TOXICOLOGICAL REVIEW

OF

ACETONITRILE

(CAS No. 75-05-8)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to acetonitrile. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of acetonitrile.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 202-566-1676.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Chemical Manager/Author

Mark Greenberg
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

Reviewers

This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval with EPA's Program Offices (the Offices for Air and Radiation, Planning and Evaluation, Prevention, Pesticides, and Toxic Substances, Research and Development, Solid Waste and Emergency Response, and Water) and the ten Regional Offices.

Internal EPA Reviewers

Katherine Anitole, Ph.D.
Risk Assessment Division
OPPTS

David Lai, Ph.D.
Risk Assessment Division
OPPTS

External Peer Reviewers

Shayne C. Gad, Ph.D.
Gad Consulting Services
Raleigh, NC

C.C. Conaway, Ph.D.
American Health Foundation
Valhalla, NY

Edward J. Sowinski, Ph.D.
Environmental Health Management and Science, Inc.

Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for acetonitrile has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Proposed Guidelines for Carcinogen Risk Assessment* (1996a), and *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategy employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Acetonitrile (ACN) is also known as cyanomethane or methyl cyanide. Some relevant physical and chemical properties of ACN are listed below (WHO, 1993):

CAS Registry number:	75-05-8
Empirical formula:	CH_3CN
Molecular weight:	41.05
Vapor pressure:	74 mmHg at 20°C
Water solubility:	infinitely soluble
Log K_{OW}:	-0.34
Conversion factor:	1 ppm = 1.68 mg/m^3, 1 mg/m^3 = 0.595 ppm (25 °C, 760 mmHg)

At room temperature, ACN is a volatile, colorless liquid with etherlike odor. It is one of the most stable nitriles. Acetonitrile has a TLV-TWA of 40 ppm (67 mg/m^3), with a short-term exposure limit (STEL) of 60 ppm (101 mg/m^3), recommended to protect against organic cyanide poisoning and injury to the respiratory tract (ACGIH, 1991).

Although nitriles are widely used to synthesize amines, amides, ketones, aldehydes, and a variety of other compounds (Kirk-Othmer Concise Encyclopedia of Chemical Technology, 1985), ACN is used primarily as a solvent. Nitriles in general are hydrolyzed in the presence of acidic conditions to form amides. Although ACN is one of the more stable nitriles, acidic hydrolysis would be expected to yield hydrogen cyanide. Hydrolysis in water has been reported as extremely slow (WHO, 1993). Willhite (1983) found that freshly prepared solutions of ACN in distilled water did not undergo any significant hydrolysis upon incubation at 37 °C for 2.5 hours.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

Inorganic cyanide has long been known to react with trivalent iron of cytochrome oxidase in mitochondria and block the reduction of oxygen needed for cellular respiration, thus leading to cytotoxic anoxia (Albaum et al., 1946). The toxicity of ACN is believed to be mediated, in part, through this mechanism. ACN is metabolized to inorganic cyanide, but the conversion occurs slowly compared to other nitriles (which may explain the delay in onset of acute symptoms). Freeman and Hayes' (1988) data suggest that the conversion to cyanide is oxygen- and NADPH-dependent, possibly mediated by P450 isozyme (2E1 or P-450j). Some investigators suggest that ACN produces cyanohydrin by a P450 reaction, which is then decomposed by catalase to release

cyanide (Ahmed et al., 1992; Feierman and Cederbaum, 1989; Willhite and Smith, 1981). Formaldehyde and formic acid are also postulated to be by-products of ACN metabolism (Ahmed et al., 1992). Cyanide can be further oxidized to thiocyanate, a less toxic compound that is excreted in urine, but one that may interfere with thyroid function (Hartung, 1981). Conversion is mediated by rhodanese, a sulfurtransferase found in liver and human nasal respiratory mucosa (Lewis et al., 1991). A minor urinary metabolite that has been detected after administration of ACN in drinking water to rats is 2-aminothiolazine-4-carboxylic acid (Swenne et al., 1996). Cyanide also can be oxidized to cyanate ion with further oxidation to formic acid (McMahon and Birnbaum, 1990).

Like hydrogen cyanide (HCN), ACN is readily absorbed from the lungs and gastrointestinal tract, and is distributed throughout the body in both humans and laboratory animals. In a group of male and female test subjects (16), 74% of inhaled ACN was absorbed when cigarette smoke was held in the mouth for 2 seconds (and not inhaled), and 91% was absorbed when smoke was inhaled (Dalhamn et al., 1968a,b). Autopsy of an individual who died 2 days following inhalation of ACN vapors showed that cyanide reaches the spleen, lungs, and kidneys, but was not detected in the liver (WHO, 1993). Oral exposure of animals resulted in metabolites found primarily in the spleen, stomach, and skin (U.S. EPA, 1985a). ACN serum concentrations were higher than cyanide levels in an individual hospitalized after oral ACN ingestion (Michaelis et al., 1991); elimination half-lives were 32 hours for ACN and 15 hours for cyanide. Cyanide was not detected in the blood of three human subjects exposed to concentrations of 40, 80, or 160 ppm for 4 hours; a slight increase in thiocyanate levels was detected in urine (Pozzani et al., 1959).

Hydrocyanic acid was found in brain, heart, kidney, and spleen of rats after inhalation of ACN vapors (Haguenoer et al., 1975). Cyanide and thiocyanate were measured in blood, liver, brain, and kidney of hamsters following oral exposure (Willhite, 1983); thiocyanate in blood, kidney, and liver was up to 10-fold higher than in the brain, while cyanide in blood and liver was generally higher than in the brain and kidney 2.5 hours after exposure to a single dose of 100, 200, or 400 mg/kg ACN. Exposure of rats to ACN in drinking water indicated that the rat has a high capacity for cyanide detoxification on both normal and low-protein diets. On a low-protein diet, cyanide detoxification is at the expense of protein catabolism (Swenne et al., 1996).

Absorption of ACN is rapid in beagle dogs exposed to 16,000 ppm ACN (26,880 mg/m^3) vapors for 4 hours, based on blood cyanide concentrations peaking and reaching steady-state concentrations of 305-433 µg/100 mL after approximately 3 hours (Pozzani et al., 1959). With longer-term exposure in monkeys inhaling 350 ppm for approximately 99 days, blood samples on the 35th day of exposure, after a 2-day rest period (i.e., a weekend), did not detect cyanide ion, but 7.6-9.2 µg cyanide ion/100 mL was measured after the 39th day of exposure (i.e., following 5 consecutive days of exposure). Thiocyanate was detected in the urine after the 2-day break, and accumulated over the 5-day exposure week (Pozzani et al., 1959). Rats exposed to 166 or 300 ppm, 7 hours/day, 5 days/week, for 90 days had almost complete urinary excretion of thiocyanate after the 2.5-day rest period each week (Pozzani et al., 1959).

ACN conversion to cyanide appears to be about 10-fold greater in rat nasal ethmoturbinate microsomes than in liver microsomes (Dahl and Waruszewski, 1989), possibly

because of high P450 content. The nasal cavity, in particular the olfactory region, has a high concentration of rhodanese (Dahl and Waruszewski, 1989; Dahl, 1989). Rhodanese has also been found in the respiratory epithelium of human nasal tissue (Lewis et al., 1991); these in vitro studies suggest that rhodanese in smokers may have reduced affinity for cyanide. Lewis and colleagues also calculate that the capacity of rat nasal tissue is sufficient to metabolize a maximum of 2,800 ppm inhaled HCN. The high concentration of rhodanese in rat nasal tissue suggests that cyanide may be detoxified more rapidly to thiocyanate than in animal species with lower amounts of the enzyme (Dahl and Waruszewski, 1989). Aminlari et al. (1994) demonstrated that dogs had a greater rhodanese activity in the nasal cavity than in the lower respiratory tract.

Whole body autoradiography in male mice injected intravenously with ACN radiolabeled with ^{14}C in the methyl group indicated that radioactivity was widely distributed throughout the body (e.g., liver, thymus, and reproductive organs). Interestingly, nonvolatile radioactivity was also observed in nasal secretions, mouth cavity, esophagus, and stomach contents (Ahmed et al., 1992). One could infer from these observations that ACN could also distribute to the stomach upon inhalation exposure.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS

Other than one case-referent study, there are no epidemiological studies of effects of ACN exposure in humans. Individuals who were acutely exposed to ACN developed effects generally attributed to metabolism of ACN to cyanide (U.S. EPA, 1985a). Case reports of acute occupational exposure to ACN indicate that workers exhibited nausea, shallow and/or irregular respiration, and impaired motor activity. An autopsy of a worker who died shortly after exposure revealed cerebral, thyroid, liver, splenic, and renal congestion (WHO, 1993). Gastric erosion has been reported in individuals who ingested ACN (Way, 1981; Ballantyne, 1983).

In a clinical study by Pozzani et al. (1959), two men who inhaled 40 ppm ACN (67 mg/m^3) for 4 hours did not develop any adverse subjective effects, and blood cyanide and urinary thiocyanate were not appreciably increased. A third subject, also exposed to the same conditions, experienced slight chest tightness the evening after exposure, and cooling sensation in the lungs the following day. Only an increase in thiocyanate in the urine was evident; no cyanide was detected in the blood. "Olfactory fatigue" was reported during exposure for all subjects. Nine days later, two of the subjects inhaled 160 ppm ACN vapor (269 mg/m^3) for 4 hours. Flushing of face and chest tightness were reported 5 days after exposure, but there were no changes in urinary thiocyanate and blood cyanide from pre-exposure values.

WHO (1993) has reported on several cases of children or adults who ingested large amounts of ACN (≈250 to 4,000 mg/kg); symptoms included vomiting, respiratory distress, confusion, convulsions, seizures, and pulmonary edema, and in some instances, death occurred. Cyanide was detected in the blood of these individuals.

In a case-referent study of medical records of Finnish women employed in state laboratories that was designed to determine if there was an association between spontaneous abortion and exposure, the odds ratio for seven cases involving exposure to ACN was close to unity (Taskinen et al., 1994). However, the small number of cases and other limitations (e.g., exposure was self-reported) of this study preclude any definitive conclusions.

4.2. PRE-CHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

The National Toxicology Program (NTP, 1996) evaluated the toxicity of ACN to the rat and mouse in both subchronic and chronic inhalation studies. In the 13-week subchronic study that served to set exposure levels for the chronic study, F344/N rats and B6C3F1 mice (10/sex/group) were exposed whole-body to ACN concentrations of 0, 100, 200, 400, 800, or 1,600 ppm (0, 168, 336, 672, 1,343, or 2,686 mg/m^3), 6 hours/day, 5 days/week (duration-adjusted concentrations were 0, 30, 60, 120, 240, and 480 mg/m^3). Purity of ACN was 99% or greater and actual concentrations were within 10% of target concentrations. Clinical observation and body weights were recorded weekly. At necropsy, brain, heart, kidney, liver, lungs, testis, and thymus weights were measured, hematology was performed, and thyroid hormone assays were conducted. All organs of animals exposed to 0, 800 ppm (males only), and 1,600 ppm were examined by histopathology. Selected organs of 800-ppm females (bone marrow, brain, lung, lymph node, ovary, spleen, thymus) and 400-ppm males (bone marrow, testes, thymus) were subjected to histopathological examination; additional organs were examined if they exhibited gross lesions.

In rats, deaths occurred at 800 ppm (1 male) and 1,600 ppm (6 males and 3 females). All but one of the deaths occurred during the first 2 weeks of exposure. There was a significant decrease in body weight gain and final body weight at 1,600 ppm (81% of control for males; 91% for females); no change occurred in the other groups. Clinical signs at the two high-concentration groups included hypoactivity and ruffled fur during the first week. Ataxia, abnormal posture, and clonic convulsions occurred in the 1,600-ppm males that died. The other groups did not exhibit any treatment-related signs. Thymus weights were significantly lower in 800- and 1,600-ppm rats (both sexes), compared to those of the controls. Significant decreases in red blood cell count, hemoglobin concentration, and hematocrit occurred in the 800 and 1,600-ppm females and 1,600-ppm males. The investigators reported that these alterations were suggestive of anemia (characterized as nonresponsive, normocytic, and normochromic) since reticulocyte counts, mean cell volume, and mean cell hemoglobin concentration were similar to controls. The 1,600-ppm females also exhibited a decrease in triiodothyronine (T3) concentration, without changes in thyroxine (T4) and thyroid-stimulating hormone (TSH) concentrations. Histopathologic effects were limited to rats that died at 800 and 1,600 ppm; effects included congestion, edema, and hemorrhage in alveoli observed in lungs (no incidence data were provided). Because of the one death at 800 ppm (in week 1), coupled with mortality in the mouse (see below) at a lower concentrations, it is prudent to regard 800 ppm as a FEL (FEL[ADJ] = 239 mg/m^3) for the rat. Because only limited histopathology (i.e., bone marrow, thymus, and testes) was conducted in animals exposed to 400 ppm (males only), the results are insufficient to identify a no-observed-adverse-effect-level (NOAEL).

In mice, deaths were observed at concentrations of 400 ppm and greater (0/10, 0/10, 0/10, 0/10, 1/10, 10/10 in males; 0/10, 0/10, 0/10, 1/10, 4/10, 10/10 in females). All animals in the 1,600-ppm groups died by week 4 of the study. Final body weight (92% of control) and body weight gain were significantly reduced at 400 ppm in males, but are not considered toxicologically significant. Hematological parameters were not evaluated in mice. Males exhibited a significant concentration-related increase in absolute ($\geq$200 ppm; $p \leq 0.05$) and relative ($\geq$100 ppm; $p \leq 0.01$) liver weight. Significant increased relative lung and kidney weights in some male groups were also observed, but changes were not concentration-related. Females had a significant increase in absolute liver weight at 800 ppm and in relative weight at $\geq$400 ppm. Histopathology revealed an increased incidence of hepatocellular vacuolation, with significance at 400 and 800 ppm ($p \leq 0.01$) (0/10, N/A, 0/10, 8/10, 7/9, and 0/10 in males; 0/10, N/A, 0/10, 7/10, 6/10, and 0/10 in females; 100 ppm males and females were not examined). No other hepatic effects were observed. Vacuolization was considered to represent increased glycogen storage by distension of previously existing clear spaces. Severity of vacuolization was characterized as moderate in the 800-ppm female group and mild in males. No hepatocellular vacuolization was observed in the 1,600-ppm animals that died during the study. The absence of this change in the 1,600-ppm animals may be indicative of an increased utilization of glycogen stores by the animals that died. Incidences of forestomach squamous epithelial hyperplasia were significantly increased in 800-ppm males and in females exposed to $\geq$200 ppm. The incidences were 0/10, 0/10, 3/10, 6/9, and 1/9 in males (100-ppm males were not examined) and 0/10, 0/10, 7/10, 8/10, 7/10, and 5/10 in females; severity was not concentration-related. Hyperkeratosis and inflammatory cell infiltrate (effects associated with hyperplasia) also occurred in the forestomach. A significant increase in focal ulcers of the forestomach was also observed in 1,600-ppm female mice. There were no effects reported for the lungs.

The study identified a NOAEL of 200 ppm (NOAEL[ADJ] = 60 mg/m^3) based on mortality. The level of 400 ppm is considered a FEL given the early death (week 2) of one female mouse in the 400 ppm group and increased mortality (one male and four females) at 800 ppm. Neither a NOAEL nor lowest-observed-adverse-effect level (LOAEL) can be identified for forestomach lesions inasmuch as grooming of contaminated fur and/or mucociliary clearance likely was the primary cause of the increased incidence in hyperplasia of the forestomach. Hyperplasia is considered adverse because it was associated with infiltration of inflammatory cells and, at the highest concentration in females, focal ulcers.

Based on the findings in the 13-week study, the two-year study was initiated with F344/N rats (56/sex/group) exposed to actual ACN concentrations of 0, 100, 200, or 400 ppm (0, 168, 336, or 672 mg/m^3), 6 hours/day, 5 days/week, for 103 weeks (duration adjusted to 30, 60, and 120 mg/m^3) and B6C3F1 mice exposed to concentrations of 0, 50, 100, or 200 ppm (0, 84, 168, or 336 mg/m^3) for 111 weeks (duration adjusted to 15, 30, and 60 mg/m^3). An interim necropsy at 15 months involved 8 rats (each sex) and 10 mice (each sex). Complete histopathological examinations were conducted on all animals at this time and hematological parameters and liver, kidney, and lung weights were measured. Clinical signs and body weight were assessed throughout the study. After 2 years, animals were necropsied and examined for gross and microscopic alterations.

At the 15-month interim necropsy for rats, hematological alterations were observed, but were significant ($p \leq 0.05$) only at the high concentration. Changes included decreased mean cell volume and mean cell hemoglobin (in both sexes), increased red cell count (males), and decreased hematocrit and hemoglobin (females); none of these effects was concentration-related. At this time, there were no neoplastic or nonneoplastic lesions observed in tissues examined that were attributable to exposure. Hematological parameters were not measured at study end.

No significant changes in survival, body weights, or clinical appearance were observed in rats following 103-week exposure to ACN. Histopathological examination revealed no neoplastic or nonneoplastic lesions in any organs of exposed females. In male rats, a statistically significant increase in the incidence of basophilic hepatic foci was observed in the 200- and 400-ppm groups (15/48, 22/47, 25/48, and 31/48), but the foci were not atypical in appearance. Thus, it is uncertain if these lesions are preneoplastic. The incidences of eosinophilic and mixed cell foci were marginally elevated in 400-ppm males, but were not statistically significant. Although there was a marginally significant positive trend in the incidence of adenoma, carcinoma, or adenoma and carcinoma (combined) in liver of male rats, no significant dose-related trend was present after incidences were adjusted for survival using the life table test. The incidences of hepatocellular adenomas and carcinomas in male rats were not significantly increased in the treated animals based on pairwise comparison with incidences in control animals. Also, the tumor incidences at 400 ppm were only slightly higher than the historical control range. Other effects observed in male rats included marginal (not concentration-related) increases in tumors in the adrenal medulla and pancreatic islets; incidences observed were within historical control range. Keratoacanthoma was observed in the skin of 400-ppm males (0/48, 1/47, 0/48, and 4/48), but was not considered treatment-related; the incidence was within the historical control range. Although an increased incidence of basophilic foci is generally considered a possible preneoplastic effect and, thus, appropriate for discussion of potential carcinogenicity, the incidence is not considered evidence of a hepatotoxic effect nor a precursor to an hepatotoxic effect. As support for this view, hydrogen cyanide (U.S. EPA, 1985b) has not been found to cause adverse liver effects in rat feeding studies nor has it been associated with liver effects in human occupational studies. Thus, a NOAEL of 400 ppm (NOAEL[ADJ] = 120 mg/m^3) was identified for the rat.

In mice, no changes in the survival of the treated animals were observed, compared to the survival in control animals. Body weights were similar for all groups, and treatment-related clinical signs were not evident. In contrast to the 13-week study, there were no concentration-related effects of liver weight, suggesting that the changes observed in the 13-week study were adaptive. At the 15-month interim sacrifice, the only nonneoplastic change observed was a significant increase in the incidence of squamous hyperplasia in the forestomach of 200-ppm females (incidences were 0/10, 1/10, 0/10, and 6/10). At terminal sacrifice, the incidence of alveolar/bronchiolar adenomas was significantly increased in male mice following administration of the high concentration ($p = 0.011$) (6/50, 9/50, 8/48, and 18/50). Combined incidences of alveolar/bronchiolar adenomas or carcinomas were also significantly increased in 200-ppm males ($p = 0.042$) (10/50, 14/50, 14/48, and 21/50). The 100-ppm males exhibited a statistically significant increased incidence of hepatocellular carcinoma (7/50, 11/50, 13/49, and 7/50) ($p = 0.038$) and combined adenoma or carcinoma (19/50, 21/50, 30/49, and 15/50)

(p = 0.013), with incidences greater than those observed in historical controls. Because the incidence of this lesion did not increase with increasing concentration, it was considered a sporadic finding. Unlike the rat study, basophilic liver cell foci were not observed in the mouse. The incidences of hepatocellular adenoma or carcinoma (combined) in females were similar to controls. No increases in the incidence of lung tumors were observed in female mice. Forestomach squamous hyperplasia was significantly increased in 200-ppm males (3/49, 3/50, 6/48, and 12/50) and in 100- and 200-ppm females (2/49, 7/50, 9/50, and 19/48); however, severity of the effect was not concentration-related. The incidence at 200 ppm equaled the highest values observed in historical controls. The incidence of squamous cell papillomas in the forestomach was slightly increased after 2 years (incidences were 0/49, 0/50, 1/48, and 2/5 in males; 1/49, 0/50, 1/50, and 3/48 in females); however, these increases were not statistically significant and were within the range of historical control values.

Although forestomach hyperplasia in mice is clearly associated with exposure to ACN, the role of inhaled concentrations in eliciting these lesions is not known. It is likely that preening activities and/or mucociliary clearance, resulting in oral ingestion of ACN, play a central role. Thus, it is not possible to identify either a NOAEL or LOAEL attributable to inhalation exposure in the chronic study. The absence of these lesions in the rat study is puzzling. In a study by Wolff et al. (1982), whole-body exposure versus nose-only exposure of rats to radiolabeled fine particles indicated that 60% of the pelt burden was calculated to be ingested following whole-body exposure.

Subchronic studies (Pozzani et al., 1959) were performed on Carworth Farms-Wistar rats (15/sex/group) exposed to 0, 166, 330, or 655 ppm ACN vapors (0, 278, 554, or 1,100 mg/m^3), 7 hours/day, 5 days/week, for 90 days (duration-adjusted to 0, 58, 115, and 229 mg/m^3). The purity of the ACN was not reported. Body, liver, and kidney weights were determined, and histopathology was performed on liver and lungs (any effects in these organs resulted in examination of brain, pancreas, spleen, trachea, and testis). Hematocrit and hemoglobin values were measured in 5 rats in the 655 ppm group and controls 4 days prior to exposure and on the 53rd, 72nd, and 89th days. The values with exposed animals were no different from controls. Exposure to ACN did not affect body or organ weights in exposed rats, and no deaths attributable to exposure were reported. Pathological effects were limited primarily to the 655-ppm group; alveolar capillary congestion, focal edema, bronchial inflammation, desquamation, and hypersecretion of mucus occurred in lungs (10/27; p = 0.001), tubular swelling in kidneys (8/27; p = 0.05), and central cloudy swelling in liver (7/27; p = 0.04). No lesions were reported for other organs at 655 ppm. In the other groups, histiocyte clumps in alveoli or atelectasis (2/28 at 166 ppm), as well as bronchitis and pneumonia (3/26 at 330 ppm) were reported. Hematocrit and hemoglobin values for five female rats (males not evaluated) were similar to controls at 655 ppm. No treatment-related tumors developed in any groups. Interpretation of study results was limited by incomplete histopathology (e.g., stomach and thymus) and a lack of details about protocol. An unambiguous NOAEL and LOAEL could not be identified.

The investigators also examined effects of 350 ppm ACN (588 mg/m^3) on three male rhesus monkeys and three male dogs (2 Basenji-Cocker hybrid and one Basenji-Chow × Springer spaniel hybrid), 7 hours/day, 5 days/week, for 91 days (duration-adjusted to 123 mg/m^3). The purity of the ACN was not reported. Controls consisted of two male Basenji-Cocker hybrid

dogs; there was no control group for monkeys. In dogs and monkeys, focal emphysema and diffuse proliferation of alveolar septa were exhibited. Monkeys also showed hemosiderin accumulation in lungs and swelling of convoluted tubules in kidneys. Interpretation of this experiment was limited by inadequate measurement of chamber concentrations and the lack of a control group for monkeys. In a separate inhalation study (7 hours/day, 5 days/week) with 4 rhesus monkeys, the one monkey (female) exposed to 2,510 ppm died on the second day, the two female monkeys exposed at 660 ppm died by day 51, and the one male monkey exposed to 330 ppm was sacrificed at day 99 of exposure. At that time it exhibited considerable excitability. Each of three monkeys in the 2,510, 660, and 330 concentration groups was found to have dural and subdural hemorrhages. The two monkeys in the 660-ppm group had focal areas of emphysema and cloudy swelling of the proximal and convoluted tubules of the kidney.

An unpublished 90-day inhalation study in the B6C3F1 mouse was conducted by Hazelton Laboratories (1983a) for the National Toxicology Program (NTP). In this study, male and female mice (10/sex/group) were exposed by inhalation to ACN concentrations (purity>99%) of 0, 25, 50, 100, 200, and 400 ppm (0, 42, 84, 168, 336, and 672 mg/m^3) for 6.5 hours/day, 5 days/week for a total of 65 exposures during a 92-day period. The duration-adjusted concentrations were 0, 8.1, 16.2, 32.5, 65, and 130 mg/m^3. Chamber atmospheres were monitored every 30 minutes using infrared spectroscopic methods. Actual mean concentrations were all within ± 15% of nominal concentrations. Histopathological examination at necropsy included all major tissues and organs, including thymus, testes, ovaries, and lungs from controls and 400-ppm group mice. Three sections of the nasal turbinates were examined from all animals in all groups. Livers were examined from mice in 100- and 200-ppm groups as well. Clinical chemistry and hematological parameters were also examined. All animals from control, 100-, 200-, and 400-ppm groups at terminal necropsy were subjected to examination of sperm motility, count, and sperm head staining. Separate groups of females were exposed in the same study to 0, 100, 200, and 400 ppm ACN for 6.5 hours/day, 5 days/week for a total of 10 exposures and used for immunotoxicology studies.

Three male mice died during the course of the study (one in each of the 50, 200, and 400-ppm groups). Mortality was not considered to be exposure-related. In contrast to the findings from a 14-day study (ImmuQuest Laboratories Inc., 1984; see Section 4.4) in the same strain of mice, there were no reported histopathological effects on the thymus. Thymus/body weight ratios were somewhat lower in the 200- and 400-ppm groups compared to controls, but were not significantly decreased. Other altered terminal organ/body weight and terminal organ/brain weight ratios were mentioned, but tabular data were not included with the final report. There were no adverse effects on sperm or in the nasal turbinates. There were no adverse liver effects observed upon histopathology. Although cytoplasmic vacuolization was observed in all animals, including those in the control group, the vacuolization in animals in the 200- and 400-ppm groups was only slightly greater than those in animals of the 100-ppm and control groups.

In the group of females examined for hematologic and immunotoxic responses, all exposure groups exhibited significant decreases in hematocrit, hemoglobin, and red blood cell counts. They were described as of low magnitude and of questionable biological significance. Lymphocyte counts were decreased only in the 200- and 400-ppm groups. IgG was significantly decreased in all exposure groups in a concentration-related manner. These decreases in IgG are

consistent with the findings in the ImmuQuest study (see Section 4.4) at these concentrations. Other tests of immune function (e.g., lymphocyte proliferation, delayed hypersensitivity, host resistance) were unaffected by exposure; thus, the depressed IgG is of uncertain significance. The hematological and hepatic effects in mice were used as the basis for deriving an oral RfD that was previously placed on IRIS. Inasmuch as (1) these hematological and hepatocellular effects were of questionable biological significance, (2) hematological parameters were unaffected in the NTP (1996) subchronic and chronic rat study (these parameters were not measured in the mouse portion of the study), and (3) hepatic vacuolization was not observed in the chronic mouse study, it was recommended that the RfD for ACN be withdrawn from IRIS.

In a study with the F344 rat using the same exposure and examination protocols, there were no adverse gross or histopathological effects (Hazelton Laboratories, 1983b).

As part of a developmental toxicity study (Mast et al., 1994), nonpregnant female Sprague-Dawley rats (10/group) were exposed for 14 consecutive days to 0, 100, 400, or 1,200 ppm ACN (168, 672, and 2,015 mg/m^3). One animal at the high concentration died; no treatment-related clinical signs or body weight changes were evident in exposed animals. Gross examination did not reveal any significant effects in the exposed animals.

Willhite (1981) exposed CD-1 mice to ACN for 60 minutes to determine the lethal concentration (LC). The LC_{50} value was determined to be 2,693 ppm (4,524 mg/m^3).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES – ORAL AND INHALATION

Mast et al. (1994) exposed positively mated female Sprague-Dawley rats (33/group) to 0, 100, 400, or 1,200 ppm (0, 168, 672, and 2,015 mg/m^3) ACN, 6 hours/day, 7 days/week during gestation days 6-19, and then sacrificed on gestational day 20. Controls consisted of 10 nonpregnant females per exposure group. The 1,200-ppm dams exhibited hypoactivity (14/33) and appeared emaciated (6/33). Deaths occurred in two 1,200-ppm dams and one 400-ppm dam. The death at 400 ppm was suggested by the investigators to be the result of a possible spontaneous cerebral hemorrhage. There was no effect on body or organ weights in pregnant dams. Fertility did not appear to be affected by ACN exposure; mean pregnancy rate for sperm-positive females was 79%. A slight increase in percentage of resorptions per litter (particularly late resorptions) was seen at the high concentration, but the effect was not significant or concentration-related. The percent of live fetuses per litter was not affected for any group, nor were there treatment-related fetal malformations. The only effect on skeletal variations was a significant increase in percent of supernumerary ribs per litter at 100 ppm, but the effect did not occur for the other groups. While ACN was readily measurable in maternal blood (determined in a separate group of nonpregnant animals), cyanide was not detectable except in the 1,200 ppm group. Considering that mortality was treatment-related at 1,200 ppm and exposure may also have played a role in the one death at 400 ppm, it is prudent to consider 400 ppm (672 mg/m^3) as an FEL. The NOAEL for developmental effects is 1,200 ppm (2,015 mg/m^3).

Pregnant Sprague-Dawley rats (20-23/group) were exposed to 0, 1,000, 1,287, 1,592, or 1,827 ppm ACN (0, 1,679, 2,161, 2,673, or 3,067 mg/m^3), 6 hours/day, on gestation days 6 to 20

(Saillenfait et al., 1993). Dams were sacrificed on gestational day 21. At 1,827 ppm, mortality occurred in 8/20 dams, and maternal body weight gain was significantly reduced from gestation days 6 to 21. There was no mortality at other concentrations. Acetonitrile did not affect fertility (i.e., no differences in number of pregnancies). A markedly increased percentage of nonlive implants per litter (resorptions and dead fetuses) and early resorptions per litter were observed at 1,827 ppm, along with a decrease (not significant) in the mean number of live fetuses per litter. One litter was completely resorbed at 1,827 ppm. No differences were observed between the other exposed groups and the controls. Acetonitrile exposure had no significant effect on the mean number of implantation sites per litter, fetal sex ratio, or fetal weights per litter. Incidences of any visceral or skeletal anomalies were not significantly different between exposed and control groups. A NOAEL of 1,592 ppm (2,673 mg/m^3) was determined for maternal and developmental toxicity and a FEL of 1,827 ppm (3,067 mg/m^3). Based on increased percentage of nonlive implants per litter, a LOAEL of 1,827 ppm was identified for developmental toxicity.

The lack of mortality in all but the animals in the highest concentration of the Saillenfait et al. (1993) study is in sharp contrast with mortality observed in the Mast et al. (1994) study, inasmuch as both studies used Sprague-Dawley rats. There is no clear explanation for these divergent effects.

There were no effects on pup birth weight, litter size, or pup viability when ACN was administered by intubation to pregnant Long-Evans Hooded female rats (Smith et al., 1987). In this teratology screen, ACN, dissolved in tricaprylin oil, was administered at 0, 50, 150, 300, and 600 mg/kg from days 7-21 of gestation. Principal parameters evaluated were pregnancy rate, litter resorption, and early neonatal death. The two highest dosing levels were maternally toxic.

Pregnant Sprague-Dawley rats (25/group) were administered gavage doses of 0, 125, 190, or 275 mg/kg-day of ACN (dissolved in distilled water) on gestation days 6-19 (Johannsen et al., 1986; IRDC, 1981; Berteau et al., 1982). Animals were sacrificed on gestation day 20. Maternal effects were seen with 275 mg/kg-day; two dams died and four dams appeared emaciated. Body weight gain from days 6 to 20 was slightly reduced (96% of control) in the 275 mg/kg-day dams. Fertility was not affected. An increase in postimplantation loss per dam occurred at the high dose; however, effect was not significant or dose-related. The number of live fetuses per dam was slightly less than the control value for the 275 mg/kg-day group (not significant), but the effect was not measured for each litter. No differences in total implantation, corpora lutea, fetal sex ratio, or fetal body weight were observed in any treated groups compared to controls. There was a slight, but not significant, increase in the number of fetuses with unossified sternebrae (5th and 6th) in the high-dose group; however, the number of litters with unossified sternebrae was not dose-related. No significant effects on fetal anomalies occurred. This study identifies a LOAEL of 275 mg/kg/day for maternal and developmental effects and a NOAEL of 190 mg/kg-day.

Inhalation and oral developmental studies were performed on pregnant hamsters exposed to ACN (Willhite, 1983). Pregnant Syrian golden hamsters were exposed via inhalation to 0, 1,800 (6 animals), 3,800 (6 animals), 5,000 (6 animals), or 8,000 (12 animals) ppm ACN (0, 3,022, 6,380, 8,395, and 13,432 mg/m^3) for 1 hour on gestation day 8 and then sacrificed on gestation day 14. Parameters of maternal and developmental toxicity were evaluated. Only those

dams that died were examined for histopathological effects. No hamsters exposed to 1,800 ppm exhibited any signs of intoxication and all offspring were normal. Histopathology was not performed on this group. One dam at 3,800 ppm died 3 hours after exposure after exhibiting dyspnea, tremors, hypersalivation, ataxia, and hypothermia. All offspring from this group were normal. At 5,000 ppm, all animals exhibited irritation and excessive salivation; one dam in this group died after displaying dyspnea, hypothermia, and tremors. Six abnormal offspring from two litters of this group exhibited exencephaly and rib fusions. In the 8,000-ppm group, clinical effects included respiratory difficulty, lethargy, ataxia, hypothermia, irritation, and gasping (4/12 dams), followed by tremors, deep coma, and death (3/12 dams). Histopathological examination of the liver, kidneys, and lungs from the dams that died in all groups did not reveal any significant treatment-related effects. Fetotoxicity occurred in offspring of dams exposed to 8,000 ppm, as evidenced by decreased fetal body weight compared to controls (not concentration-related). Five of the nine surviving litters at 8,000 ppm developed severe axial skeletal dysraphic disorders; one fetus exhibited extrathoracic ectopia cordis with accompanying defects in the sternum. This study identifies a maternal NOAEL of 1,800 ppm (3,022 mg/m^3) and an FEL of 3,800 ppm (6,380 mg/m^3). The NOAEL (3,800 ppm [6,380 mg/m^3]) and LOAEL (5,000 ppm [8,395 mg/m^3]) identified for developmental effects occurred at or exceeded the maternal FEL.

The effects of ingested ACN on pregnant hamsters and their fetuses were examined (Willhite, 1983). Pregnant Syrian golden hamsters (6-12/group) received gavage doses of 0, 100, 200, 300, or 400 mg/kg ACN (in distilled water) on gestation day 8, and were sacrificed on gestation day 15. Parameters of maternal and developmental toxicity were evaluated. Maternal effects (not specified) were evident in dams given 300 and 400 mg/kg, and death occurred in 1/6 and 4/12 dams in these groups, respectively. There was a significant reduction in body weight gain in dams (gestation days 8-15) given up to 300 mg/kg ACN compared to controls (no incidence data provided); however, this effect was not seen at 400 mg/kg. Slight, but significant, decrease in fetal body weight ($p < 0.05$) was seen in all exposed groups; however, the effect was not dose-related. A significant increase in the number of resorptions per group (not dose-related) was noted in dams exposed to 200 or 400 mg/kg (incidences were 0, 6%, 12%, 0, and 22%). A significant increase in the number of malformed offspring per group occurred in dams exposed to 300 or 400 mg/kg ACN (0, 10%, 0, 19%, and 18%). The most common effect was rib fusions. The reason for the nonlinear effects was not explained by the investigators. A concurrent group was administered 300 mg/kg thiosulfate by intraperitoneal injection 20 minutes prior to inhalation, and repeated every 2 hours for the next 10 hours. Treatment with thiosulfate prevented both maternal and developmental toxicity, indicating a causal connection between cyanide and the effects observed. This study identifies a NOAEL of 200 mg/kg and LOAEL of 300 mg/kg for maternal toxicity. The NOAEL for fetotoxicity could not be established on the basis of the nonlinear effects in the fetuses.

Pregnant New Zealand White SPF rabbits (25/group) were administered 0, 2, 15, or 30 mg/kg-day of ACN by gavage during gestation days 6-18, and sacrificed on gestation day 29 (Argus Research Laboratories, Inc., 1984). Dams were examined for clinical signs, body weight changes, feed consumption, and changes in absolute liver weight. Examination was performed to identify any gross lesions, which were then retained for histopathology. In the high-dose group, 5 dams died ($p = 0.025$) between days 12-19 and 2 aborted on either gestation day 23 or 27 (both effects attributed to treatment). Clinical signs in these affected rabbits included ataxia, anorexia,

decreased motor activity, bradypnea, dyspnea, impaired righting reflex, and colored exudate in cage pan. A decrease in body weight was observed in 30-mg/kg-day dams between gestation days 15 and 19 ($p \leq 0.01$), but the weight increased significantly after end of administration (i.e., gestation days 19-24) and was higher than control weights on days 24-29. Necropsy revealed thin stomach walls in the cardiac region in those rabbits that died, and this observation was considered treatment-related. Exposure to ACN did not appear to cause reproductive dysfunction, although a slight, but not significant, increase in resorptions per litter was observed. Developmental effects were seen at the high dose. The average number of live fetuses per litter was significantly decreased ($p = 0.011$) at 30 mg/kg-day. There was no significant increase in the incidence of fetal malformations or anomalies with ACN exposure. Based on maternal toxicity, this study identifies 30 mg/kg/day as a FEL (mortality). A NOAEL of 15 mg/kg/day is given for maternal toxicity. A NOAEL of 15 mg/kg/day is identified for developmental toxicity (decreased live fetuses per litter).

Morrissey et al. (1988) evaluated reproductive endpoints in the male rat and mouse exposed to nominal concentrations of 100, 200, and 400 ppm in the Hazelton (1983a,b) studies. No treatment effects were observed on the weights of right cauda epididymis, right epididymis, and right testis, nor were any effects found on sperm motility in the mouse. Similarly, a lack of effect on these endpoints were found in the rat; the weight of the right epididymis was not evaluated. In neither species were effects on sperm density or percent of abnormal sperm evaluated.

4.4. OTHER STUDIES

4.4.1. Acute Data

There are limited acute data on the oral and inhalation exposure of ACN to humans and animals. In humans, qualitative information is based primarily on case reports (see Section 4.1). In animals, oral LD_{50}s have been reported for the mouse (269-453 mg/kg) and the rat (2,230-4,050 mg/kg), inhalation LC_{50}s (1-2 hours) for the mouse (2,300-5,700 ppm), and LC_{50}s (4 hours) for the rat (16,000 ppm) (WHO, 1993).

In an unpublished subacute study (ImmuQuest Laboratories, Inc., 1984), B6C3F1 female mice were exposed to 0, 100, 200, or 400 ppm ACN, 6 hours/day, 5 days/week, for 10 days during a 14-day period. Gas chromatographic analysis indicated the test compound had a purity exceeding 99%. Chamber concentrations were monitored by infrared spectroscopy every 30 minutes during each 6-hour exposure. No treatment-related clinical signs were evident. Statistically significant decreases ($p < 0.05$) in red and white blood cell counts, hematocrit, and hemoglobin at the two highest concentrations were reported. However, mean values at these two concentrations seemed to be marginally lower than controls and may be of questionable biological significance. Necropsy revealed thymic atrophy in the 200- and 400-ppm groups (incidence not stated); the effect corresponded to reduction (not significant) in thymus/brain weight ratio (absolute organ weight and thymus-to-body-weight ratio were not reported). The number of mice examined histopathologically was not stated and appears to be no greater than six per exposure group, based on information presented for other endpoints. Serum IgG levels

were significantly decreased in a concentration-related manner (26%, 33%, and 48% decrease, respectively, of controls), but linear regression analysis indicated no concentration-related trends with IgM and IgA. Tests of B-cell function were unchanged from controls.

In the same report, reference was made to a concurrent 90-day study in which a separate group of B6C3F1 mice were exposed to the same concentrations. No data per se were presented for this study. However, thymic atrophy was mentioned for the 200 and 400 ppm groups of the 90-day study as had been for the 14-day study. No incidence or severity data were reported. A statement referring to slight vacuolization of hepatocytes and hydropic degeneration was the only information presented on histopathological findings in liver; alanine aminotransferase (AST) and aspartate aminotransferase (ALT) levels were within the normal range. It is likely that the 90-day study referred to is the Hazelton (1983a), since the principal investigator was a co-author of the ImmuQuest report. Of significance is the lack of effect of ACN on thymus in the Hazelton (1983a) report, in contrast to the statement in the ImmuQuest report. Thus, the significance of an effect of ACN on the thymus without further corroboration is doubtful.

4.4.2. Genotoxicity

The overall data indicate that ACN is not a point mutagen, but does interfere with chromosome segregation. Acetonitrile at concentrations up to 10,000 µg/plate was negative in *Salmonella typhimurium* strains TA1535, TA1537, TA97, TA98, and TA100 in the absence of S9 and in the presence of rat or hamster S9 induced with Aroclor 1254 (Mortelmans et al., 1986; NTP, 1996). Negative results were also obtained in a preincubation *S. typhimurium* assay and a reverse mutation assay in *Saccharomyces cerevisiae* D7, conducted in the presence and absence of S9 from rats induced with ACN or phenobarbitone (Schlegelmilch et al., 1988), although the bacterial assay was limited by the use of stationary cultures.

Sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells were significantly increased in the absence of S9 (only at 5,000 µg/mL), but there was no effect in the presence of S9 (Galloway et al., 1987; NTP, 1996). Gene conversions in *S. cerevisiae* were increased in the absence of S9, but not in the presence of S9 (Schlegelmilch et al., 1988). Both of these assays measure repair of DNA damage, rather than persistent DNA damage.

Chromosome aberrations were significantly elevated in CHO cells at 5,000 µg/mL in the presence of rat S9 but the trend test was borderline ($p = 0.016$); there was no effect in the absence of S9 (Galloway et al., 1987; NTP, 1996). Although the types of chromosome aberrations were not reported, Galloway et al. (1987) reported that both simple and complex aberrations were elevated at the high dose. The ability of ACN to induce mutations at the HGPRT gene locus in CHO cells in vitro was investigated both in the presence and absence of rat liver S9 (Bioassay Systems Corporation, 1984). There were no significant differences between treated and control cells over a concentration range of 0.1 to 30 mg/mL. Micronucleated normochromatic erythrocytes (NCEs) were increased in the peripheral blood of female mice, but not in males, in a micronucleus assay conducted in conjunction with a 13-week inhalation toxicity experiment (NTP, 1996). Although the micronucleus assay is usually conducted in polychromatic erythrocytes (PCEs), MacGregor et al. (1990) showed that micronucleated peripheral blood PCEs and NCEs are at steady state following dosing for 45-90 days. Schlegelmilch et al. (1988) found

that intraperitoneal injection of mice with ACN at 60% of the oral LD_{50} was weakly clastogenic, with micronucleated polychromatic erythrocytes significantly increased at 24 hours. When the mice were induced by injection of low doses of ACN for 7 days, and then challenged with 60% of the oral LD_{50}, an increase in micronucleated PCEs was not observed until 72 hours after the challenge dose. Positive micronucleus assays can indicate either clastogenic activity or interference with chromosome segregation. ACN (131 ppm for up to 70 minutes) also induced aneuploidy (both chromosome gain and chromosome loss) in treated mature oocytes of *Drosophila melanogaster* females exposed either as larvae or as adults (Osgood et al., 1991a,b). Toxicity and sterility were induced by the 70-minute exposure. When *S. cerevisiae* was exposed to 5% ACN, it induced mitotic aneuploidy (Zimmerman et al., 1985); the investigators suggested that the induction of aneuploidy by ACN in the absence of point mutations or recombination resulted from interference with tubulin assembly and the formation of microtubules in the spindle apparatus. More recently, Sehgal et al. (1990) obtained in vitro evidence that ACN does inhibit microtubule assembly in taxol-purified *Drosophila* or mouse microtubules, further indicating that ACN has potential to induce aneuploidy.

Although ACN is largely negative in gene mutation assays and produces only marginal effects in chromosome aberration assays, the potential of ACN to interfere with chromosome segregation both in vivo and in vitro has been demonstrated in *D. melanogaster*.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION—ORAL AND INHALATION

The database for ACN lacks subchronic and chronic oral toxicity studies in animals. Human data are limited to case reports of acute ACN exposure with little information on exposure level.

The noncancer inhalation toxicity of ACN in the rat and mouse is overtly demonstrated in subchronic studies at levels of 400 ppm and higher, at which lethality takes place (NTP, 1996). The two-year studies (NTP, 1996), conducted at lower concentrations, did not demonstrate adverse effects, clinically or by histopathology, in any of the organ systems examined in either the rat or mouse.

The lethal effects of exposure to ACN are believed to be associated with the production of cyanide leading to respiratory paralysis and inhibition of CNS processes. At these lethal levels, clinical signs included ataxia, convulsions, and abnormal posture (NTP, 1996). Disturbances in blood chemistry and decreased thymus weight (rats only) were associated with levels of 800 ppm and higher. (Concentration-related decreases in hemocrits and red blood cell counts were, however, reported in both the ImmuQuest and Hazelton mouse studies at lower concentrations). Abnormal histopathology was largely relegated to the lungs and included congestion, hemorrhage, and edema (NTP, 1996; Pozzani et al., 1959).

The absence of thymic atrophy in the mouse in both the NTP (1996) study and the Hazelton (1983a) study are in direct contrast to a statement (incidence/severity not reported) in the unpublished study by ImmuQuest Laboratories (1984) that thymic atrophy was observed in

B6C3F1 mice exposed to 200 and 400 ppm ACN for either 14 or 90 days. The reference to the latter 90-day study is most likely to the Hazelton (1983a) study, since one of the authors of the ImmuQuest report was the principal investigator for the Hazelton study.

Evidence of abnormal histopathology in other organs at levels below those at which lethality occurred was not apparent in the NTP study. Thus, the concentration-response relationship is quite steep. Lethality in the rat may be strain-dependent. In the subchronic study by the NTP (1996), 5/6 F344 rats exposed to 1,600 ppm died during weeks 1 and 2, whereas there were no deaths in pregnant Sprague-Dawley rats exposed to 1,592 ppm during a similar time period (Saillenfait et al., 1993). In addition, no deaths were reported in Carworth Farms-Wistar rats at 655 ppm (Pozzani et al., 1959). Developmental endpoints also have been associated with concentrations higher than those that caused lethality (Mast et al., 1994; Saillenfait et al., 1993; Willhite, 1983). The effects of ACN exposure on reproductive endpoints in either species prior to mating and through parturition has not been examined.

Mice may be more sensitive than rats to ACN toxicity, evidenced by the increase in forestomach hyperplasia and ulcers, effects not observed in the rat (NTP, 1996). The forestomach lesions observed in the subchronic and chronic studies conducted by the NTP are critical noncancer effects given the case reports of gastric erosion in humans who ingested ACN (Way, 1981; Ballantyne, 1983). However, the role that inhalation exposure plays in the occurrence of the lesions is unknown, and may be minor compared to ingestion as a result of grooming of contaminated fur and/or mucociliary clearance. Moreover, a potential role of inhalation can be envisioned given the detection of label as early as 5 minutes post-injection in nasal secretions, esophagus, and stomach contents after intravenous administration of C14-ACN to mice (Ahmed et al., 1992). Because of the uncertainties surrounding the contribution of oral ingestion versus inhalation, quantifiable levels of ACN via inhalation exposure cannot be ascertained for this endpoint.

The subchronic data (NTP, 1996) for the rat and mouse do indicate a NOAEL of 200 ppm (NOAEL[ADJ] = 60 mg/m^3) and an FEL of 400 ppm (FEL[ADJ] = 120 mg/m^3) for lethality. However, an unambiguous NOAEL pertaining to ACN inhalation cannot be determined because of the possible role of inhalation in causing forestomach lesions.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CLASSIFICATION

The National Toxicology Program (1996) concluded that the evidence for carcinogenicity via inhalation of ACN in F344/N rats was equivocal: "a causal relationship between ACN exposure and liver neoplasia in male rats is uncertain." Although there was a statistically significant positive trend in the incidence of hepatocellular adenomas, carcinomas, and adenomas and carcinomas (combined) in male rats only, the incidences were not statistically significant by pairwise comparison or by life table analysis. In addition, the incidence of adenomas and carcinomas combined in the 400 ppm group was only slightly higher than the historical range for inhalation study controls. Male rats exhibited an increase incidence of basophilic foci in liver that was statistically significant in the 200- and 400-ppm groups. Although the appearance of these foci was not atypical, as those more closely related to the carcinogenic process (Harada

et al., 1989), altered hepatocellular foci are generally considered to be preneoplastic (Williams and Enzman, 1998; Pitot, 1990). There was no evidence of carcinogenicity in female rats or in either male or female B6C3F1 mice.

The data from the NTP study coupled with the overall lack of genotoxicity potential strongly suggest that the carcinogenic potential of ACN in the rat and mouse is low. However, because of uncertainty as to the significance of (1) positive trend test of hepatocellular carcinomas/adenomas (combined) in male rats compared to controls, (2) the relevance of the basophilic cell foci, and (3) the lack of effect in the mouse, the proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996) are clear in stating that the carcinogenic potential of a substance (e.g., ACN) for humans following inhalation, oral or dermal exposure "cannot be determined because the existing evidence is composed of conflicting data (e.g., some evidence is suggestive of carcinogenic effects, but other equally pertinent evidence does not confirm any concern)." Similarly, when these data are assessed under the existing Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1987), ACN would be classified as Group D - Not Classifiable as to Human Carcinogenicity. There is an absence of human evidence and the animal evidence is equivocal.

4.7. SUSCEPTIBLE POPULATIONS

Sensitive populations may include individuals who generate increased concentrations of cyanide because of induction of the cytochrome P450 isoform, CYP2E1.

4.7.1. Possible Childhood Susceptibility

There are a few case reports of accidental ingestion of products containing ACN by children or infants (WHO, 1993). The symptoms in these cases are similar to those experienced by adults in accidental or intentional ingestion of ACN-containing products. The few developmental effects seen in oral and inhalation exposures of laboratory animals below levels that cause maternal toxicity suggest that children are not likely to be susceptible to developmental effects induced by ACN during prenatal exposure.

4.7.2. Possible Gender Differences

The World Health Organization (1993) reported two cases in which adult females accidentally ingested products containing ACN. The symptoms reported were similar to those reported for males.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

The oral reference dose and supporting information previously on IRIS have been withdrawn. The oral reference dose (RfD), derived via a route-to-route extrapolation, had been

based on the judgement that the observation of decreased red blood cells and hepatic lesions (i.e., vacuolization) in the unpublished Hazelton Laboratories (1983) 90-day inhalation study were adverse. The decreases in red blood cells are not considered adverse in the present U.S. EPA assessment. Although blood chemistry was not evaluated in mice in the current NTP studies (1996) and thus represents a shortcoming in the protocol, the Hazelton investigators had described these effects as being of "low magnitude and questionable biological significance." The vacuolization noted by these investigators was described as "slightly more pronounced ... as compared to the control mice." Similar findings were noted in the NTP (1996) study. The vacuolization is not judged adverse.

Although the available information was inadequate for developing an oral RfD, the derivation of a developmental toxicity RfD (RfD_{DT}) was considered due to developmental toxicity reported in oral developmental studies reported in hamsters, rats, and rabbits (Argus Research Laboratories, Inc., 1984; IRDC, 1981; Johannsen et al., 1986; Willhite, 1983). Based on the available developmental studies, the most sensitive developmental endpoint (decreased average number of live fetuses per litter), was reported for rabbits administered ACN by gavage during gestational days 6-18 (Argus Research Laboratories, 1984). This effect occurred at the highest dose tested in the study (LOAEL = 30 mg/kg-day); however, the high mortality (20%) in dams also indicated a FEL for this dose level. Because the deaths occurred with a short duration of exposure and dosing errors were not reported, it is likely that the observed effects were chemical-related. The other oral developmental studies in hamsters and rats were not appropriate for the development of an RfD_{DT}. No developmental toxicity was seen at any dose up to 275 mg/kg/day in the rat study (Johannsen et al., 1986), and the dose-response data in the hamster study (Willhite, 1983) were too inconsistent to determine whether there was an effect, and if so, at what level. In light of these inconsistencies, and because no study identified a LOAEL in the absence of a maternal FEL, the derivation of an RfD_{DT} was not attempted.

The NTP (1996) 2-year inhalation study in the mouse was not used for route-to-route extrapolation to an oral scenario because the quantitative contribution of inhalation and ingestion of ACN to the occurrence of forestomach lesions could not be delineated. Therefore, no oral dose-response assessment was performed for this compound.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

Inhalation data on subchronic and chronic toxicity of ACN are available from several studies: (1) the 1996 NTP study on F344 rats and B6C3F1 mice, (2) the 1984 ImmuQuest Laboratories study on mice, and (3) the 1983 Hazelton studies on the rat and mouse. The subchronic inhalation study by Pozzani et al. (1959) reported respiratory effects in dogs and mice at higher concentrations, and interpretation was limited because of inadequate study details. The developmental toxicity endpoints (Mast et al., 1994; Saillenfait et al., 1993; Willhite, 1983) also occurred at much higher inhalation concentrations (LOAELs $\geq$1,200 ppm) than those of the subchronic and chronic studies.

The NTP subchronic study in the mouse, supported by the results of the chronic study, was chosen as the principal study, with mortality of 1/10 females exposed to 400 ppm (duration-adjusted concentration = 120 mg/m^3) as the critical effect. The increases in absolute and relative liver weights in males and the incidence of hepatic vacuolization are not considered biologically significant hepatocellular adverse findings. Hepatic vacuolization also was not considered a biologically significant finding in either the ImmuQuest study or in the Hazelton studies. Because the incidence of forestomach squamous hyperplasia in females was increased above controls at 100 and 200 ppm (chronic) and at 200 ppm and above (subchronic) coupled with uncertainty as to the role of inhalation in the development of this lesion, there is no unambiguous NOAEL in either inhalation study conducted by the NTP. The absence of forestomach lesions in the rat cannot be explained. There is no information to suggest that differences between species in grooming behavior account for the forestomach lesions. Wolff et al. (1982) found that rats exposed whole-body ingested 60% of the pelt burden of a radiolabeled aerosol through preening.

Although an FEL would ordinarily not be chosen as a point of departure for RfC derivation, the choice of mortality in this instance appears appropriate. In both sexes of two laboratory animal species, there were no observed nonneoplastic adverse effects clearly associated with inhalation exposure at three exposure levels (chronic study) below those (400 ppm) that resulted in mortality. This steep exposure-response relationship is consistent with exposure-response data for other cyanide-containing chemicals. In most cases, abnormal lung and brain pathology and clinical signs of respiratory distress are features concomitant with mortality.

Thymic atrophy cited in the ImmuQuest Laboratories (1984) report was not selected as a critical effect because it was not corroborated in any of the other studies with the mouse.

5.2.2. Methods of Analysis

The RfC was derived according to procedures identified in U.S. EPA (1994b) using the regional deposited gas ratio (RDGR). Acetonitrile is considered to be a category 2 gas because (1) it has high water solubility, (2) is metabolized to reactive cyanide in the liver, but may be rapidly detoxified to thiocyanate, and (3) does not react directly with respiratory tract tissues. The RDGR (extrarespiratory effects) for category 2 gases is equivalent to 1 (U.S. EPA, 1994b). The RDGR, when multiplied with the NOAEL for mortality adjusted for duration, served to derive a human equivalent concentration (HEC). A benchmark concentration analysis (U.S. EPA, 1995) was not applied because there were only two data points.

5.2.3. RfC Derivation - Including Application of Uncertainty Factors (UF) and Modifying Factors (MF)

The following uncertainty factors (UFs) are applied to the NOAEL(ADJ) of 60 mg/m^3: 3 for interspecies variation, because dosimetric adjustments were applied; 10 for intraspecies variation; and 3 for database insufficiencies. The total UF = 3 × 3 × 10 = 100. No uncertainty factor was applied to the use of a subchronic study because lethality did not occur in the longer-term mouse or rat study at lower levels. Therefore, although this endpoint is of concern

based on the subchronic study, increased exposure would not be expected to increase the sensitivity to this endpoint given the known metabolism of cyanide-containing compounds.

A partial UF of $10^{1/2}$ (3), instead of a full factor of 10, was used for database insufficiencies because of the lack of data on reproductive endpoints involving exposure of laboratory animals before and during mating through parturition. A full factor of 10 was not considered necessary because (1) there is no evidence to suggest that ACN accumulates in the body, (2) the developmental effects observed seem to be marginal, and (3) these effects occur at concentrations lethal to dams. However, a modifying factor (MF) of 10 was applied to account for the uncertain role that inhalation may play in causing forestomach lesions. These lesions were found in mice subchronically and chronically exposed via inhalation and are likely to be a result of grooming of contaminated fur although inhalation cannot be ruled out.

RfC = 60 mg/m^3 × RDGR (1) = 60 mg/m^3 (HEC)
60 mg/m^3 ÷ [UF × MF] = 60 ÷ [100 × 10] = 6E-2 mg/m^3.

5.3. CANCER ASSESSMENT

Because the evidence of carcinogenic potential of ACN is equivocal in laboratory animals (see Section 4.6) and there are no data for humans, a quantitative dose-response assessment cannot be made.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

ACN is principally used in manufacturing operations as an intermediate in closed systems to produce other organic chemicals. Because it is sometimes used in noncaptive processes, it does have the potential to reach ambient air and water. In water, it is only slowly hydrolyzed at neutral pH. At acidic pH, it would be expected to yield hydrogen cyanide. Its half-life in air depends on the extent of its reaction with hydroxyl radicals, the principal scavenging mechanism (NTP, 1996). It also is one component of tobacco smoke.

ACN is readily absorbed in the respiratory tract and distributes throughout the body, where it is metabolized in the liver. Metabolism is known to result in formation of cyanide and thiocyanate, with formaldehyde and formic acid as additional postulated metabolites. Hydrocyanic acid has been detected in various organs (e.g., brain and heart) of the rat upon inhalation. Cyanide and thiocyanate have been detected in various organs and the blood of hamsters upon oral exposure. However, in clinical studies of three individuals who inhaled ACN for 4 hours, no cyanide was detected in blood.

There is little direct information concerning the nature of effects that ACN causes in humans. Case reports of adults or children who ingested ACN indicate that symptoms included

respiratory distress (e.g., pulmonary edema), vomiting, confusion, convulsions, gastric erosion, and seizures. Case reports involving occupational exposure involved a similar spectrum of symptoms. Autopsy findings from an occupationally exposed individual revealed brain, kidney, thyroid, and liver involvement. From these reports alone, it is clear that the respiratory tract, the central nervous system, and other organs can be adversely affected. Some of the effects observed in the human case reports have also been noted in studies with laboratory animals. Inhalation exposure of rats over 13 weeks indicated that ACN at high concentration causes pulmonary edema, congestion, and hemorrhage leading to death. At these concentrations, ataxia and clonic convulsions were observed as well as decreases in hemoglobin and thymus weight. When exposed over 2 years to lower concentrations, male rats (only) exhibited a positive trend in liver tumors, but the incidence was not significant when compared pairwise to controls or after adjustment for survival. In female mice exposed to high concentrations for 13 weeks, the principal finding was focal ulcers in the forestomach. Forestomach hyperplasia was observed in males. Other than increases in liver weight, there were no histopathological effects in the liver, nor were there adverse lung effects. Effects on hematological parameters were not studied. In a lifetime study in the mouse at lower concentrations, there were no clinical effects or effects in the liver. Forestomach hyperplasia was increased in incidence, but severity of the lesion was not concentration-related. The incidence of pulmonary adenomas/carcinomas observed in males was not considered to be treatment-related.

Oral and inhalation exposure of pregnant rats and hamsters during gestation to ACN revealed that developmental effects occurred only at levels at or exceeding those at which maternal adverse effects were observed. In one study that examined the effect of inhalation exposure on male rat and mouse reproductive parameters, there were no observed effects on sperm motility or sperm density. Although two-generation studies have not been performed, and this represents a database deficiency, there is no indication that ACN at levels expected in the environment poses a risk of developmental effects.

Because the carcinogenic potential in both the rat and mouse is low, coupled with an overall lack of genotoxicity potential, the carcinogenic potential in humans is expected to be low. However, there is uncertainty in this area inasmuch as there was a positive trend for hepatocellular adenomas/carcinomas (combined) in the male rat as well as an increase in the incidence of basophilic liver foci, of which the latter may represent a preneoplastic effect.

6.2. DOSE RESPONSE

Quantitative estimates of human risk as a result of exposure to low levels of ACN are based on exposures of laboratory animals because no human data are available. The human chronic concentration of inhaled ACN considered to be safe (the RfC) is 6E-2 mg/m^3. The RfC is based on subchronic and chronic inhalation studies in the rat (NTP, 1996) in which thorough histopathological analyses were performed. Because only limited evaluation of hematological endpoints was carried out in the rat and none in the mouse, coupled with no examination of the effects on ventilatory parameters or CNS endpoints, the scientific quality of these studies is considered medium. The confidence in the overall database also is medium inasmuch as two-generation studies were not performed. Although acceptable developmental studies were carried out (via inhalation) in two species, rat and rabbit, with adverse effects occurring at levels at or

exceeding the level that caused severe maternal effects, reproductive endpoints have not been thoroughly evaluated. This represents a database deficiency. In addition, the potential of ACN inhalation to induce gastric lesions is unknown, but is a consideration given the possible role of inhaled ACN in causing forestomach lesions in the mouse. The neurological symptoms and respiratory distress in humans after acute high-level exposures suggest that effects observed in laboratory animals are consistent with those observed in humans.

No dose-response assessment was performed for oral exposure to ACN. The database lacked subchronic and chronic studies for laboratory animals and there was only limited information from case reports of human ingestion. The NTP (1996) inhalation study was not used in a route-to-route extrapolation because the contributions of inhalation and ingestion to the occurrence of forestomach lesions could not be quantified. Similarly, a route-to-route extrapolation from inhalation to other endpoints could not be conducted because of lack of mechanistic data.

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APPENDIX A. EXTERNAL PEER REVIEW--SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for Acetonitrile have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists performed accordance with EPA guidance on peer review (U.S. EPA, 1992). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

(1) General Comments

A. The carcinogenic potential of ACN

Comment: All three reviewers agreed that the characterization of carcinogenic potential is appropriate. All reviewers agreed that "equivocal" is the appropriate designation. Two of the reviewers were of the opinion that the significance of liver tumors in rodents is of uncertain relevance to human risk. One reviewer intimated that a better discussion of the significance of basophilic liver foci could be presented and cited a recent article by Williams and Enzman (1998) as support for an association of these foci with carcinogenicity.

Response to Comment: Information pertaining to this reference and to the publication of Harada et al. (1989) was added to both the Toxicological Review and cancer summary to indicate that basophilic liver foci are generally considered to be preneoplastic (Williams and Enzman, 1998), but that the foci in the NTP mouse study are not atypical in appearance, a characteristic that is often associated with progression to cancer (Harada et al., 1989).

B. Selection of the most appropriate critical effect for RfC calculation

Comment: All three reviewers agreed that mortality is the most appropriate choice for the critical effect given the data presented.

C. Discussion pertaining to "Supporting/Additional or Other" studies

Comment: Two reviewers thought the Hazelton (1983a) and ImmuQuest Laboratories (1984) should be discussed more fully. In particular, the Hazelton (1983a) should not be discounted without presenting a clear rationale. One reviewer suggested that a discussion of this study appear in the summary document under I.B.4.

Response to Comment: A full discussion of the unpublished Hazelton (1983a) 90-day inhalation study was included and the results, in part, were contrasted with those of the unpublished 14-day ImmuQuest Laboratories (1984) study, presented under "Other Studies."

The shortcomings of the ImmuQuest study were presented. A discussion of Hazelton (1983a) was also presented in Section I.B.4 of the summary document.

D. Data that should be considered in developing uncertainty and modifying factors

Comment: Two reviewers agreed that the selection of uncertainty and modifying factors was appropriate. One reviewer was uncomfortable with the identification of 400 ppm as an FEL considering there was only one mouse death at this concentration. It was proposed that 800 ppm be identified as the FEL for risk assessment purposes.

Response to Comment: It is possible that the one death at 400 ppm was unrelated to treatment. However, given the steep concentration-response characteristics of ACN and the sudden onset of mortality in both the rat and mouse, it was considered appropriate to regard 400 ppm as treatment-related.

E. The rationale for the weight-of-evidence and confidence statements

Comment: One reviewer suggested that the relevance of liver tumors in the rat to humans should be presented here and that the significance of the preneoplastic lesions should be addressed more fully. A second reviewer indicated that genotoxicity data should be included. The third reviewer stated that the underlying assumptions and limitations are sufficiently apparent.

Response to Comment: EPA recognizes the ongoing scientific debate regarding the relevance of rodent tumors to humans. However, in this case the overall weight of evidence is inadequate for determining the human cancer potential for acetonitrile, given the lack of evidence seen in the rat and the marginal effects seen in the mouse. Therefore, EPA believes that an expanded discussion of the significance of preneoplastic basophilic foci is unneeded.

(2) Comments on Chemical-Specific Questions

Question 1: Is it appropriate to use mortality as a point-of-departure in the derivation of the RfC?

All reviewers agreed that there are no more sensitive endpoints to use in RfC derivation.

Question 2: Is the rationale given for discounting the Hazelton (1983a) study sufficient?

All reviewers agreed that a complete discussion of this study should be presented.

Response to Comment: In the draft presented to the reviewers a discussion of the results of this unpublished study was not presented because NTP had elected not to use or cite the study, but repeated it. Therefore, a comment was included to suggest that the study did not meet NTP acceptance criteria. In view of the fact that the reasons NTP did not choose to utilize this study were not expressed, EPA adopted the reviewers' suggestion to include a discussion of the conduct of the study and its results.

Question 3: Do you agree that forestomach lesions in mice cannot be used as the critical effect because of uncertainties pertaining to oral ingestion?

One reviewer stated that "the discussion provided is a good example of the use of scientific knowledge in the risk assessment process." This reviewer indicated that the lesions have little relevance to human effects reported after inhalation exposure. The other two reviewers agreed that the discussion presented on forestomach lesions clearly indicates why these lesions cannot be used in derivation of the RfC.

Question 4: Was it appropriate to discount the ImmuQuest Laboratories (1984) results in the choice of the principal study?

All reviewers were of the opinion that a more complete discussion of the strengths and limitations of this study should be presented. One indicated more weight should be given.

Response to Comment: A full discussion of this study is now presented in the Toxicological Review and in the RfC summary document. In these discussions, it is indicated that the lack of study results to support the statement of thymic atrophy in the mouse and the lack of such histopathological results in either the subchronic NTP (1996) or Hazelton (1983a) studies limit the significance of this undocumented and uncorroborated finding.

Question 5: Would you agree that the NTP chronic study cannot be used to derive an oral RfD?

All reviewers were in agreement that an oral RfD cannot be derived because of the uncertainties in the role of inhalation and oral ingestion in causing forestomach lesions.