tumors of the brain were not increased at 20 ppm, the incidence of tumors plus proliferative changes "suggestive of early tumors" was elevated in female rats exposed at this level. The incidence of some tumors associated with aging in this strain of rats declined with exposure to acrylonitrile as a result of early treatment related mortality. It is apparent from this study (Quast et al., 1980b) that inhalation exposure of rats to acrylonitrile results in many of the same kinds of tumors as were observed by Quast et al. (1980a) following exposure of rats to acrylonitrile in the drinking water.

In a limited study, Maltoni et al. (1977) exposed rats by gavage to a single dose of 5 mg/kg of acrylonitrile dissolved in olive oil, 3 times a week, for 52 weeks (40 rats of each sex were used in the treated and control groups). On spontaneous death, a moderate increase in tumors of the mammary region and forestomach of female rats was noted. All other tissues examined (the same tissues examined in the Maltoni et al., 1977 inhalation study, as described earlier) showed no increase in tumor incidence. Although this study was very limited, with only a single exposure level and a relatively short exposure period, the results give further support to the conclusion that acrylonitrile is an animal carcinogen by showing increases in specific tumor types in the treated animals that were similar to those observed in other studies.

13.5.2 Cell Transformation

Acrylonitrile has been shown to transform cells in culture and to enhance the rate of transformation in cells that were previously infected with a transforming virus (Parent and Casto, 1979). Primary cultures of hamster embryo cells were treated with 25, 50, 100, and 200 μg/ml of acrylonitrile 5 hours after cultures were exposed to 200 focus-forming units of simian adenovirus SA7. Following the 48-hour treatment period, cells were plated to determine survival number and transformed colonies. Transformation rates 8.9 and 8.4 times greater

than those in cells infected with virus only were observed at treatment levels of 200 and 100 μg/ml acrylonitrile, respectively. At these concentrations of acrylonitrile, cell survival was poor, with surviving fractions of 7% (200 μg/ml) and 21% (100 μg/ml) of control plates; thus, there was a possibility that the treatment could have imparted survival advantages to preexisting transformed cells as compared with normal cells. Only a slight increase in transformation rate was detectable when the cells were treated with acrylonitrile prior to viral infection. The authors also reported on a simple transformation assay in which the cells were treated for 6 days (fresh media that contained acrylonitrile was supplied after three days) with 100, 50, 25, and 12 μg/ml acrylonitrile (these cells were not infected with SA7 virus). A slight increase of 3 transformed foci per 9 dishes and 2 transformed foci per 6 dishes occurred at 100 and 50 μg/ml, respectively. The control value was zero colonies in seven dishes.

13.5.3 Nucleic Acid Interactions

Guengerich et al. (1981) used microsomal preparations from rat liver to study the binding of radiolabeled acrylonitrile to protein and DNA. Protein adducts were detected at the same level, both in the presence and absence of NADPH, indicating direct alkylation of protein by acrylonitrile. When the microsomes were obtained from rats induced with either phenobarbital or β-naphthaflavone, however, a net increase in protein adduct formation was observed in the presence of NADPH. Binding of acrylonitrile to DNA was strictly dependent upon the presence of NADPH in the assay system, indicating that microsomal mediated metabolism was required for DNA binding. Microsomes isolated from rat brain were incapable of mediating the binding of acrylonitrile to DNA, and of microsomes obtained from six human liver autopsy specimens, only one was capable of mediating acrylonitrile binding to DNA. Microsomal preparation from rat liver was shown to metabolize acrylonitrile to the epoxide

2-cyanoethylene oxide, and this epoxide was shown to non-enzymatically bind to both DNA and protein. It was suggested that the microsomal NADPH-mediated binding of acrylonitrile to DNA occurred through the formation of the intermediate 2-cyanoethylene oxide.

Ofengand (1971) used acrylonitrile to cyanoethylate nucleotides in tRNA in an investigation of tRNA function. Only 3 rare nucleotides reacted at an appreciable rate; these were, in order of decreasing reactivity, pseudouridine, 4-thiouridine, and inosine. The reaction conditions employed with isolated tRNA were 1 M acrylonitrile at 30°C and high ionic strength for maintaining native structure, and the same concentration of acrylonitrile at high temperature (60°C) and low ionic strength for denaturing conditions. Cyanoethylation occurred predominantly with the three nucleotides mentioned. The other more common nucleotides also reacted under these conditions, although at a considerably slower rate (Ofengand, 1967). It is not known whether these reactions occur under physiological conditions and at concentrations of acrylonitrile that would not be lethal to the organism.

Parent and Casto (1979) have reported that single strand DNA breaks occur following exposure of hamster embryo cells in vitro to 200 and 400 µg acrylonitrile/ml. The cells were treated for 18 hours in low serum media, and DNA damage was assessed by changes in the sedimentation pattern in alkaline sucrose gradients. It was concluded that the observed shift to lighter molecules was indicative of acrylonitrile's having reacted with DNA and caused strand breakage or alkaline lability of the DNA molecule. Data concerning the acute toxicity of acrylonitrile in this cell system were not presented. Both concentration levels of acrylonitrile were probably toxic to the cells, since 200 µg/ml acrylonitrile used in the cell transformation assays described earlier caused reductions in cell survival as measured by plating efficiency. If appreciable cell death had

occurred, the shift in sedimentation pattern could be accounted for by normal cell autolysis. Further investigations are needed to determine whether acrylonitrile can cyanoethylate nucleic acids in vivo and whether acrylonitrile or a metabolite of acrylonitrile can react with DNA and cause single strand breaks.

13.5.4 Epidemiologic Studies

There are no community studies available on associations between exposure to ambient levels of acrylonitrile and the development of disease; however, an occupational epidemiology study that involved workers exposed to acrylonitrile at a DuPont textile fibers plant in Camden, South Carolina, was conducted by E.I. DuPont deNemours and Co., Inc. (O'Berg, 1980). The cohort consisted of 1345 male workers identified through work history cards as being possibly exposed to acrylonitrile between the years 1950 and 1966. A cut-off date of 1966 was chosen in order that a follow-up period extending to 1976 would allow for a 10-year latency period. Risk of excess cancer in exposed workers was determined by comparison with company experience using both the DuPont Mortality File (begun in 1957) and the DuPont Cancer Registry (begun in 1956)*. The use of company experience was deemed more appropriate than the use of national or regional experience in order to eliminate the "healthy worker effect." The exact exposure of the study population to acrylonitrile could only be crudely estimated as low, median or high, by the use of job descriptions, since monitoring data was not available.

In the cohort exposed to acrylonitrile, there were 25 cases of cancer by 1976 which included 8 lung, 3 colon, 3 prostrate, 2 bladder, 1 thyroid, 1 nasopharynx, 1 penis, 1 esophagus, 1 brain, 1 leukemia, 1 lymphosarcoma, 1 Hodgkin's

*The use of both registries, for example, leads to apparent inconsistencies. According to O'Berg, 4.4 respiratory cancer cases were expected in this cohort using the DuPont controls. However, using the same controls, O'Berg predicts 6.1 respiratory cancer deaths. A telephone conversation with O'Berg confirmed that this inconsistency was due to different methods of following the cohort for cases and deaths. Cases were decribed only for active employees, while deaths included retirees.

disease, and 1 malignant melanoma. The expected number of cancers for all sites was 20.5 versus the observed of 25, and the expected number of the most common cancer, lung cancer, was 4 versus an observed of 8. These excess respiratory cancers** were found primarily among wage roll employees who had worked during plant start up, 1950 to 1952 and had been exposed for at least 6 months. For these employees there were eight cases of respiratory cancer versus 2.6 expected ($P < 0.01$). Furthermore, most of this above excess occurred during the latest follow-up period, 1970 to 1976, when there were six cases of respiratory cancer versus 1.5 expected ($P < 0.01$). Total cancer cases in this latest follow-up period for this group were also significant, 17 versus 5.6 ($P < 0.01$).

A trend toward increased risks was seen not only with increased follow-up time but also with severity of exposure. In wage roll workers with at least moderate exposure and follow-up time > 10 years, the observed and expected numbers of cancer cases was 13 and 5.5, respectively. Furthermore, half of this excess cancer was due to respiratory cancer, 5 versus 1.4 ($P < 0.05$). Thus, this study provides some evidence that acrylonitrile is carcinogenic to humans. However, because of the known relationship between smoking and lung cancer, further analysis is attempted concerning the role of smoking behavior in these findings. (Seven of the eight lung cancer cases were reported tc be smokers by their supervisors or associates; the eighth was unknown.) Dr. Bruce Karrh of Du Pont stated that there were pathology slides for five of the eight respiratory cancer cases. He further stated that these were identified as 4 squamous cell carcinomas, and 1 oat cell carcinoma. Of the 3 remaining respiratory cancers, for whom slides were unavailable, he stated 2 were bronchogenic, and the other

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**This analysis on respiratory cancer and the following possible interaction of smoking is taken from the document prepared by the Carcinogen Assessment Group, Office of Health and Environmental Effects, Office of Research and Development, EPA (4/1/1981).

unknown. These cell types are generally believed to be associated with both chemicals and smoking by most pathologists.

In an attempt to investigate the impact of smoking on the risk of developing lung cancer in this population, the DuPont Company provided additional data to the Carcinogen Assessment Group (CAG) regarding the smoking habits of 32 of the 36 cancer cases reported on in this plant (some of these were not in the study cohort), as well as data on the smoking habits of a matched group of non-cases in the plant. Of the 32 cancer cases, 22 were cancers other than lung, and 16 or 73% of these were smokers. The smoking habits of the matched group of 36 noncancer controls from the same plant were also provided. They were matched on a three to one basis to certain selected cancers occurring in the study on the basis of age, payroll classification, date of first exposure, and date of termination. It was found that 25 or 69% were smokers in this group.

Based on this information, we can estimate that 70% of the plant population were smokers, and 30% nonsmokers. Of the 70% who were smokers, it is assumed that 50% were "moderate" smokers while 20% were "heavy" smokers. Assume also that the relative risk of lung cancer is 1, 10, and 20, respectively, for the nonsmokers, moderate, and heavy smokers (Doll and Hill, 1952; Hammond, 1975). The relative incidence of lung cancer in the plant population is then

$$I_p = 1(0.30)I_o + 10(0.50)I_o + 20\ (0.20)I_o = 9.3\ I_o$$

where I_o equals the risk of lung cancer in a nonsmoking population (Axelson, 1978).

Based upon a nationwide survey (U.S. Health, Education, and Welfare, 1973) which found that 62% of the male blue collar workers smoked in 1966, we assume that the overall company population had 40% nonsmokers. In addition, we assume

that the distribution of moderate and heavy smokers is the same as above, so that moderate and heavy smokers constitute 43% and 17% of the population, respectively. The computed incidence of lung cancer in this comparison population is then

$$I_g = 1(0.40)I_o + 10(0.43)I_o + 20(0.17)I_o = 8.1\ I_o$$

where I_g is the incidence in the company population. Hence, the relative contribution of smoking to the risk of lung cancer in the cohort is the ratio of the two incidence rates:

$$I_p/I_g = 9.3\ I_o/8.1\ I_o = 1.15$$

Therefore, because of the slightly higher proportion of smokers in the study cohort relative to the reference company population, the number of respiratory cancer cases would be about 15% higher than the 1.4 cases expected without considering smoking differences, or 1.4 x 1.15 = 1.61 cases. Assuming a Poisson distribution of cases, the probability of seeing 5 cases or more when only 1.61 is expected is only 0.024. Therefore, after the adjustment for smoking differences, the respiratory cancer rate in the study cohort exposed to acrylonitrile for over 6 months is significantly higher than that of the reference population.

In an attempt to assess whether the excess respiratory cancer rates could be ascribed to a higher ratio of heavy to moderate smokers in the acrylonitrile exposed cohort than that in the reference population, the following calculation was done. In equation (1) for I_p the ratio of heavy to moderate smokers was increased from 0.2/0.5 = 1/2.5, keeping the total fraction of smokers at 0.7, until the point was reached where the probability of the observation of five

cases versus the expected number, 1.4 x (I_p/I_g), was decreased from 0.024, as above, to 0.05. The result is that in order for a higher ratio of heavy to moderate smokers in the cohort to account (at the 0.05 level of significance) for the observed excess respiratory cancer rate, that ratio would have to be 1.6/1. Our judgement is that such a marked excess of heavy versus moderate smokers would not likely occur in the acrylonitrile workers, since for blue collar workers there are generally about 2.5 times more moderate than heavy smokers. In conclusion, the observations by O'Berg of a statistically significant excess of respiratory cancer in workers exposed to acrylonitrile and followed up for more than 10 years constitute significant evidence that acrylonitrile is likely to be a human carcinogen, although smoking at least as a contributing factor cannot be completely ruled out at this time.

A second epidemiologic study was conducted for the B.F. Goodrich Company and the United Rubber, Cork, Linoleum, and Plastic Workers of America (Federal Register, 1978b). This retrospective cancer morbidity and mortality study included some workers with exposure to acrylonitrile. Among workers with potential exposure to acrylonitrile (extent of exposure not known), there was a slight overall excess in the number of deaths from cancer observed compared to the number expected (the data used for determining expected number were not specified): lung cancer deaths (7 observed/4.4 expected); genitourinary cancer deaths (2 observed/1.6 expected deaths, and 6 observed/3.1 expected incidences); and Hodgkin's disease deaths (2 observed/ 0.3 expected). Deaths from all causes (cancer and non-cancer) were not significantly elevated in the worker study group. Because the workers in this study had potential for exposure to other carcinogens, OSHA concluded (Federal Register, 1978b) that the study could not be used to support an association between acrylonitrile exposure and human cancer development.

On April 23rd and 24th, 1980, a conference sponsored by the Association of Plastics Manufacturers in Europe was held to review several recently completed studies on the epidemiology of acrylonitrile. Six groups from Europe and the United States presented the results of their respective investigations on the association of cancer incidence or mortality with acrylonitrile exposure. According to the minutes of the meeting, deficiencies in most of the studies prevented a definitive analysis of the results (Baxter, written communication, 1980). These deficiencies involved problems with cohort identification and selection, limited study population sizes, lack of exposure data, potential exposures to carcinogens other than acrylonitrile, and lack of data on smoking habits. Nevertheless, there was some indication from at least two studies that cancers of the lung and brain may be present in excess.

A further analysis of these data was attempted by combining the data for Monsanto workers in the United States with data for acrylonitrile workers in the United Kingdom (Zack, written communication, 1980). The combined data were categorized according to length of time since first exposure to acrylonitrile; that is, less than 10 years, 10 to 14 years, and greater than 15 years. In the less than 10 years category, elevated standardized mortality ratios (SMR) for site-specific cancers were found for stomach (SMR = 286), colon (SMR = 200), and respiratory tract (SMR = 154). In the 10 to 14 years category, excesses were seen for cancers of the stomach (SMR = 286) and brain (SMR = 500). Only cancers of the brain showed a trend of marked increase in the SMR with time since first exposure to acrylonitrile. These results are indicative of the need to continue and to expand epidemiological studies in defined populations.

13.5.5 Conclusions

The weight of evidence from the above studies points toward the likely carcinogenicity of acrylonitrile. The evidence in support of acrylonitrile

being a carcinogen is based on positive results in short term in vitro mutagenicity assays, long term in vivo carcinogenicity assays in laboratory animals and the epidemiology study by O'Berg (1980). A quantitative risk assessment evaluation by the U.S. EPA Cancer Assessment Group (1981) for acrylonitrile carcinogenic effects also appears in their report contained in the Appendix.

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EXTERNAL REVIEW DRAFT
February 24, 1982

THE CARCINOGEN ASSESSMENT GROUP'S

CARCINOGEN ASSESSMENT

OF

ACRYLONITRILE

Roy E. Albert, M.D.
Chairman

PARTICIPATING MEMBERS

Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.
Steven Bayard, Ph.D.
David L. Bayliss, M.S.
Chao W. Chen, Ph.D.
Margaret M.L. Chu, Ph.D.
Herman J. Gibb, B.S., M.P.H.
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert E. McGaughy, Ph.D.
Dharm V. Singh, D.V.M., Ph.D.
Todd W. Thorslund, Sc.D.
Vicki Vaughan-Dellarco, Ph.D.

*Reproductive Effects Assessment Group

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This document has been reviewed and approved by the Chairman and staff of the Carcinogen Assessment Group, Office of Health and Environmental Assessment, U.S. Environmental Protection Agency. It has not been formally released by the EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implication.

CONTENTS

I. SUMMARY AND CONCLUSIONS

QUALITATIVE ASSESSMENT

Much information on the carcinogenicity of acrylonitrile has been obtained in the last few years. This includes: three epidemiology studies; seven lifetime cancer bioassays in rats; several mutagenicity studies in bacteria, Drosophila, and rodents; chromosome anaylsis with known exposure to acrylonitrile in humans; and numerous metabolic studies.

Only one of the three epidemiology studies was technically adequate. It involved a cohort of 1,345 workers exposed to acrylonitrile between 1950 and 1966 at the Du Pont Chemical Company (O'Berg 1980). Among workers with at least a moderate exposure and a follow-up time greater than 10 years, there was a statistically significant excess of cancer cases with half of this excess, also significant, due to respiratory cancer. Since at least seven of the eight people with respiratory cancer were reported to be smokers, an analysis of the available information on the smoking patterns was done, with the conclusion that smoking differences between the acrylonitrile-exposed cohort and the reference population probably could not have accounted for the observed excess cancer rates. It is possible, however, that smokers could have a higher sensitivity to the carcinogenic effects of acrylonitrile than nonsmokers.

Seven cancer bioassay studies have been performed in which acrylonitrile was administered to rats: four in drinking water, one by gastric intubation, and two by inhalation.

Quast et al. (1980a) administered acrylonitrile in drinking water to Sprague-Dawley rats for 2 years at dose levels of 35, 100, and 300 ppm. A statistically significant incidence of tumors was observed in the central

nervous system, Zymbal gland (all doses), stomach, tongue, and small intestine in both male and female rats, as well as in the mammary gland of female rats.

The occurrence of central nervous system and Zymbal gland tumors in Sprague-Dawley rats was further confirmed in four other studies, a three-generation reproduction study performed at Litton-Bionetics by Beliles et al. (1980); three Biodynamics, Inc. studies (1980a, b, c) in which acrylonitrile was administered in drinking water and via gastric intubation, and an inhalation study by Quast et al. (1980b).

A second inhalation study by Maltoni et al. (1977) exposed rats to atmosphere containing 5, 10, 20, and 40 ppm acrylonitrile 4 hr/day, 5 days/week, for 12 months. Marginal increases in tumors of the mammary gland in females and the forestomach in males were observed, although the sensitivity of this test was limited by the relatively low dose levels and the short duration of exposure.

Acrylonitrile is readily absorbed in mammalian species both by oral and inhalation routes. It is metabolized ultimately to CO_2 and thiocyanate. The proposed metabolic pathway suggests that an epoxide of acrylonitrile is formed as a transient metabolite, and epoxides, as a group, are regarded as having carcinogenic potential.

Acryonitrile caused point mutations in bacteria (Salmonella typhimurium and Escherichia coli). In Salmonella, it was only detected as mutagenic in the presence of mammalian liver enzyme activation and most effective in base-pair substitution sensitive strains. Therefore, it appears that a potentially mutagenic and carcinogenic metabolite(s) of acrylonitrile primarily interacts with and causes DNA base-pair substitution.

In summary, there is evidence that acrylonitrile is a human carcinogen. This position is based on: 1) findings of respiratory cancer in the O'Berg study of Du Pont workers, 2) three positive drinking water rat bioassays and one positive rat gastric intubation study, and one positive rat inhalation bioassay study, 3) the positive mutagenicity findings in bacteria, 4) the metabolic evidence of a probable directly carcinogenic metabolite of acrylonitrile, and 5) acrylonitrile's structural similarity to vinyl chloride, a known human carcinogen.

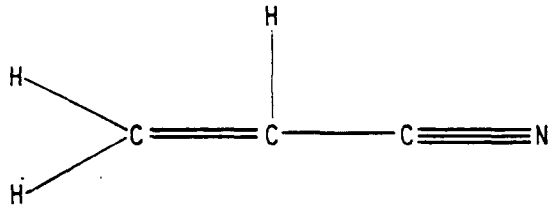
QUANTITATIVE ASSESSMENT

A quantitative cancer risk assessment is calculated based on both the inhalation and drinking water studies of Quast et al. (1980a, b). The lifetime risk of cancer associated with 1 ug/m^3 of acrylonitrile exposure by inhalation is estimated as 1.5×10^{-5} based on the inhalation study and 1.0×10^{-5} based on the drinking water study. These estimates are close to the value of 6.8×10^{-5} based on the O'Berg (1980) epidemiology study.

II. INTRODUCTION

Acrylonitrile is an explosive, flammable liquid having a boiling point of 77°C and a vapor pressure of 80 mm Hg (20°C). Its molecular weight is 53, and its molecular structure (CH_2 = CH - CN) resembles that of vinyl chloride (CH_2 = CH - Cl), a chemical known to cause animal and human cancer as shown by Maltoni et al. (1977), Lee et al. (1978), and O'Berg (1980).

The structural formula of acrylonitrile is shown below.



Acrylonitrile is soluble in water, acetone, and benzene. It undergoes reactions at both the nitrile group and the double bond (Maltoni et al. 1977).

Acrylonitrile production in the United States is in excess of 1.5 billion pounds per year. Principally, it is used as an intermediate in the manufacture of a wide variety of acrylic fibers, plastics, and in synthetic rubber. Other major uses of acrylonitrile include the manufacture of acrylonitrile-butadiene-styrene (ABS) and styrene-acrylonitrile (SAN) resins, nitrile elastomers, and latexes. Acrylonitrile has been used as a fumigant; however, all U.S. registrations for this use were voluntarily withdrawn as of August 8, 1978 (Federal Register, Vol. 43, p. 35099). The U.S. Food and Drug Administration has recently banned the use of an acrylonitrile resin in the production of soft drink bottles (Chemical and Engineering News, Sept. 12, 1977; Jan. 23, 1978) but its use is still allowed in other food packaging. The

National Institute for Occupational Safety and Health (NIOSH) estimates that 125,000 persons are potentially exposed to acrylonitrile in the workplace (NIOSH 1977).

The major sources of acrylonitrile emissions are monomer and polymer production facilities. The acrylonitrile emissions from these facilities have been estimated to be 10,872 metric tons per year (Suta 1979).

The estimated population exposured to airborne acrylonitrile has been reported as 2.6 million persons exposed to annual average concentrations of 0.05 to 15 ug/m^3 (Suta 1979).

III. METABOLISM

ABSORPTION, DISTRIBUTION, AND EXCRETION

Young et al. (1977) studied extensively the pharmacokinetic and metabolic fate of acrylonitrile in male Sprague-Dawley rats by administering [1-^{14}C] or [2,3-^{14}C]* acrylonitrile with different routes and dose levels. When acrylonitrile was orally administered to rats, essentially all of the dose was absorbed. After 72 hours, the percentage of recovered radioactivity accounted for 82% and 104% of the administered dose at 0.1 and 10 mg/kg, respectively (Table 1). The recovery of administered radioactivity in the urine and body was significantly dose-related, while both doses resulted in a 5% recovery of radioactivity in the feces. Thus, at least 95% of the dose had been absorbed.

The absorption of acrylonitrile through inhalation was also investigated by Young et al. (1977). Animals were exposed to acrylonitrile vapor in a "nose only" chamber at concentrations of 5 ppm and 100 ppm. The results presented in

*The acrylonitrile (AN) radiotracers, either [1-^{14}C] AN or [2,3-^{14}C] AN, were obtained from American Radiochemical Corp., Sanford, Fla. and Monsanto Corp., St. Louis, Mo. and have the specific activities of 1.15 and 3.3 mCi/mmol, respectively.

TABLE 1. RECOVERY OF RADIOACTIVITY FROM RATS GIVEN SINGLE ORAL DOSES OF 0.1 and 10 mg/kg ^{14}C-ACRYLONITRILE[a] (Young et al. 1977)

	Percentage of Dose, mean ± standard deviation	
	0.1 mg/kg	10 mg/kg
Radioactivity in urine	34.22 ± 6.26[b]	66.68 ± 10.60[b]
Radioactivity in feces	5.36 ± 1.43	5.22 ± 1.17
Expired air		
Organics in carbon[c]	0.09 ± 0.09	0.11 ± 0.06
Organics in solvent[d]	0.19 ± 0.19	0.20 ± 0.16
H^{14}CN[e]	0.07 ± 0.05	0.08 ± 0.03
$^{14}CO_2$[f]	4.56 ± 1.82	3.93 ± 1.79
Body	37.02 ± 6.09[b]	26.61 ± 5.91[b]
Carcass	24.24 ± 5.02[b]	16.04 ± 1.87[b]
Skin	12.78 ± 1.17	10.57 ± 4.55
Cage wash[g]	0.86 ± 0.37	1.22 ± 0.41
Total recovery[h]	82.37 ± 9.64[b]	104.04 ± 14.40[b]

[a]Two groups of rats housed individually in glass metabolism cages were given 0.1 mg/kg (4 rats) or 10 mg/kg (5 rats); excreta were collected at 8-hour intervals for 72 hours.

[b]The mean values for the two dose levels are different at the P = 0.05 level of significance.

[c]Pittsburg activated coconut charcoal, 12 x 30 mesh.

[d]trap = 2-methoxyethanol

[e]trap = 0.02 M Ag_2SO_4 in 0.1 N H_2SO_4

[f]trap = 5 M ethanolamine in 2-methoxyethanol

[g]water-acetone

[h]The mean and standard deviation of the recovery were calculated from the total recovery of the dose in individual rats.

Table 2 indicate that the urinary excretion of acrylonitrile is slightly dose-dependent (i.e., the higher the dose, the higher the recovery in urine), but a slightly smaller percentage was recovered in the expired air as CO_2 when the dose was increased.

Rogaczowska (1975) studied dermal absorption of acrylonitrile vapor and found that the penetration rate of acrylonitrile vapor through the skin is about 1% in relation to the quantity absorbed via the lungs.

The plasma concentration of acrylonitrile (ug Eq/ml plasma) as a function of time, routes of administration (oral and intravenous), and dose levels (0.1, 1, and 30 mg/kg) has been investigated by Young et al. (1977). Following oral and intravenous administration of ^{14}C-acrylonitrile, typical plasma concentration versus time curves were observed. The biphasic disappearance of radioactivity indicated a pharmacokinetic two-compartment open model for elimination. The half-life values calculated by linear regression analysis ranged from 3.5 to 5.8 hours and 50 to 77 hours for the two phases, respectively.

Freshour et al. (1980) studied the pharmacokinetic profile of acrylonitrile after intravenous or oral administration of acrylonitrile to male Fischer 344 rats. The plasma concentration of acrylonitrile versus time determined after 30 mg/kg intravenous administration was characteristic of a one-compartment model with first-order elimination, but a biphasic elimination was observed following a 30 mg/kg oral administration. The half-life of first-order elimination ranged from 7.8 to 13.9 minutes after 30 mg/kg intravenous and oral doses, and the half-life for terminal phase after 30 mg/kg oral administration was 85 to 120 minutes.

Tissue distribution of radioactivity (acrylonitrile and its metabolites) was determined in rats given a single oral or intravenous dose of ^{14}C-acrylonitrile at 0.1 and 10 mg/kg (Young et al. 1977). Acrylonitrile and its metabolites were distributed to all tissues examined (lung, kidney, liver,

TABLE 2. RECOVERY OF RADIOACTIVITY FROM RATS EXPOSED BY INHALATION TO 5 OR 100 ppm ^{14}C-ACRYLONITRILE FOR 6 HOURS[a] (Young et al. 1977)

	Percentage of Recovered Dose[b]	
	5 ppm	100 ppm
Urine	68.50 ± 9.38[c]	82.17 ± 4.21[c]
Feces	3.94 ± 0.97	3.15 ± 0.82
$^{14}CO_2$	6.07 ± 1.58[c]	2.60 ± 0.83[c]
Body	18.43 ± 4.68[c]	11.24 ± 2.85[c]
Cage wash	2.95 ± 3.95	0.85 ± 0.58
Total dose in ug equivalents	172.92 ± 28.35	2556.65 ± 672.10
Total recovery of radioactivity	95%	100%

[a]Rats were exposed in a "nose only" chamber under dynamic air flow conditions for 6 hours. After exposure, rats were housed in glass metabolism cages and excreta were collected for 220 hours.

[b]Values are the mean ± standard deviation for four rats per exposure level.

[c]The mean values for the two exposure levels are different at the P = 0.05 level of significance.

[d]Calculated mean value of 5 ppm corresponds to 0.7 mg/kg body weight; 100 ppm to 10.2 mg/kg body weight. The values were reported in the Ambient Water Quality Criteria Document (June 15, 1979).

stomach, skin, blood, and brain); notably high levels of radioactivity were observed in stomach, skin, and red blood cells regardless of route and dose level. Analysis of the stomach following intravenous equivalent administration showed that the radioactivity had increased from 30.33 ug equivalent of acrylonitrile at 5 minutes to 68.64 ug at 24 hours. The specific retention of acrylonitrile and its metabolites in the stomach seems in part due to enterogastric circulation (Young et al. 1977). The accumulation of radioactivity in the blood was mainly due to covalent binding of acrylonitrile to macromolecules and lipids in the red blood cells (Ahmed and Patel 1979).

In other tissues, the amount of radioactivity declined rapidly with time because of excretion (Young et al. 1977, Ahmed and Patel 1979).

METABOLISM

Gut et al. (1975) investigated the metabolism of acrylonitrile in Wistar rats, albino mice, and Chinese hamsters. The authors observed that the extent of conversion of acrylonitrile to cyanide was dependent on the route of administration, decreasing in the following order: oral (20%), intraperitoneal (i.p.) or subcutaneous (s.c.) (2 to 4%), and intravenous (i.v.) (1%). Thus, the more slowly acrylonitrile enters the system, the more extensively it is converted to cyanide. This suggests that conversion of acrylonitrile to cyanide involves metabolic processes competing with blood protein binding and nonenzymatic cyanoethylation. Pretreatment with phenobarbital did not significantly influence elimination of thiocyanate (SCN) in the urine after acrylonitrile administration; however, simultaneous administration of thiosulfate and acrylonitrile significantly increased the metabolized portion (SCN) of acrylonitrile given to rats by twofold and mice by threefold. Pretreatment with Aroclor 1254 was found to greatly enhance the toxicity of

acrylonitrile, and to cause a threefold increase in the cyanide level in the blood of treated rats. Gut et al. (1975) found acrylonitrile to be strongly bound in blood. Acrylonitrile was metabolized to SCN more effectively by mice than by rats followed by oral, intraperitoneal, and intravenous administration. Possible differences in the mechanism of acrylonitrile toxicity in rats and mice are indicated by the greater metabolism of acrylonitrile to SCN and the larger decrease in its acute toxicity by thiosulfate in mice compared to rats. Gut et al. (1975) concluded that cyanide may play a more important role in the toxicity of acrylonitrile in mice than it does in rats.

An in vitro study conducted by Boyland and Chasseaud (1967) implicates that acrylonitrile conjugates with glutathione (GSH) via GSH transferase enzyme. Although uptake of acrylonitrile gives rise to a slight increase in cyanomethemoglobin, combined therapy with nitrite and thiosulfate affords partial protection against its toxic action. Recently, the presence of cyanoethylated mercapturic acid in rat urine was confirmed (Ahmed 1980, personal communication; cited in Water Qualtity Criteria Document, U.S. Environmental Protection Agency 1980). These facts suggest that acrylonitrile toxicity is due in part to the acrylonitrile itself or other unknown metabolites rather than just the cyanide functional group. Czajkowska (1971) detected only traces of unchanged acrylonitrile in the urine of acrylonitrile-treated rats. This study, as well as the study conducted by Young et al. (1977), suggests that the major portion of the compound is altered in the body to other metabolites or conjugates as shown in the proposed pathways for acrylonitrile biotransformation in Figure 1. Cyanoethylation products of cell macromolecules and of circulating nucleophiles can be detected in tissue fractions and in biologic fluids. If the proposed pathway is correct, cyanoethylated glutathione conjugates should be

recoverable in bile and urine. One, in fact, has been found--cyanoethylated mercapturic acid. Oxidation by the mixed-function oxidases could lead to formation of an epoxide.

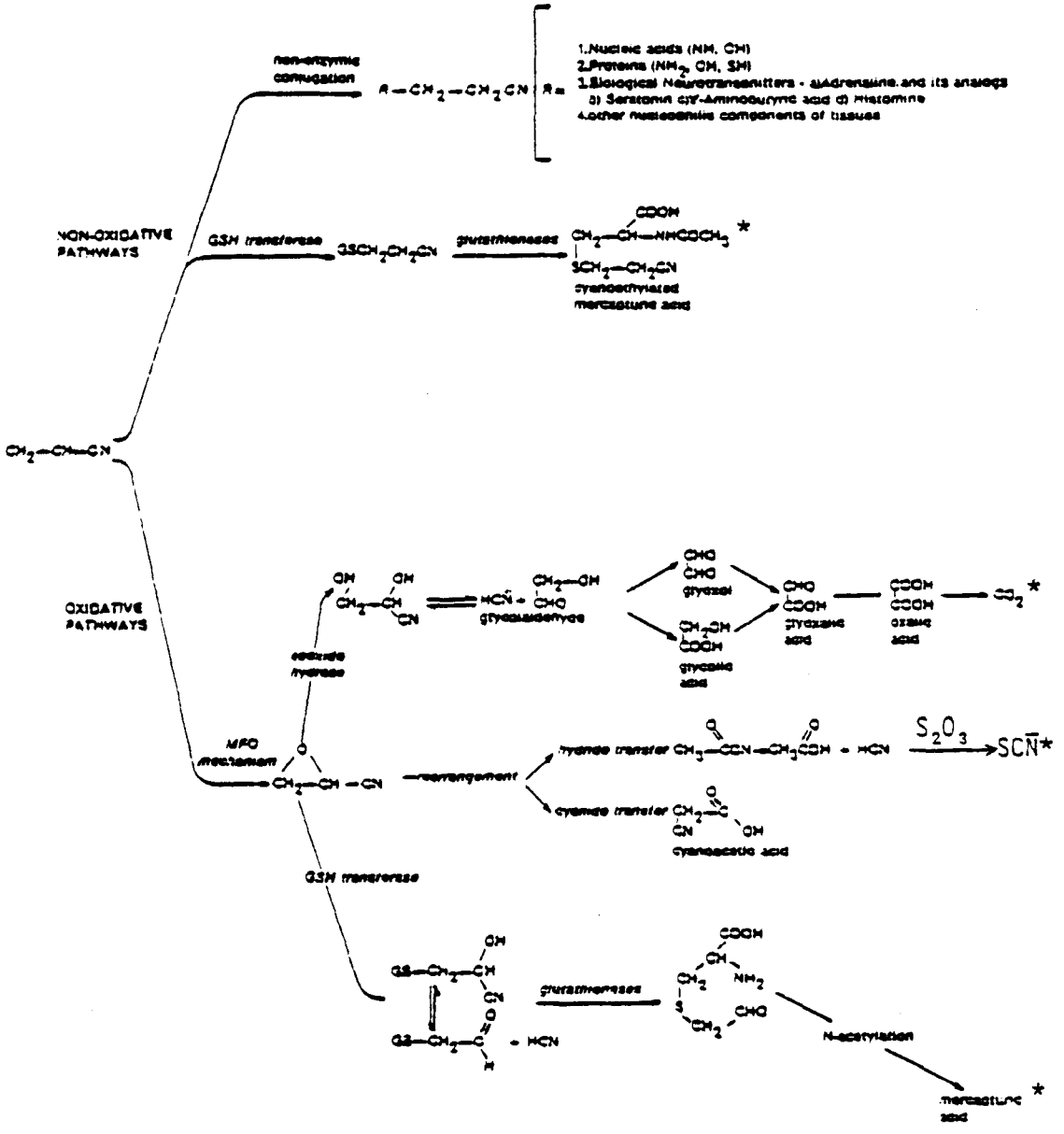
On February 13, 1981, the Monsanto Chemical Company submitted a summary of preliminary results from metabolic studies with acrylonitrile. Their conclusions are based on results which were not available to the Carcinogen Assessment Group (CAG) in detail; therefore, the CAG was unable to evaluate or assess the significance of these results. Only Monsanto's conclusions are stated here.

1. There was no detectable binding of acrylonitrile to rat DNA in studies with intact animals.
2. In these same studies, no evidence of alkylated adenine or guanine was observed.
3. detectable, low-level binding to DNA in rat liver cells but not rat brain cells was observed in vitro.
4. extensive binding to protein has been observed in all systems.

The CAG has communicated with Monsanto to obtain detailed information. The final assessment of this study will be made when the information becomes available to us.

In summary, acrylonitrile is readily absorbed both by oral and inhalation administration. It is metabolized ultimately to CO_2 and thiocyanate. The proposed metabolic pathway suggests the formation of an epoxide, and epoxides, as a group, are regarded as having carcinogenic potential.

Figure 1. Proposed pathways for acrylonitrile biotransformation. (U. S. Environmental Protection Agency 1980)



*These metabolites are tentatively identified in rat urine and radiolabeled CO_2 in the expired air (Langvardt et al. 1980).

IV. MUTAGENICITY* AND CELL TRANSFORMATION

Acrylonitrile has been evaluated in several different test systems for mutagenic activity (namely in bacteria, plants, Drosophila, mice, and rats). The results of the available studies are discussed below and each study is summarized in Table 3.

MUTAGENICITY STUDIES IN BACTERIA

Acrylonitrile has been shown to cause point mutations in bacteria. Venitt et al. (1977) evaluated the mutagenicity of acrylonitrile (purity > 99%, impurities not reported) in a plate incorporation assay with the tryptophan-dependent Escherichia coli strains WP2 (repair proficient), WP2 uvrA (lacks excision repair), WP2 uvrApolA (lacks excision repair and DNA polymerase I), and WP2 lexA (deficient in an error-prone pathway). In the absence of rat liver enzyme activation, acrylonitrile produced a weak dose-related mutagenic response in all strains except WP2 lexA when examined at concentrations of 0, 75, and 150 umoles/plate. It should be pointed out that acrylonitrile is volatile and in this test no precautions to prevent evaporation were reported. If evaporation occurred under the standard test conditions, the weak responses observed would not reflect the mutagenic potency of acrylonitrile. The responses reported were as follows: WP2 was slightly more sensitive to the mutagenic effect of acrylonitrile; a fourfold increase in the spontaneous level was produced by 150 umole compared with a threefold increase for WP2 urvA and a twofold increase for WP2 urvApolA. The authors reported that doses above 150 umole per plate caused a decline in the mutagenic response, which was explained by increasing toxicity (reduction in bacterial lawn observed).

*Prepared by the Reproductive Effects Assessment Group.

TABLE 3. MUTAGENICITY TESTS OF ACRYLONITRILE

A. BACTERIA

Reference	Test System	Strain	Activation System	Concentration	Result*	Comment
Connor et al. 1979	Body fluid analysis: bile from rats, Salmonella typhimurium as indicator organism; plate incorporation assay	TA 1535	In vivo male Sprague-Dawley rats	45 mg/kg acrylonitrile (Source: Aldrich Chemical Co.) in saline injected i.v. into femoral vein, 200 ul of bile tested/plate	Negative	1. One dose and one bacterial strain tested. 2. No information on toxicity of dose given to the animals. 3. Number of animals used not reported. 4. Number of revertants not reported. 5. Purity of chemical not given.
DeMeester et al. 1978	Salmonella test: plate test, fluctuation assay	TA 1530 TA 1535 TA 1537 TA 1538 TA 98 TA 100 TA 1950 his G46 TA 1978 TA 1532 TA 1975	S9 mix prepared from livers of styrene, Aroclor 1254, acrylonitrile, methyl-3 cholanthrene, phenobarbital -induced male rats (Wistar); Aroclor 1254- and phenobarbital -induced female mice (NMRI); uninduced male beagle dog	Tested in atmosphere at ± 0.2% for 1 hour, purity reported as 99% (impurities not described). Source: Aldrich Europe	Positive in the presence of metabolic activation in both frameshift -sensitive and base-pair substitution sensitive strains, with most pronounced effects on base-pair substitution sensitive strains. Negative in the absence of metabolic activation	1. No concurrent positive controls reported. 2. Single dose tested; thus, no dose-response curve. 3. Method to determine toxicity not described.

*Results described in text.

(continued on following page)

TABLE 3. (continued)

A. BACTERIA

Reference	Test System	Strain	Activation System	Concentration	Result*	Comment
Lambotte-Vandepaer et al. 1980	Body fluid analysis: urine from rats and mice, Salmonella typhimurium as indicator organism; plate incorporation assay	TA 1530	In vivo adult male Wistar rats and adult male NMRI mice; in vitro, S9 mix from Aroclor 1254-induced mice (strain not reported)	30 mg/kg (purity > 99%, impurities not described; Source: Aldrich Europe) in saline injected i.p.; urine collected 24 hours	Positive:	1. Single dose tested, thus no dose-response curve determined. 2. No concurrent positive control data reported.
McMahon et al. 1979	Reversion in Salmonella typhimurium and Escherichia coli; gradient plate test				Reported as positive for both Salmonella and Escherichia	1. Purity and source of chemical not given. 2. Authors did not indicate if mutagenic activity was microsome-related. 3. Concentration(s) where activity was observed not reported. 4. Bacteria strains, which were reverted, were not given. 5. Information on toxicity not reported.
Milvy and Wolff 1977	Salmonella test: spot assay, liquid suspension, plate test (in vapor phase)	TA 1535 TA 1538 TA 1978	Aroclor 1254-induced male Swiss-Webster mice/S9 mix	Purity reports as 99+%, (Source: Aldrich Chemical Co.) spot test: 5 and 10, ul, liquid suspension: 5, 10, 20 ul for 30 min. to 4 hrs at 2 to 300 ul	Liquid suspension and vapor: positive only in the presence of metabolic activation in both frameshift and base-pair substitution sensitive strains; negative results using spot test and testing in the absence of metabolic activation	1. No clear dose-response observed with vapor exposure. 2. Neither the actual revertant counts per plate nor the standard deviation per dose was given; thus the extent of variation is not known.

*Results described in text.

(continued on following page)

TABLE 3. (continued)

A. BACTERIA

Reference	Test System	Strain	Activation System	Concentration	Result*	Comment
Venitt et al. 1977	Escherichia coli test: plate incorporation, fluctuation assay	WP2 WP2 urvA WP2 urvApolA WP2 lexA WP2P (pKM 101)	Aroclor 1254-induced CB hooded male rats, S9 mix, 0.5 ml/plate	0, 75, 150 umole/plate for plate incorporation assay $4x10^{-4}$M to $2x10^{-3}$M for fluctuation assay. Dissolved in DMSO, (purity > 99% as determined by GLC; Source: Kochlight Lab)	Weak positive in the absence of metabolic activation for all strains except WP2 lexA	1. Dose-related response. 2. No concurrent positive control reported. 3. Data in the presence of metabolic activation not reported. 4. No precautions to prevent evaporation were reported.
	Salmonella test: plate incorporation, fluctuation assay	TA 100 TA 1538 TA 98 TA 1535 his G46 his D3052			Reported as negative	1. Data were not presented. 2. Protocol was not described.

*Results described in text.

(continued on following page)

Table 3. (continued)

B. DROSOPHILA

Reference	Test System	Dose	Result: Brood I (postmeiotic)	Result: Brood II (meiotic and premeiotic)	Total	Comment
Benes and Sram 1969	Sex-linked recessive lethal test in Drosophila melanogaster	Injection of a 0.1% solution into abdomen	572/2 (0.35%)	725/4 (0.55%)	0.46%	1. Toxicity not given. 2. Sufficient sample size not analyzed. 3. Purity and source of chemical was not given. 4. No concurrent positive controls and information concerning negative controls not given; appears to be historical. 5. Post-mating times not given.
		spontaneous frequency	41,921/58 (0.14%)			

(continued on following page)

TABLE 3. (continued)

C. MAMMALIAN IN VIVO CYTOGENETICS

Reference	Test System	Strai		Concentration	Result	Comment
Leonard et al. 1981	Bone marrow assay (micronuclei and chromosome aberration analysis)	NMRI	le mice	Mice give single i.p. injection of 20 or 30 mg/kg and cells examined 6, 18, 24, 48, and 72 hours for chromosome analysis and 24, 30, and 48 hours for micronucleus analysis.	Negative	1. Aberrations were scored as gaps, breaks, and rearrangements.
	Dominant lethal assay	NMRI	le mice	Mice given i.p. treatment of 30 mg/kg followed by weekly matings (1 to 5 weeks). Source: Aldrich Europe (purity not given)	Negative	1. Background control frequency was very high (33.6% postimplantation loss).
Rabello-Gay and Ahmed 1980	Bone marrow assay	Albin mice, Dawle	Swiss prague-rats	Mice given by gavage 4, 15, 30 days at 7, 14, and 20 mg/kg/day; rats exposed orally to 16 daily doses of 40 mg/kg/day (purity 99.5%). Source: Aldrich Chem. Co.	Negative	1. Aberrations were scored as gaps, isogaps, breaks, fragments, and translocations.
Theiss and Fleig 1978	Examination of lymphocytes from workers with known exposure to acrylonitrile			Atmospheric monitoring data indicated a typical exposure level of 5 ppm between 1963 and 1974, and a level of 1.5 ppm between 1975 and 1977	Negative	1. Cohort was exposed to acrylonitrile for an average of 15.3 years. 2. Aberrations scored included gaps and isogaps; other aberrations scored not defined in the report.

(continued on following page)

TABLE 3. (continued)

D. PRIMARY DNA DAMAGE TESTS

Reference	Test System	Strain	Activation System	Concentration	Result	Comment
Parent and Casto 1979	Detection of single strand DNA breaks by alkaline sucrose gradient	Primary Syrian Golden hamster embryo cells *in vitro*	None	Cells incubated for 18 hours at 50, 100, 200, and 400 ug of acrylonitrile/ml (> 99% pure; Source: ATdrich Chem Co.), and contains 30-45 ppm 1-hydroxy-4-methoxybenzene 0.3% water, < 0.5% acetonitrile.	DNA breakage 200 and 400 u ml but not at 5(r 100 ug/ml.	1. Toxicity data not given; thus, it is unclear whether the results are due to nonspecific toxicity.

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Because of the toxic effects and weak mutagenicity of acrylonitrile in the plate test, Venitt et al. (1977) conducted a fluctuation test (which is a sensitive assay for detecting low levels of mutagens) at a concentration range of 4×10^{-4} M to 2×10^{-3} M. The results of the fluctuation tests confirmed the mutagenicity detected in WP2, WP2 uvrA (results based on one experiment and data not presented), and WP2 urvApolA by the plate assay. In contrast to the results derived from the plate tests, WP2 uvrApolA was more sensitive to the mutagenic effects of acrylonitrile than WP2; the responses of WP2 and WP2 uvrA were reported as similar. Acrylonitrile again tested negative in WP2 lexA. The lack of a mutagenic effect in WP2 lexA suggests that acrylonitrile may produce mutations by mis-repair DNA damage, which is believed to be associated by the generation of DNA strand breaks (Green and Muriel 1976). In addition, the reported observation that WP2 uvrA was not more mutable than WP2 suggests that the induced DNA damage is not subject to excision repair. When the resistance factor pKM 101 was transferred to WP2 (strain designated WP2P) to increase the sensitivity of this strain, WP2P (pKM 101) was reported to be more sensitive to the mutagenic effects of acrylonitrile than WP2. A dose-related increase in mutagenic activity was observed at a concentration range as low as 4 to 40 umoles per plate in the plate test. These results further support the notion that acrylonitrile is producing mutations via mis-repair DNA damage because Salmonella strains containing the pKM 101 plasmid have been shown to be more sensitive to the mutagenic effects of chemical mutagens which are error-prone DNA repair-dependent (McCann et al. 1975). Metabolic activation by an exogenous S-9 system did not enhance the mutagenic activity of acrylonitrile (data not presented); thus, acrylonitrile, according to the authors, is primarily detected as a direct-acting mutagen in these Escherichia coli strains.

Also, Venitt et al. (1977) reported that acrylonitrile was not detected as mutagenic for Salmonella typhimurium strains his G46, his D3052, TA 1535, TA 100, TA 1538, and TA 98 using either plate incorporation assays or fluctuation tests. Although mutagenic activity was reported to be detected in strain his G46 using a fluctuation test, the results were reported as "erratic and statistically nonsignificant." The authors did not present the protocol; thus it is not known if mammalian liver activation was employed in the Salmonella assays or if precautions to prevent evaporation were taken. In addition, they did not present data to support their negative conclusions for Salmonella.

McMahon et al. (1979) reported that acrylonitrile (purity not reported) was mutagenic in both Escherichia coli and Salmonella typhimurium strains when screened in a qualitative gradient plate assay, but they do not indicate if acrylonitrile required metabolic activation for activity, the concentration(s) at which activity was observed, and which bacterial strains were reverted.

Milvey and Wolff (1977) used three methods of exposure to examine the mutagenicity of acrylonitrile (99% purity, impurities not reported) in the Salmonella/microsome assay. In a spot test with liver enzyme activation, strain TA 1535 (base-pair substitution-sensitive) was not detectably reverted by 5 or 10 ul of acrylonitrile. However, because the authors indicated there was no zone of growth inhibition, in this test the chemical exposure to the indicator organism may not have been adequate. When 20 ul (volume of reaction mix not reported) of acrylonitrile was preincubated at 37°C with TA 1535, the authors reported approximately a threefold increase in the spontaneous level of revertants. The authors noted "large statistical fluctuations" with the test results, but give no indication of the extent of variability by reporting the actual revertant counts for each plate or the standard deviation of the means. This variation in plate counts could be explained by acrylonitrile's

volatility at 37°C. When precautions were reported to have been taken to prevent excessive evaporation of acrylonitrile, the authors reported that the lowest acrylonitrile exposure, where mutagenic activity (1.9-fold increase over spontaneous level, standard deviation of results not given) was detected in TA 1535, was 2 ul in a commercial thin layer chromatographic desiccant chamber for 4 hours. The authors estimated this exposure to be 57 ppm. Acrylonitrile was also reported as weakly active on the frameshift-sensitive strain TA 1538 (tested as vapor at 200 ul for 2 hours). For these data, no indication of the variability was provided by reporting the actual revertant counts for each plate or the standard deviation of the mean. The authors emphasized that the mutagenic effects of the acrylonitrile in Salmonella were observed only in the presence of metabolic activation (S-9 prepared from livers of Aroclor 1254-induced male Swiss-Webster mice). (Data were not shown in the absence of S-9 activation.) In general, the main deficiency of this report is the lack of available data to conduct a statistical analysis for determination of the significance of the results. In addition, Venitt (1978) pointed out errors of calculation found in this report. Although there are deficiencies in the report by Milvey and Wolff (1977), their data suggest a weak mutagenic effect in Salmonella. However, the studies by DeMeester et al. (1978) provide more convincing evidence for the mutagenicity of acrylonitrile in Salmonella typhimurium.

Unlike the results for Escherichia coli, DeMeester et al. (1978) reported that the mutagenicity of acrylonitrile in Salmonella was detected only in the presence of an in vitro liver microsome-activation system. This difference is not necessarily contradictory because these are two different bacterial test systems, which may have different sensitivities. DeMeester et al. (1978) injected 0.15 liters of gaseous acrylonitrile (99% purity, impurities not

reported) into a desiccator and exposed the cells for one hour. Using a gas chromatograph, they measured the concentration of acrylonitrile to be about 0.2% in the atmosphere. The concentration of acrylonitrile on the test plates was also measured by freezing the plate agar and analyzing the concentration of acrylonitrile by gas chromatography. This was found to be about 200 ug/plate. Toxicity of 0.15 l of gaseous acrylonitrile for one hour was reported as weak (cell survival 80% to 100%), and the toxicity was reported to be high when bacteria was exposed to 0.15 l of gaseous acrylonitrile for 2 hours or to 0.24 l for one hour (method to measure toxicity not described). Reversion to histidine prototrophy was reported for the base-pair substitution-sensitive strains TA 1538, TA 1535, TA 1530, TA 100, TA 1950, and for the frameshift-sensitive strains TA 1978, TA 98, in the presence of microsomal activation (Arcolor 1254-induced rats, 300 ul S-9/ml mix). Acrylonitrile had the most pronounced effect on the base-pair substitution-sensitive strains TA 1535, TA 1950, with TA 1530 exhibiting the highest number of revertants over the spontaneous number (approximately 6- to 9-fold increases in the spontaneous level of revertants). This would be consistent with the properties of an alkylating agent. The frameshift-sensitive strains TA 98 and TA 1978 and the base-pair substitution-sensitive strain TA 100 were only weakly reverted (approximately a twofold or less increase over spontaneous level). Negative results were found with the strains TA 1975, TA 1532, TA 1537 and his G46. A fluctuation test confirmed the sensitivity of TA 1530 to the mutagenic effects of acrylonitrile at a concentration as low as 2.5 ug/ml ($P < 0.001$). Negative results were reported in all strains when acrylonitrile was tested in a "classical" plate incorporation assay or tested in liquid medium (data not presented in report).

DeMeester et al. (1978) also reported that certain procedures of induction of liver enzymes applied to animals influence the mutagenic activity of acrylonitrile. Using six tester strains of Salmonella (TA 1530, TA 1535, TA 1950, TA 100, TA 1978, TA 98), it was observed that liver enzymes (S-9 mix) from Aroclor 1254- or 3-methyl-cholanthrene-induced rats were most efficient for detecting the mutagenicity of acrylonitrile, whereas uninduced, acrylonitrile-induced, and phenobarbital-induced rat liver enzymes were found to have a lower effect. Two other vinylic compounds, butadiene and styrene, were also able to enhance the mutagenicity of acrylonitrile, particularly towards strains TA 1530 and TA 1950. Acrylonitrile was also detected as mutagenic in the presence of S-9 mix prepared from phenobarbital- or Aroclor 1254-induced mice or uninduced beagle dogs. In all experiments, negative results were reported without S-9 mix or S-9 homogenate minus cofactors (data not shown). The results of various S-9 activation systems is of practical significance in assaying the mutagenicity of acrylonitrile in vitro; how these data relate to the in vivo situation is uncertain.

In addition to acrylonitrile being activated by in vitro S-9 systems, Lambotte-Vandepaer et al. (1980) examined the ability of the whole mammal to metabolize acrylonitrile. These authors reported that urine (0.1 ml per plate) from both rats (adult male Wistar) and mice (NMRI) treated with a single intraperitoneal dose of acrylonitrile (30 mg/kg; purity 99%, impurities not reported) was mutagenic in Salmonella strain TA 1530 (9-fold increase in the spontaneous level of revertants in urine for mice and 13-fold increase for rats). S-9 mix (from Arcolor-1254 treated mice) added to the test plates caused a fivefold reduction in the mutagenicity of urine from acrylonitrile-treated rats. Only a slight reduction (1.3-fold) was seen with the urine of acrylonitrile-treated mice. When animals were pretreated with both

phenobarbital and acrylonitrile, the urine from treated rats produced no detectable mutagenicity and the mutagenicity of urine from mice was reduced. The addition of β-glucuronidase to the test plates only slightly enhanced (approximately 1.4-fold) the mutagenic effects of urine collected from acrylonitrile-treated rats and mice (β-glucuronidase is added to cleave possible conjugates). β-glucuronidase had a marked effect on the mutagenicity of urine from rats treated with phenobarbital and acrylonitrile (an eightfold increase in mutagenic activity observed). The authors indicated that XAD-2 resin concentrates of urine from all treatment groups were not detected as mutagenic (data not presented), suggesting that the mutagenic metabolite(s) was very hydrophilic. Therefore, the urine from acrylonitrile-exposed rats and mice was mutagenic and glucuronoconjugation appears to play a minor role in the deactivation of the acrylonitrile derivative(s) (except in the case of phenobarbital acrylonitrile-treated rats, where a greater effect was found).

Conner et al. (1979) also examined whole mammal activation of acrylonitrile. These authors tested the mutagenicity of bile obtained from rats (male Sprague-Dawley) treated with acrylonitrile in Salmonella. Rats were given a single i.p. injection of acrylonitrile (45 mg/kg); bile was collected for 6 hours at one-hour intervals and tested at 200 ul/plate in a plate assay with β-glucuronidase using TA 1535 (base-pair substitution-sensitive). Negative results were reported. However, data were not presented to support this conclusion. Also, it should be noted that known mutagens (dimethylnitrosamine, 3-methyl-cholanthrene), which require metabolic activation, and the direct-acting mutagen methyl methanesulfonate tested as negative in this study. The results of this study do not reduce the weight of the positive results reported in the other bacterial studies.

MUTAGENICITY STUDIES IN DROSOPHILA MELANOGASTER

Although acrylonitrile has been shown to cause point mutations in bacteria, its ability to act at the level of the gene in a eucaryotic organism has not been sufficiently examined. Only one eucaryotic gene mutation study by Benes and Sram (1969) was available. These authors examined the occurrence of sex-linked recessive lethal mutations in Drosophila melanogaster following injection of 0.2 ul of a solution of acrylonitrile (0.1% concentration) into the abdomen of male flies. Although the authors reported negative results, the data are considered suggestive of an increase in the mutation frequency over that of the spontaneous frequency by a factor of three. The compound, however, has not been adequately tested in this system and re-testing is necessary to determine whether or not acrylonitrile is mutagenic in Drosophila. Several deficiencies in the report were found: 1) only a small sample of flies were tested, 2) no information on whether clustering of mutations occurred, 3) the purity of the test material was not described, 4) post-mating days not given, and 5) no information was reported for concurrent positive or historical negative controls.

OTHER STUDIES RELATED TO THE MUTAGENICITY OF ACRYLONITRILE

Other tests have been conducted which do not measure mutation per se but may indicate a potential of acrylonitrile to cause mutations. Chemical-adduct formation in DNA is a critical event in mutagenesis. When a mutagenic agent reacts with DNA, it may ultimately cause single strand breaks.

Parent and Casto (1979) reported that acrylonitrile caused single strand DNA breaks in primary Syrian golden hamster embryo cells in vitro as detected by alkaline sucrose gradient sedimentation. When cells were treated for 18 hours at 200 or 400 ug of acrylonitrile/ml, a shift in sedimentation patterns

was observed. This did not occur at 50 ug/ml or 100 ug/ml. The authors stated that these shifts are comparable to those produced by known carcinogens and are not seen with non-carcinogenic chemicals. They concluded that the results are suggestive of carcinogenicity. However, because the toxicity of the concentrations tested was not given, it is uncertain if the DNA damage observed at 200 or 400 ug/ml is simply a reflection of nonspecific toxicity. In addition, these data were generated in the absence of an exogenous metabolic activation system. These authors also conducted cell transformation studies which will be discussed later on page 31.

Acrylonitrile has been shown to cyanoethylate ring nitrogen atoms of certain minor tRNA nucleosides, and, at a slower rate, ribothymidine and thymidine (Ofengand 1967, 1971). Guengerich et al. (1981) provided evidence that acrylonitrile can not only alkylate RNA but also DNA. The extent of binding to DNA was found to be less than with RNA. These authors studied the in vitro metabolism of acrylonitrile to 2-cyanoethylene oxide. These authors found that there was a high level of binding of labeled ^{14}C-acrylonitrile to rat liver microsomal protein (3.8 $\pm$ 0.5 nmole/mg/hr) and a much lower level of binding to calf thymus DNA (0.004 $\pm$ 0.002 nmole/mg/hr) in the absence of NADPH. This low level binding is difficult to intrepret because there were no standards reported to determine the detection limit of their DNA binding. In the presence of reported rat liver microsomes and NADPH, the binding to DNA was enhanced (0.207 $\pm$ 0.017 mmole/mg/hr), but brain microsomes and NADPH did not increase the low level of binding to DNA (0.004 $\pm$ 0.001 nmole/mg/hr). It is not clear if rat brain microsomes do not effectively metabolize acrylonitrile in vitro, because the authors did not include positive controls to ensure that the rat brain microsome preparation was functional. A slight increase was seen in binding to DNA when liver microsomes were derived from

rats pretreated with inducers of cytochrome P-450 (phenobarbital and β-naphthoflavone). Human liver microsomes derived from six autopsy samples did not enhance the binding of the acrylonitrile metabolite(s) to DNA. There were no adequate controls, however, to determine their cytochrome P-450 activity.

Glutathione S-transferase played a role in the deactivation of acrylonitrile *in vitro*. A rat liver cytosol preparation conjugated 2-cyanoethylene oxide at a greater rate than it conjugated acrylonitrile. Human liver and rat brain cytosol preparations reacted with 2-cyanoethylene oxide at a much lower rate than rat liver, and not at a detectable rate with acrylonitrile. Therefore, *in vitro*, rat brain and human liver cytosol preparations conjugated acrylonitrile's metabolite 2-cyanoethylene oxide at a lower rate than rat liver. 2-Cyanoethylene oxide appears to have a long half-life (2 hours from these studies). Thus, if it reached the brain via blood circulation, it may not be effectively inactivated. Because these are *in vitro* studies, whole mammal tests are needed to elucidate the *in vivo* events. However, these studies do demonstrate that an exogenous liver activation system enhances the amount of acyronitrile binding to DNA *in vitro*. Thus, these results are consistent with the positive mutagenicity studies in bacteria.

CHROMOSOMAL ABERRATION STUDIES

The ability of acrylonitrile to act at the level of the chromosome *in vivo* has also been investigated. Acrylonitrile was not detected as clastogenic in two independent bone marrow studies (Rabello-Gay and Ahmed 1980, Leonard et al. 1981). Rabello-Gay and Ahmed (1980) tested acrylonitrile (purity reported as 99.5%, impurities not identified) for chromosomal effects in mice (albino

Swiss) and rats (Sprague-Dawley). No increase in the incidence of chromosome aberrations (gaps, breaks, fragments, Robertsonian translation) were found in mouse bone marrow cells when male mice were given acrylonitrile by gavage for 4, 15, and 30 days at doses of 7, 14, and 21 mg/kg/day. (In a second experiment mice received 10, 15, and 20 mg/kg/day.) The toxicity of 7, 14, and 20 mg/kg/day given orally was reported to correspond to 0.25, 0.50, and 0.75 of the LD_{50}, respectively. Mortality for the acrylontrile-treated animals was reported (3 of the 72 animals died). The chemical also was found to be negative when rats were exposed orally to 16 daily doses of 40 mg/kg/day (reported to represent one-half of the LD_{50}).

Leonard et al. (1981) also evaluated the clastogenicity of acrylonitrile (purity not given) in mouse bone marrow cells in vivo. These authors conducted both chromosome aberration and micronuclei analyses. The percentage of chromosome aberrations or micronuclei formation did not differ between acrylonitrile-treated animals and negative control animals. Male mice (NMRI) were injected intraperitoneally with a single acute dose of 20 or 30 mg acrylonitrile/kg of body weight.* For chromosome aberration analysis (gaps, breaks, fragments, rearrangements), 200 cells (4 animals and 50 cells/animal) were examined 6, 18, 24, 48, and 72 hours after each treatment. The background control frequency was 0.5% cells with chromosome anomalies (1 gap/200 cells) and the acrylonitrile-treated cells did not exceed 1.5% cells with chromosome anomalies (3 gaps/200 cells). For micronuclei analysis, polychromatic erythroblasts were sampled 24, 48, or 72 hours after initial treatment. The background frequency was 1.8% cells with micronuclei and acrylonitrile-treated cells did not exceed 2.5% with micronuclei. No dose-related effects were seen in these studies.

*The authors indicated that 30 mg/kg was the maximum dose, which allows survival of mice for several weeks.

Thiess and Fleig (1978) examined the clastogenic effects of lymphocytes of 18 workers who had been exposed to acrylonitrile. The workers had been exposed for an average of 15.3 years, and were compared with 18 age-matched workers who had no known exposure to acrylonitrile or any other compounds that were suspected of causing chromosomal damage. Although no information is available concerning the exact levels of exposure in the past, atmospheric monitoring data betweeen 1963 and 1974 indicated a typical exposure level of 5 ppm for acrylonitrile, and atmospheric monitoring data between 1975 and 1977 indicated an average exposure level of 1.5 ppm. However, individual workers may have been exposed to higher concentrations during specific operations. The workers could have been exposed on the job to styrene, butadiene, ethylbenzene, butylacrylate, and diphyl (a mixture of diphenyl and diphenyl oxide ether). For each subject, 100 metaphases were examined. The type of chromosome aberrations was not indicated in the report except for gaps and isogaps. Chromosome aberrations of the exposed group were 1.8 $\pm$ 1.3% (excluding gaps) and 5.5 $\pm$ 2.5% (including gaps and isogaps), while chromosome aberrations in the control group were 2.0 $\pm$ 1.6% (excluding gaps) and 5.1 $\pm$ 2.4% (including gaps and isogaps). Therefore, no apparent chromosomal damage was detected in these workers as a result of their exposure to acrylonitrile or to the other compounds present in their work environment.

Loveless (1951) and Kihlman (1961) reported negative findings for chromosome effects in plants (*Vicia faba*). However, data were not reported in these articles and details of the protocol were not presented. Thus, the authors' conclusions cannot be evaluated.

SUMMARY

In summary, there is evidence that acrylonitrile caused point mutations in

bacteria (Salmonella and Escherichia). The covalent binding of acrylonitrile to calf thymus DNA in vitro is enhanced when exogenous liver microsomal activation is added. This result would be consistent with the Salmonella mutagenicity studies. Acrylonitrile was not detected as clastogenic in bone marrow cells of rats and mice. Nevertheless, because acrylonitrile causes point mutations in bacteria and binds DNA in vitro, it may cause gene mutations in other organisms. If the metabolism and pharmacokinetics of this chemical substance in humans results in metabolic products that can interact with DNA, as is the case for the test systems employed in the reported studies, it may cause somatic mutations in humans as well. Further testing, however, is needed in eukaryotic organisms to confirm the mutagenic activity observed in bacteria. For example, it would be appropriate to conduct a gene mutation test using mammalian cells in culture and measure alkylation of macromolecules such as DNA in whole mammals.

CELL TRANSFORMATION

Parent and Casto (1979) reported the effect of acrylonitrile on Syrian golden hamster embryo cells (HEC), and found that acrylonitrile (ACN) transforms cells in culture and enhances the transformation of cells previously affected with simian adenovirus SA7, a colony transforming oncogenic virus.

ACN from Aldrich Chemical Co. (Milwaukee, Wis.) was used in this experiment. The purity was stated to be greater than or equal to 99%. The impurities include about 0.3% water, less than 0.5% acetonitrile, and 30 to 45 ppm 1-hydroxy-4-methoxybenzene. In this experiment cultures of primary Syrian golden HEC were prepared by trypsinization of decapitated and eviscerated embryos after 13-14 days of gestation. Cells were plated into 60-mm-diameter

Lux plastic dishes at a density of 5 x 10^6 cells/dish with modified Dulbecco's medium and 10% fetal bovine serum (Reheis Chemical Co., Kankakee, Ill.) and incubated at 37°C for 3 days in 5% CO_2. ACN was dissolved in 100 mg acetone/ml and diluted in complete medium to give the final concentrations.

In this viral transformation enhancement assay, HEC were exposed to ACN in concentrations of 0, 25, 50, 100, and 200 ug/ml. Treatment of HEC with ACN for 18 hours before SA7 inoculation resulted in only slight but significant enhancement to 1.8-fold (Table 4). When cells were treated with ACN 5 hours after they were inoculated with virus (Table 4), a significant enhancement of 8.9- and 8.4-fold was observed at 200 and 100 ug ACN/ml, respectively. Treatment with 200 ug ACN/ml reduced the cloning efficiency to less than 10%, but the number of SA7 foci only decreased from 41 in control to 26 in treated cells. The increased enhancement found when cells were chemically treated after virus inoculation was observed with several other chemicals.

When HEC were treated for 6 days with ACN (chemical transformation) without added virus, foci of morphologically transformed cells were observed that were similar to those described previously with known chemical carcinogens. At 100 ug ACN/ml, three foci were observed on nine dishes and two foci on six dishes at 50 ug ACN/ml; BP treatment resulted in three foci on four dishes at 1.25 ug/ml and two foci on ten dishes at 0.62 ug/ml (Table 5). No foci were observed on medium or solvent control dishes.

The observation that acrylonitrile transforms cells adds support to the animal and human evidence that acrylonitrile may be carcinogenic.

TABLE 4. ENHANCEMENT OF SA7 TRANSFORMATION BY TREATMENT OF HEC WITH ACN[a] (Parent and Casto 1979)

Time of ACN treatment	ACN ug/ml	Surviving fraction[b]	SA7 foci[c]	Enhancement ratio[d]
18 hr before SA7	200	0.08	1	0.3
	100	0.34	32	1.8
	50	0.62	41	1.3
	25	0.75	45	1.2
	0	1.00	50	1.0
18 hr before SA7	200	0.18	6	1.6
	100	0.46	19	2.1
	50	1.07	31	1.5
	25	1.02	37	1.8
	0	1.00	20	1.0
5 hr after SA7	200	0.07	26	8.9
	100	0.21	75	8.4
	50	0.60	37	1.5
	25	0.69	41	1.4
	0	1.00	41	1.0

[a]Chemical dilutions were added to mass cultures of HEC 18 hr before or 5 hr after treatment with SA7. Virus was absorbed 3 hr, and the cells were transferred for survival (500-700 cells/dish) and for transformation assays (200,000-300,000 cells/dish).

[b]Determined from plates receiving 500-700 cells. Number of colonies from virus-treated and chemically-treated cells was divided by the number of colonies from virus-inoculated control cells to give the surviving fraction. Cloning efficiency of control cells was 10-15%.

[c]Number of foci from 10^6 plated cells.

[d]Enhancement ratio was determined by dividing the TF of untreated cells (TF = SA7 foci x reciprocal of the surviving fraction) by that obtained from control cells. Underlined values are statistically significant at the 5% level.

TABLE 5. TRANSFORMATION OF HEC BY ACN
(Parent and Casto 1979)

Treatment[a]	ACN ug/ml	Surviving fraction[b]	Foci/dishes
ACN	100	0.06	3/9
	50	0.76	2/6
	25	0.84	0/6
	12	1.06	0/5
BP	1.25	0.78	3/4
	0.62	0.94	2/10
Control	----	1.00	0/7

[a]Chemicals were added to tertiary HEC plated 24 hr earlier with 50,000 (transformation) or 1,000 (survival) cells/dish. Fresh medium with chemical was added after 3 days and removed after 6 days. Colonies were fixed and stained at 9 days for survival assays; focus assays for transformation were done 25 days after treatment was indicated.

[b]Determined from dishes receiving 1,000 cells. Number of colonies from treated plates was divided by the number from control dishes.

V. TOXICITY

Dudley and Neal (1942) reported that a 4-hour exposure by inhalation to 635 ppm acrylonitrile was fatal to rats, while a 4-hour exposure at a lower level, 100 ppm, was fatal to dogs. Subsequent animal experiments have shown that acrylonitrile is acutely toxic by all routes of administration including inhalation, oral, subcutaneous, and cutaneous exposure. The toxic effect levels for different species are presented in Table 6. These levels are varied between species (Wilson and McCormick 1949). Mice are most sensitive to acrylonitrile and suffer a severe decrease in body weight with a slight change in blood pressure (Hashimoto 1962). Benes and Cerna (1959) observed that rats have higher resistance to acrylonitrile exposure; they developed delayed symptoms and high levels of thiocyanate in urine and blood. Dudley et al. (1942) reported that inhalation exposure of rats to 56 ppm x 4 hours, 5 days/week, for 8 weeks, resulted in irritation of the respiratory mucous membrane with hyperemia, lung edema, alveolar thickening, and hemosiderosis of the spleen. Central nervous system disorders were also observed.

TABLE 6. TOXIC LEVELS OF ACRYLONITRILE FOR DIFFERENT SPECIES
(Registry of Toxic Effects of Chemical Substances 1977)

Species	Route	Effect	Dose
Man	Inhalation[a]	TDLo[b]	16 ppm/20 min
Rat	Oral	LD_{50}[c]	82 mg/kg
	Inhalation	LCLo[d]	500 ppm/4 hr
	Subcutaneous	LD_{50}	96 mg/kg
Mouse	Oral	LD_{50}	27 mg/kg
	Inhalation[a]	LCLo	784 ppm/hr
	Intraperitoneal	LDLo[e]	10 mg/kg
Dog	Inhalation	LCLo	110 ppm/4 hr
Cat	Inhalation	LCLo	600 ppm/4 hr
Rabbit	Oral	LD_{50}	93 mg/kg
	Inhalation	LCLo	258 ppm/4 hr
	Skin	LD_{50}	280 mg/kg
Guinea Pig	Oral	LD_{50}	50 mg/kg
	Inhalation	LD_{50}	576 ppm/4 hr
	Skin[a]	LD_{50}	250 mg/kg

[a]Taken from the Registry of Toxic Effects of Chemical Substances, 1975 edition.

[b]TDLo = Lowest published toxic concentration.

[c]LD_{50} = Dose fatal to 50% of the test animals.

[d]LCLo = Lowest published lethal concentration.

[e]LDLo = Lowest published lethal dose.

VI. CARCINOGENICITY

ANIMAL DATA

Seven studies in which acrylonitrile was administered to rats will be discussed. In four studies (three cancer bioassays and one three-generation reproductive study), the route of administration was via drinking water, one study was via gavage, and two (cancer bioassays) were via inhalation.

Drinking Water Studies

Dow Chemical Company (Quast et al. 1980a)--

Quast et al. (1980a) of the Dow Chemical Company performed a 2-year chronic study under the auspices of the Chemical Manufacturers Association in which acrylonitrile was incorporated in the drinking water of rats. In this study 6- to 8-week-old male and female Sprague-Dawley rats (48 animals of each sex at each exposure level and 80 animals of each sex in the control group) were used. For the first 21 days, the concentrations of acrylonitrile given were 35, 85, and 210 ppm, however, the two higher concentrations were subsequently raised to 100 and 300 ppm. Acrylonitrile used in this study was produced by E.I. du Pont de Nemours and Company, Inc. Its purity was greater than 99%. After 9 months of treatment, the animals at the two higher doses showed signs of toxicity as indicated by decreased self-grooming and an unthrifty appearance. There was also a dose-related decrease in food consumption (except in male rats at the lowest exposure level), and concomitant dose-related decrease in water consumption. Using water consumption data and the weight of the rats, the calculated mean amount of acrylonitrile ingested for males was 3.42, 8.53, and 21.18 mg/kg/day, and for females, 4.36, 10.76, and 24.97 mg/kg/day for the 35, 100, and 300 ppm exposure levels, respectively.

Cumulative mortality data for male and female rats at all exposure levels are presented in Tables 7 and 8. The results indicate that early mortality was observed at all dose levels in females.

On death or at necropsy at 24 months, histopathologic examination was performed on complete sets of tissues from the control and high dose animals and on selected target tissues and tissues with grossly recognized tumorous changes from the other exposure groups.

Statistically significant increases in tumor incidence at multiple sites in male and female rats exposed to acrylonitrile were observed as described in Tables 9 and 10. Increases in the incidence of specific tumor types with respect to individual treatment groups are summarized as follows: central nervous system tumors in males and females in all treatment groups; Zymbal gland tumors in females in all treatment groups and in males in the high dose group; tumors in the nonglandular portion of the stomach in males in all treatment groups and in females in the mid and high dose groups; tongue tumors in males in all treatment groups and in females in the mid and high dose groups; mammary gland tumors in females in the low and mid dose groups; tumors in the small intestine in females in the mid and high dose groups.

The central nervous system tumors included a statistically significant increase in the incidence of astrocytomas. There was also a statistically significant increase in the incidence of glial cell proliferations, suggestive of early tumors, which were observed most frequently in the cerebral cortex. Zymbal gland tumors were observed in the ear canal, and these tumors were usually ulcerated and, in some animals, caused displacement of the lower jaw that resulted in the inhibition of food consumption. In the nonglandular portion of the stomach, both papillomas and carcinomas were found with a

TABLE 7. CUMULATIVE MORTALITY DATA OF MALE RATS MAINTAINED FOR 2 YEARS ON DRINKING WATER CONTAINING ACRYLONITRILE
(Quast et al. 1980a)

Days on test	Control No. dead (% dead)	35 ppm No. dead (% dead)	100 ppm No. dead (% dead)	300 ppm No. dead (% dead)
0-30	0	0	0	0
31-60	0	0	0	0
61-90	0	0	0	0
91-120	0	0	1(2.1)	0
121-150	0	0	1(2.1)	0
151-180	1(1.3)	0	1(2.1)	0
181-210	1(1.3)	1(2.1)	1(2.1)	0
211-240	1(1.3)	1(2.1)	1(2.1)	0
241-270	2(2.5)	1(2.1)	1(2.1)	2(4.2)
271-300	3(3.8)	1(2.1)	1(2.1)	2(4.2)
301-330	6(7.5)	2(4.3)	1(2.1)	2(4.2)
331-360	7(8.8)	2(4.3)	1(2.1)	4(8.3)
361-390	7(8.8)	3(6.4)	1(2.1)	7(14.6)
391-420	8(10.0)	5(10.6)	2(4.2)	8(16.7)
421-450	11(13.8)	7(14.9)	5(10.4)	11(22.9)
451-480	15(18.8)	8(17.0)	7(14.6)	15(31.3)
481-510	21(26.3)	13(27.7)	11(22.9)	22(45.8)[a]
511-540	29(36.3)	14(29.8)	16(33.3)	29(60.4)[a]
541-570	33(41.3)	20(42.6)	18(37.5)	32(66.7)[a]
571-600	40(50.0)	23(48.9)	23(47.9)	34(70.8)[a]
601-630	48(60.0)	27(57.4)	26(54.2)	37(77.1)[a]
631-660	54(67.5)	33(70.2)	33(68.8)	40(83.3)[a]
661-690	67(83.8)	39(83.0)	35(72.9)	48(100)[a]
691-720	70(87.5)	41(87.2)	40(83.3)	48(100)[a]
721-745	73(91.3)	42(89.4)	43(89.6)	48(100)[a]
Total number of rats	80(100%)	47(100%)	48(100%)	48(100%)

[a]Significantly different from controls by Fisher's Exact Probability Test, $P < 0.05$.

TABLE 8. CUMULATIVE MORTALITY DATA OF FEMALE RATS MAINTAINED FOR 2 YEARS ON DRINKING WATER CONTAINING ACRYLONITRILE
(Quast et al. 1980a)

Days on test	Control No. dead (% dead)	35 ppm No. dead (% dead)	100 ppm No. dead (% dead)	300 ppm No. dead (% dead)
0-30	0	0	0	0
31-60	0	0	0	0
61-90	0	0	0	0
91-120	0	0	0	0
121-150	0	0	0	1(2.1)
151-180	0	0	0	1(2.1)
181-210	1(1.3)	0	1(2.1)	1(2.1)
211-240	1(1.3)	0	1(2.1)	1(2.1)
241-270	1(1.3)	0	2(4.2)	3(6.3)
271-300	1(1.3)	0	3(6.3)	4(8.3)
301-330	1(1.3)	1(2.1)	3(6.3)	9(18.8)[a]
331-360	1(1.3)	1(2.1)	3(6.3)	14(29.2)[a]
361-390	1(1.3)	3(6.3)	5(10.4)[a]	16(33.3)[a]
391-420	3(3.8)	3(6.3)	6(12.5)	20(41.7)[a]
421-450	3(3.8)	5(10.4)	6(12.5)	24(50.0)[a]
451-480	6(7.5)	7(14.6)	11(22.9)[a]	31(64.6)[a]
481-510	9(11.3)	10(20.8)	13(27.1)[a]	35(72.9)[a]
511-540	11(13.8)	12(25.0)	17(35.4)[a]	37(77.1)[a]
541-570	18(22.5)	20(41.7)[a]	27(56.3)[a]	42(87.5)[a]
571-600	22(27.5)	24(50.0)[a]	34(70.8)[a]	45(93.8)[a]
601-630	34(42.5)	27(56.3)	38(79.2)[a]	46(95.8)[a]
631-660	37(46.3)	33(68.8)[a]	42(87.5)[a]	46(95.8)[a]
661-690	45(56.3)	38(79.2)[a]	44(91.7)[a]	48(100)[a]
691-720	54(67.5)	42(87.5)[a]	46(95.8)[a]	48(100)[a]
721-745	60(75.0)	44(91.7)[a]	47(97.9)[a]	48(100)[a]
Total number of rats	80(100%)	48(100%)	48(100%)	48(100%)

[a]Significantly different from controls by Fisher's Exact Probability Test, $P < 0.05$.

TABLE 9. HISTOPATHOLOGIC DIAGNOSES AND TUMOR INCIDENCES IN MALE RATS MAINTAINED FOR 2 YEARS ON DRINKING WATER CONTAINING ACRYLONITRILE (Quast et al. 1980a)

Diagnoses	Tumor Incidences at Various Dose Levels[a] 0 ppm (%)	35 ppm (%)	100 ppm (%)	300 ppm (%)
Ear Canal Gland (Zymbal gland) carcinoma	3/80 (3.8)	4/47 (8.5)	3/48 (6.3)	15/48 (31.3) P = 2.57×10^{-5}[b]
Tongue squamous cell tumors (papilloma and/or carcinoma)	1/75 (1.3)	2/7 (28.6) P = 0.018[b]	4/9 (44.4) P = 3.10×10^{-4}[b]	5/40 (12.5) P = 0.019[b]
Stomach - Nonglandular portion papilloma, squamous cell	0/80 (0.0)	2/46 (4.3)	20/48 (41.7) P = 1.40×10^{-10}[b]	34/47 (72.3) P = 1.59×10^{-20}[b]
Stomach - Nonglandular portion squamous cell tumors papilloma and/or carcinoma	0/80 (0.0)	3/46 (6.5) P = 0.047[b]	23/48 (47.9) P = 2.24×10^{-12}[b]	39/47 (83.0) P = 3.95×10^{-25}[b]
Brain and/or Spinal Cord focal or multifocal glial cell proliferation suggestive of early tumor	0/80 (0.0)	4/47 (8.5) P = 0.017[b]	4/48 (8.3) P = 0.018[b]	8/48 (16.7) P = 2.64×10^{-4}[b]
Brain and/or Spinal Cord glial cell tumor (astrocytoma)	1/80 (1.3)	8/47 (17.0) P = 1.5×10^{-3}[b]	19/48 (39.6) P = 7.86×10^{-9}[b]	23/48 (47.9) P = 4.16×10^{-11}[b]
Brain and/or Spinal Cord glial cell tumor (benign and/or malignant)	1/80 (1.3)	12/47 (25.5) P = 2.27×10^{-5}[b]	22/48 (45.8) P = 1.61×10^{-10}[b]	30/48 (62.5) P = 1.21×10^{-15}[b]

[a]Tumor incidence expressed as number of rats bearing certain tumor types over the number of rats examined microscopically for that particular organ.

[b]P-values calculated using the one-tailed Fisher Exact Test.

TABLE 10. ISTOPATHOLOGIC DIAGNOSES AND TUMOR INCIDENCES IN FEMALE RATS MAINTAIN D FOR 2 YEARS ON DRINKING WATER CONTAINING ACRYLONITRILE (Quast et al. 1980a)

Diagnoses	Tumor Incidences at Various Dose Levels[a]			
	(ppm (%)	35 ppm (%)	100 ppm (%)	300 ppm (%)
Mammary Gland fibroadenoma/ adenofibroma	5 /80 (70.0)	41/48 (85.4) P = 0.037[b]	39/48 (81.3)	25/48 (52.1)
Mammary Gland benign and/or malignant mammary gland tumors	5 /80 (71.3)	42/48 (87.5) P = 0.026[b]	42/48 (87.5) P = 0.026[b]	35/48 (72.9)
Ear Canal Gland (Zymbal gland) Adenoma	/80 (0.0)	1/48 (2.0)	3/48 (6.3) P = 0.051[b]	4/48 (8.3) P = 0.018[b]
Ear Canal Gland (Zymbal gland) carcinoma	/80 (1.3)	4/48 (8.3)	6/48 (12.5) P = 0.011[b]	14/48 (29.2) P = 3.00 x 10^{-6}[b]
Ear Canal Gland (Zymbal gland) benign and/or malignant tumors	/80 (1.3)	5/48 (10.4) P = 0.028[b]	8/48 (16.7) P = 1.67 x 10^{-3}[b]	18/48 (37.5) P = 2.72 x 10^{-8}[b]
Tongue carcinoma, squamous cell	/78 (0.0)	1/5 (20.0)	0/3 (0.0)	10/45 (22.2) P = 2.13 x 10^{-5}[b]
Tongue papilloma and/or carcinoma, squamous cell	/78 (0.0)	1/5 (20.0)	2/3 (66.7) P = 9.26 x 10^{-4}[b]	12/45 (26.7) P = 2.00 x 10^{-6}[b]
Stomach - Nonglandular papilloma, squamous cell	/80 (1.3)	1/47 (2.1)	12/48 (25.0) P = 2.72 x 10^{-5}[b]	29/48 (60.4) P = 6.03 x 10^{-15}[b]

[a]Tumor incidence expressed as n nber of rats bearing certain tumor types over the number of rats examined microscopically for that particular organ.

[b]P-values calculated using the ne-tailed Fisher Exact Test.

(continued on the following page)

TABLE 10. (continued)

Diagnoses	0 ppm (%)	Tumor Incidences at Various 35 ppm (%)	ıse Levels[a] 100 ppm	%)	300 ppm (%)
Stomach - Nonglandular carcinoma, squamous cell	0/80 (0.0)	0/47 (0.0)	0/4	0.0)	12/48 (25.0) P = 2.94 x 10^{-6}[b]
Stomach - Nonglandular papilloma and/or carinoma, squamous cell	1/80 (1.3)	1/47 (2.1)	12/4 P = 2.	25.0) x 10^{-5}[b]	30/48 (62.5) P = 1.21 x 10^{-15}[b]
Small intestine cystadenocarcinoma, mucinous	0/80 (0.0)	1/7 (14.3)	4/1 P = 1.	36.4) x 10^{-4}[b]	4/48 (8.3) P = 0.018[b]
Brain and/or Spinal Cord focal or multifocal glial cell proliferation suggestive of early tumor	1/80 (1.3)	5/48 (10.4) P = 0.028[b]	4/4	8.3)	7/48 (14.6) P = 4.38 x 10^{-3}[b]
Brain and/or Spinal Cord focal or multifocal glial cell tumor (astrocytoma)	0/80 (0.0)	17/48 (35.4) P = 6.90 x 10^{-9}[b]	22/4 P = 9.	45.8) x 10^{-12}[b]	24/48 (50.0) P = 5.34 x 10^{-13}[b]
Brain and/or Spinal Cord focal or multifocal glial cell tumors (benign and/or malignant)	1/80 (1.3)	20/48 (41.7) P = 2.21 x 10^{-9}[b]	25/4 P = 2.	52.1) x 10^{-12}[b]	31/48 (64.6) P = 2.31 x 10^{-16}[b]

[a]Tumor incidence expressed as number of rats bearing certain tumor types (er the number of rats examined microscopically for that particular organ.

[b]p-values calculated using the one-tailed Fisher Exact Test.

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progression from hyperplasia and hyperkeratosis to papilloma and, finally, to carcinoma. Tongue tumors were diagnosed as squamous cell papillomas and carcinomas, and tumors in the small intestine were identified as cystadenocarcinomas.

Biodynamics, Inc. Study in Sprague-Dawley Rats (1980a)--

A report entitled "A Twenty-four Month Oral Toxicity/Carcinogenicity Study of Acrylonitrile Administered to Spartan Sprague-Dawley Rats in the Drinking Water," dated June 30, 1980, was submitted to the U.S. Environmental Protection Agency by the Monsanto Company, St. Louis, Missouri. This study, conducted by Biodynamics, Inc. for the Monsanto Company, was designed to evaluate the toxicity/carcinogenicity of acrylonitrile. Acrylonitrile (100% pure, supplied by the Monsanto Company) was administered in the drinking water to 100 Sprague-Dawley rats of each sex at dose levels of 0, 1, and 100 ppm. Interim necropsies were performed at 6, 12, and 18 months (10/sex/group). The study was terminated early due to low survival rates; females were sacrificed at 19 months and males were sacrificed at 22 months.

Body weights for the high-dose males and females were consistently lower than the weights of controls; body weight differences between controls and treated male rats were less than 10%, while the body weight differences for females were less than 8%. Body weights for the low-dose males and females were generally comparable to the controls throughout the study. Reduced water intake (test substance) was consistently noted in the high-dose group. Water intake for the low-dose group was generally comparable to the controls throughout the study. Slight, but consistent decreases in hemoglobin concentration, hematocrit, and erythrocyle counts were noted for the high-dose males and females.

Histopathology evaluation revealed an increased incidence of astrocytomas of the brain and spinal cord, carcinomas and adenomas of the Zymbal gland or ear canal, and squamous cell carcinomas and papillomas of the forestomach in the high-dose males and females (Table 11). Increased incidences of the aforementioned tumor types were observed predominantly in animals dying, sacrificed in a moribund condition, or at sacrifice intervals after the 12th month of the study, although the increases in the incidences of astrocytomas of the brain and carcinomas of the Zymbal gland or ear canal were noted earlier in the high-dose female after the sixth month of the study.

In conclusion, the carcinogenic effect of acrylonitrile administered to rats in drinking water further reconfirmed the earlier findings of Dow and Litton-Bionetics.

TABLE 11. TUMOR INCIDENCES IN SPRAGUE-DAWLEY RATS FED ACRYLONITRILE IN DRINKING WATER (Biodynamics, Inc. 1980)

Dose Level (ppm)	Sex	Brain Astrocytoma	Spinal Cord Astrocytoma	Zymbal Gland/ Ear Canal Carcinomas	Stomach Papilloma/ Carcinoma
0	M	2/98 (2%)	---	1/100(1%)	3/98 (3%)
	F	0/99 (0%)	0/96(0%)	0/99 (0%)	1/100(1%)
1	M	3/95 (3%)	---	0/91 (0%)	3/98 (3%)
	F	1/100(1%)	0/99(0%)	0/95 (0%)	4/99 (4%)
100	M	23/97(24%)[a]	---	14/93(15%)[a]	12/97(12%)[a]
	F	32/97(33%)[a]	7/98(7%)[a]	7/98(7%)[a]	7/99(7%)[a]

[a]Statistically significant at P > 0.05.

Biodynamics, Inc. Study in Fischer 344 Rats (1980b)--

A report entitled "A Twenty-four month Oral Toxicity/Carcinogenicity Study of Acrylonitrile Administered to Fischer 344 Rats in Drinking Water," dated December 12, 1980, was submitted to the U.S. Environmental Protection Agency by the Monsanto Company, St. Louis, Missouri. This study, conducted by Biodynamics Inc. for the Monsanto Company, was designed to evaluate the toxicity/carcinogenicity of acrylonitrile. Acrylonitrile (100% pure supplied by the Monsanto Company) was administered in the drinking water to 100 Fischer 344 rats of each sex at dose levels of 1, 3, 10, 30, and 100 ppm the control group contained 200 animals/sex. Interim necropsies were performed at 6, 12, and 18 months (20/sex from the control group and 10/sex from each treatment group each time interval). This study was orignially designed to be 24 months in duration; however, to ensure at least 10 animals/sex/group for histopathological evaluation at termination, all females were sacrificed at 23 months due to low survival. The males were continued on test until the 26th month when similar survival levels were reached. Mortality in the males and females receiving 100 ppm was markedly greater than controls, while mortality in the 10 ppm males and in the females receiving 3 and 30 ppm was also somewhat greater than controls. Food consumption was comparable for all groups on a g/kg/day basis. Liquid consumption for the females receiving 100 ppm was slightly lower than controls on a ml/kg/day basis, while values for the males in this group were comparable to or greater than controls.

Slight, but generally consistent reductions in hemogoblin, hematocrit, and erythrocyte counts were noted for the females receiving 100 ppm throughout the study.

Histopathological evaluation presented in Table 12 revealed an increased incidence of malignant tumor-bearing animals in the groups receiving 10, 30, and 100 ppm. The observed tumors were: astrocytomas of the central nervous system (brain and/or spinal cord) and squamous cell carcinomas of the ear canal, as well as mammary gland carcinomas in the females receiving 100 ppm.

In summary, the ingestion of acrylonitrile via drinking water at doses of 10, 30, and 100 resulted in an increased incidence of certain tumors. The target organ specificity (central nervous system, ear canals) confirms the similar earlier findings of the drinking water study in rats by the Dow Chemical Company.