been some confusion as to the dose used. The dose reported in the abstract is correct, i.e., 25  $\mu$ l of a 4% solution of acrylic acid was added to 75  $\mu$ l of acetone. The final concentration is 1 mg of acrylic acid in 100  $\mu$ l of solution. This is the dose administered (dermal application) 3 times/week. Subsequent to publication of the abstract I had reported use of 100  $\mu$ l of a 4% solution (i.e., 4 mg/100  $\mu$ l). This was based on my misinterpretation of a phone conversation with Dr. Segal. I have reviewed the original data and have determined that the 100  $\mu$ l of a 1% solution is correct as published.

In Dr. Segal's letter (May 12, 1986) in the "Bioassay - Topical Applications" section, the DMBA/acrylic acid group results were reported as "1 squamous cell carcinoma and 3 papillomas." This should have read "1 squamous cell carcinoma and 4 papillomas in 4 animals." One animal developed two papillomas. In our analysis we used the number of animals with tumors rather than total number of tumors.

Lastly, I wish to mention that the apparent increase in leukemia in the treated group, as compared to the control group, should be interpreted cautiously. There is considerable spontaneous variability in the incidence

of leukemia in this strain of mouse. An 80% incidence of leukemia is somewhat commonly observed in untreated animals. As a consequence, I feel that less weight should be given to the observation of leukemia, in this strain of mouse, than for a tumor type with a more stable background incidence. In addition, it is my impression that induction of leukemia is a somewhat common response to a variety of chemicals which induce no other neoplastic response. However, this has not been systematically assessed in the New York laboratory. In a study run concurrently with the acrylic acid study, mice treated with topically applied acrylonitrile experienced a similar incidence of leukemia as did the acrylic acid treated animals.

Life table analysis for leukemia was not performed. This type of analysis may shed additional light on this issue. As Dr. Segal has indicated, a subsequent study of acrylic acid using subcutaneous injection as the route of exposure has been conducted. At this time, these data have not been tabulated for leukemia incidence.

I apologize for this misunderstanding. Please contact me if I can be of further assistance.

Sincerely,

*Ila L. Cote*

Ila L. Cote, Ph.D.

cc: J. Cleary  
A. Segal  
J. Solomon  
D. Williams  
M. Winrow

APPENDIX E

A. Segal (5/21/86)

TESTING FOR CARCINOGENICITY OF ACRYLONITRILE (AN) AND ACRYLIC ACID (AA) FEMALE ICR/HA MICE.

Testing for complete carcinogenesis, initiating or promoting activity was begun 11/1/51 for acrylonitrile and the related compound acrylic acid.

Complete carcinogenicity studies. For the mouse skin application experiments, 40 female ICR/HA mice (Harlan Industries, Madison, WI) (6-8 weeks old) are being used per group. The maximum tolerated dose is being used and dry acetone will be the vehicle of choice. The conditions used are essentially identical to previous mouse skin carcinogenicity testing in the Laboratory of Organic Chemistry and Carcinogenesis. The backs of mice were clipped 2 days before the initial experiment and thereafter as needed for the duration of the experiment. Solutions containing the maximum tolerated dose of AN or AA dissolved in 0.1 ml acetone is applied to the dorsal skin 3X weekly by micropipette. All treatments are being continued for the lifespans of the animals. The dosages used were determined by short term (4-6 weeks) toxicity evaluations in groups of 5 mice. Doses are 20 ug of AN and 10 ug of AA. Animals are observed regularly and tumors recorded. Only tumors persisting for 30 days or more are counted in the cumulative totals. Animals bearing tumors that appear grossly to be carcinomas will be killed approximately 2 months after the tumors are classified as malignant or when animals are moribund. All animals will be autopsied. Specimens from tumors and any abnormal tissues will be excised, fixed in 10% formalin, blocked in paraffin, stained with hematoxylin and eosin and confirmed histologically. Included in the experimental protocol are a control group receiving acetone only and a group given no treatment.

Two-stage carcinogenicity studies. Testing for Initiating Activity of AN and AA in mouse skin. The doses used for AN and AA respectively were applied once to the dorsal skins of mice (40 mice/group) in 0.1 ml acetone. Fourteen days later the application of the tumor promoter phorbol myristate acetate (PMA) (2.5 ug/0.1 ml acetone) to the dorsal skins was begun (3X/week for the lifespans of the animals). Included in the experimental protocol are a control group receiving AN or AA only, PMA only, acetone only and a group given no treatment. All other procedures are described above.

Testing for promoting activity of AN and AA. Mice (40/group) were initiated by a single application of 20 ug of 7,12-dimethylbenz(a)anthracene (DMBA) in 0.1 ml of dry acetone. Fourteen days later application was begun of AN and AA respectively to the dorsal skins of mice in dry acetone at the doses used in the complete carcinogenesis studies. This will continue for the lifespans of the animals. Included in the experimental protocol are a control group receiving DMBA alone, a control group receiving AN alone, a control group receiving AA alone, a control group receiving acetone alone and a group receiving no treatment. All other procedures are as described above.

Results. To date no skin lesions have appeared in experimental or control groups. Three premature deaths have occurred. Upon autopsy two animals (one in the AN complete carcinogen group and one in the PMA only control group) were observed to have severely enlarged thymuses, suggestive of the thymic lymphomas which occur spontaneously in this strain of mouse. No gross abnormalities were observed in the third animal (AN initiation-PMA promotion

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group). Tissue from these animals awaits histologic examination.

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