Acetonitrile; CASRN 75-05-8

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR Acetonitrile

File First on-line 09/30/1987

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	withdrawn; qualitative discussion	03/03/1999*
Inhalation RfC (I.B.)	yes	03/03/1999*
Carcinogenicity Assessment (II.)	yes	03/03/1999*

*A comprehensive review of toxicological studies was completed (07/27/05) - please see sections I.A.6., I.B.6., and II.D.2. for more information.

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

IA Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Acetonitrile (ACN)
CASRN — 75-05-8
Last Revised — 03/03/1999

The oral Reference Dose (RFD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. 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 effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the US EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

An oral RFD for acetonitrile is not available at this time. There are no oral studies involving ACN that have looked at all relevant endpoints. The oral RFD and supporting information previously on IRIS have been withdrawn. The oral RFD, derived via a route-to-route extrapolation, had been based on the judgment that the decreased red blood cells and hepatic lesions (i.e., vacuolization) observed in the unpublished Hazleton Laboratories (1983; referred to in previous IRIS summary as NTP, 1983) 90-day inhalation study were adverse. The decreases in red blood cells are not considered adverse in the present US EPA assessment. Although blood chemistry was not evaluated in mice in the current NTP studies (1996), which represents a shortcoming in the protocol, the Hazleton 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. Route-to-route extrapolation for forestomach lesions observed in the 1996 mouse study was not considered appropriate because of the uncertain contribution of either inhalation or oral exposure to this endpoint. Similarly, route-to-route extrapolation for other endpoints was not considered because of uncertainties in the mechanisms of action. A developmental RFD was considered, but developmental toxicity occurred at or above levels at which frank toxicity in dams was observed.

I.A.1. Oral RFD Summary

Not Applicable

I.A.2. Principal and Supporting Studies (Oral RFD)

Not Applicable

I.A.3. Uncertainty and Modifying Factors (Oral RFD)

Not Applicable

I.A.4. Additional Studies/Comments (Oral RFD)

Not Applicable

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

I.A.5. Confidence in the Oral RFD

Not Applicable

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

I.A.6. EPA Documentation and Review of the Oral RFD

Source Document — US EPA. (1999) Toxicological review of acetonitrile in support of summary information on the Integrated Risk Information System (IRIS).

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS Summary. A record of these comments is included as an appendix to the Toxicological Review of Acetonitrile in Support of Summary Information (a PDF document) on the Integrated Risk Information System (IRIS) (US EPA, 1999). ***To review this appendix, exit to the toxicological review, Appendix A, External Peer Review -- Summary of Comments and Disposition (PDF)***.

Agency Consensus Date — 1/26/1999

A comprehensive review of toxicological studies published through July 2005 indicated that there is insufficient health effects data to derive an RfD for Acetonitrile at this time. For more information, IRIS users may contact the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

I.A.7. EPA Contacts (Oral RFD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (Internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Acetonitrile (ACN)
CASRN — 75-05-8
Chemical Formula -- CH_3CN
Last Revised — 03/03/1999

The inhalation Reference Concentration (RfC) is analogous to the oral RFD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/cu.m. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Inhalation RfCs were derived according to the Interim Methods for Development of Inhalation Reference Doses (EPA/600/8-88/066F August 1989) and subsequently, according to Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994). RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the US EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.B.1. Inhalation RfC Summary

Critical Effect	Exposures*	UF	MF	RfC
Mortality **Mouse subchronic/ chronic inhalation studies** **NTP, 1996**	NOAEL: 336 mg/m^3 (200 ppm) NOAEL(ADJ): 60 mg/m^3 NOAEL(HEC): 60 mg/m^3 FEL: 672 mg/m^3 (400 PPM) FEL(ADJ): 120 mg/m^3 FEL(HEC): 120 mg/m^3	100	10	6E-2 mg/m^3

Note: FEL = frank effect level; ADJ = duration-adjusted concentration; HEC = Human equivalent concentration

*Conversion Factors and Assumptions — MW = 41.05. Assuming 25 C and 760 mmHg, NOAEL (mg/m^3) = 200 PPM × 41.05/24.45 = 336 mg/m^3. NOAEL(ADJ) = NOAEL (mg/m^3) × 6 hours/24 hours × 5/7 days = 60 mg/m^3. The NOAEL(HEC) was calculated for a category 2 gas and extrarespiratory (systemic) effects assuming periodicity was attained. ACN is considered to be a category 2 gas because it has high water solubility, is metabolized to reactive cyanide in the liver but may be detoxified rapidly to thiocyanate, and does not react directly with respiratory tract tissues. The RGDR[1] for a category 2 gas is assumed to be 1 (US EPA, 1994). NOAEL(HEC) = NOAEL(ADJ) × 1 = 60 mg/m^3. Therefore, the NOAEL(ADJ) of 60 mg/m^3 will be considered equivalent to the HEC at this time.

[1]$RGDR_{ER}$ equation (Equation 4-48, p. 4-59 of US EPA, 1994) is currently undergoing EPA reevaluation, so no conversion will be made using the $RGDR_{ER}$ equation until the analysis has been completed.

I.B.2. Principal and Supporting Studies (Inhalation RfC)

National Toxicology Program (NTP). (1996) Toxicology and carcinogenesis studies of acetonitrile (CAS No. 75-05-8) in F344/N rats and B6C3F1 mice (inhalation studies). NTP TR 447.

In a 13-week study, B6C3F1 mice (10/sex/group) inhaled ACN at concentrations of 0,0 100, 200, 400, 800, or 1,600 PPM (0, 168, 336, 672, 1,343, or 2,686 mg/m^3, respectively [author's conversion]), 6 hours/day, 5 days/week (duration adjusted to 0, 30, 60, 120, 240, and 480 mg/m^3). Clinical observation and body weights were recorded weekly. At necropsy, brain, heart, kidney, liver, lungs, testis, and thymus weights were measured. Hematology parameters were not measured. This represents a shortcoming of the study protocol. Complete histopathological examinations were performed on all organs in both sexes at 0, 800 PPM, and 1,600 PPM Organs examined in lower dose groups included adrenals (200 and 400 PPM females), liver and stomach (200 and 400 PPM males and females), lung (400 PPM females), and thymus (400 PPM females).

Mortality was observed at concentrations of 400 PPM and greater (0/10, 0/10, 0/10, 0/10,1/10, and 10/10 in males; 0/10, 0/10, 0/10, 1/10, 4/10, and 10/10 in females). All animals in the 1,600-PPM groups died by week 4 of the study. There were no effects reported for the lungs. Final body weight (92% of control) and body weight gain were significantly reduced at 400 PPM in males, but are not considered toxicologically significant. Males exhibited a significant concentration-related increase in absolute (>= 200 PPM; $p <= 0.05$) and relative (>= 100 PPM; $p <= 0.01$) liver weight. Significantly increased relative lung and kidney weights in some male groups also were observed, but changes were not concentration-related. Females had a significant increase in absolute liver weight at 800 PPM and in relative weight at >= 400

PPM Histopathology revealed an increased incidence of hepatocellular vacuolation, with significance at 400 and 800 PPM (p <= 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, 0/10 in females; livers in the 100 PPM groups were not examined microscopically). The extent of vacuolization, characterized by NTP as a "slight distension of preexisting cytoplasmic clear spaces," was greater in the 800-PPM groups compared to the vacuolization observed in the 400-PPM groups. 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. The authors considered the vacuolization to represent increased glycogen storage. Vacuolization is not considered adverse. Incidences of forestomach squamous epithelial hyperplasia were significantly increased in 800-PPM males and in females exposed to >= 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 also was observed in 1,600-PPM female mice. Forestomach hyperplasia is considered adverse because it was associated with infiltration of inflammatory cells and, at the highest concentration in females, focal ulcers. Grooming of contaminated fur or mucociliary clearance followed by ingestion likely played a central role in the increased incidence in hyperplasia of the forestomach. However, a role for inhalation cannot be ruled out and represents an area of uncertainty. 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 ingested following whole-body exposure.

A NOAEL of 200 PPM (NOAEL[ADJ] = 60 mg/m^3) can be identified in this study, based on mortality as an endpoint. The data for increased absolute liver weight in males in the absence of other liver effects at this level are not considered adverse findings. The level of 400 PPM is considered a FEL, given the early death (week 2) of one female (considered treatment-related) followed by increased mortality at higher concentrations.

B6C3F1 mice were exposed to concentrations of 0, 50, 100, or 200 PPM (0, 84, 168, or 336 mg/m^3, respectively) for 111 weeks (duration-adjusted to 15, 30, or 60 mg/m^3) for 6 hours/day, 5 days/week. The exposures selected were based on the results of the 13-week study. Ten male and 10 female mice also were evaluated at 15 months, at which time liver, kidney (right), and lung weights were measured. Hematological parameters were not measured; this represents a shortcoming in the study protocol. Clinical signs and body weight were assessed throughout the study. After 2 years, animals were necropsied and examined for gross and microscopic alterations. No differences in the survival of the treated animals were observed that differed with those of the 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 on liver weight, suggesting that the changes observed in the 13-week study were adaptive. Forestomach squamous hyperplasia, the only nonneoplastic effect, 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), although the incidence at 50 PPM in females also was elevated; however, the severity of the effect was not concentration-related. At the 15-month interim sacrifice, there was a significant increase in the incidence of squamous hyperplasia in the forestomach of females administered 200 PPM ACN (incidences were 0/10, 1/10, 0/10, and 6/10). Because of the uncertainty of the role of inhalation in causing the forestomach lesions, neither a NOAEL nor a LOAEL can be identified for this endpoint. Thus, in the absence of unambiguous adverse effects of inhalation, only a NOAEL (200 PPM) can be identified in this study.

I.B.3. Uncertainty and Modifying Factors (Inhalation RfC)

UF = 100. A factor of 3 ($10^{½}$) was used for interspecies extrapolation, a full factor of 10 was used to protect sensitive human subpopulations, and 3 was applied for database deficiencies (e.g., reproductive endpoints, hematology in mice). Because two factors of 3 coalesce to a 10, a total uncertainty factor of 100 results.

No uncertainty factor was applied to the use of a subchronic study because there was no mortality in the longer term mouse study. 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. A partial UF of 3, instead of a full factor of 10, was used for database insufficiencies in the areas of limited data on reproductive endpoints involving exposure of laboratory animals before and during mating through parturition and the absence of hematological measurements in either mouse study. A full factor of 10 was not considered necessary for the following reasons: (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 that are lethal to dams.

MF = 10. A modifying factor of 10 was applied because of the uncertain role that inhalation may have played in the development of the concentration-related increase in the incidence of forestomach lesions in both male and female mice. A potential role of inhalation can be envisioned. Ahmed et al. (1992) administered ^{14}C-ACN to mice via intravenous injection. As early as 5 minutes postinjection, label was detected in nasal secretions, esophagus, and stomach contents.

I.B.4. Additional Studies/Comments (Inhalation RfC)

NTP (1996) also conducted 13-week and 2-year inhalation studies in 344/N rats. In the 13-week study, rats (10/sex/group) were exposed whole-body to concentrations of 0, 100, 200, 400, 800, or 1,600 PPM (0, 168, 336, 672, 1,343, or 2,686 mg/m^3, respectively), 6 hours/day, 5 days/week (duration adjusted to 0, 30, 60, 120, 240, or 480 mg/m^3, respectively). Complete histopathological examinations were performed on the 0-, 800- (excluding females), and 1,600-PPM rats. Bone marrow, testes, and thymus were examined in 400-PPM males only. Deaths occurred at 800 PPM (1 male) and 1,600 PPM (6 males and 3 females). 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 in the two high-concentration groups included hypoactivity and ruffled fur, with ataxia, abnormal posture, and clonic convulsions in the 1,600-PPM males that died. The other groups did not exhibit any treatment-related signs. Thymus weights were significantly lower in the 800- and 1,600-PPM animals (both sexes) compared to those of the control. Significant decreases in red blood cell count, hemoglobin concentration, and hematocrit occurred in the 800- and 1,600-PPM females and the 1,600-PPM males. The investigators reported that alterations were suggestive of anemia (characterized as nonresponsive, normocytic, and normochromic because of unaffected reticulocyte counts, mean cell volume, and mean cell hemoglobin concentration). The 1,600-PPM females also exhibited a decrease in triiodothyronine concentration, without changes in thyroxine and thyroid-stimulating hormone concentrations. Histopathologic effects were limited to animals who 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 concentration, it is prudent to regard 800 PPM as a FEL [FEL(ADJ) = 240 mg/m^3]. Because of limited histopathological examinations in the lower concentration groups, the reported results are insufficient to identify a NOAEL.

In the 2-year study, F344/N rats (56/sex/group) were exposed to ACN concentrations of 0, 100, 200, or 400 PPM (0, 168, 336, or 672 mg/m^3, respectively), 6 hours/day, 5 days/week, for 103 weeks (duration adjusted to 0, 30, 60, or 120 mg/m^3, respectively). The exposures selected were based on the results of the 13-week study. Eight male and eight female rats also were evaluated at 15 months, with hematological parameters and liver, kidney, and lung weights measured. Hematology was not performed at study end and is considered a shortcoming of the study protocol. Clinical signs and body weight were assessed throughout the study. After 2 years, animals were necropsied and examined for gross and microscopic alterations.

No significant changes in survival, body weights, or clinical appearance were observed in rats following the 103-week exposure to ACN. At the 15-month interim sacrifice, hematological alterations were observed, but were significant ($p <= 0.05$) 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, and the changes appear minimal. There were no adverse effects seen on histopathology of the lungs or other organs. Histopathological examination revealed no nonneoplastic lesions in any organs of exposed females. Although a statistically significant increase in the incidence of basophilic foci was observed in the 200- and 400-PPM groups (15/48, 22/47, 25/48, and 31/48) of male rats, basophilic foci generally are considered possible preneoplastic effects. Thus, a NOAEL of 400 PPM [NOAEL(ADJ) = 120 mg/m^3] was identified for the rat.

An unpublished 90-day inhalation study in the B6C3F1 mouse was conducted by Hazelton Laboratories for NTP (Hazelton, 1983). 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, respectively) 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, respectively. Histopathological examination at necropsy included all major tissues and organs, including thymus, testes, ovaries, and lungs from controls and the 400-PPM group mice. Three sections of the nasal turbinates were examined from all animals in all groups. Livers were examined from mice in the 100- and 200-PPM groups as well. Clinical chemistry and hematological parameters also were examined. All animals from the control and 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 mice died during the course of the study. Mortality was not considered to be exposure-related (one male in each of the exposure groups). 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. There were no adverse effects on sperm or in the nasal turbinates.

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. Immunoglobulin G (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 Laboratories study (1984; see below) at these concentrations. Other tests of immune function (e.g., lymphocyte proliferation, delayed hypersensitivity, host resistance) were unaffected by exposure, thus the depressed IgG are of uncertain significance.

In an unpublished subacute study (ImmuQuest Laboratories, 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 control. It was stated that thymic atrophy in the 200- and 400-PPM groups (incidence/severity not stated) was observed; 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. Inasmuch as histologic evidence of thymic atrophy was not observed in either the NTP (1996) subchronic/chronic study or the Hazleton (1983) subchronic study, the statement presented in the ImmuQuest Laboratories (1984) report remains uncorroborated and is not sufficient evidence for use in RfC derivation. 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 immunoglobulin M and A. Tests of B-cell function were unchanged from controls.

Subchronic studies were performed on rats, monkeys, and dogs by Pozzani et al. (1959). Carworth Farms-Wistar rats (15/sex/group) were exposed to 0, 166, 330, or 655 PPM ACN vapors (0, 279, 554, or 1,100 mg/m^3, respectively), 7 hours/day, 5 days/week, for 90 days (duration adjusted to 0, 58, 115, or 229 mg/m^3, respectively) (Pozzani et al., 1959). Body weight and 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). Exposure to ACN did not affect body and organ weights in exposed animals. 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. No treatment-related tumors developed in any groups. Because the study was limited by a lack of details on protocol and quantitative results, an unambiguous NOAEL and LOAEL could not be identified.

Pozzani et al. (1959) also examined effects of 350 PPM ACN (588 mg/m^3) on three male rhesus monkeys and three male dogs (two Basenji-Cocker hybrids and one Basenji-Chow × Springer Spaniel hybrid), 7 hours/day, 5 days/week, for 91 days (duration adjusted to 123

mg/m^3). Controls consisted of two male Basenji-Cocker hybrids; 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. This experiment was limited because of inadequate study protocol and results and the absence of a control group for monkeys.

Nonpregnant female Sprague-Dawley rats (10/group) were exposed for 14 consecutive days to 0, 100, 400, or 1,200 PPM ACN (0, 168, 672, and 2,015 mg/m^3, respectively) (Mast et al., 1994). One dam 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 dams. Dams were not examined for histopathological effects. Thus, a NOAEL cannot be determined.

In the developmental toxicity portion of this study, sperm-positive female Sprague-Dawley rats (33/group) were exposed to 0, 100, 400, or 1,200 PPM (0, 168, 672, or 2,015 mg/m^3, respectively) ACN, 6 hours/day, 7 days/week, during gestational days 6 to 19, and then sacrificed on gestational day 20. 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. There was no effect on body or organ weights. Fertility did not appear to be affected by ACN exposure. A slight increase in percentage of resorptions per litter (particularly late resorptions) was seen at the high concentration; however, the effect was not significant or concentration-related. The percent of live fetuses per litter was not affected for any group. There were no treatment-related fetal malformations. The only effect on 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. Because exposure to ACN may 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. Inasmuch as developmental effects were not observed, a NOAEL of 1,200 PPM (2,015 mg/m^3) can be identified.

Pregnant Sprague-Dawley rats (20 to 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 3,068 mg/m^3, respectively), 6 hours/day, on gestational days 6 to 20 (not duration-adjusted) (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 gestational days 6 to 21. ACN 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. No differences were observed between the other exposed groups and the controls. ACN 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 was determined for maternal and developmental toxicity. A FEL of 1,827 PPM was determined for maternal deaths. Based on increased percentage of nonlive implants per litter, a LOAEL of 1,827 PPM (3,068 mg/m^3) and a NOAEL of 1,592 PPM (2,673 mg/m^3) were identified for the developmental endpoint.

Pregnant Syrian golden hamsters (6 to 12/group) were exposed to 0, 1,800, 3,800, 5,000, or 8,000 PPM ACN (0, 3,022, 6,380, 8,395, or 13,432 mg/m^3, respectively) for 1 hour on gestational day 8 and then sacrificed on gestational day 14 (Willhite, 1983). Parameters of maternal and developmental toxicity were evaluated. There were no effects of exposure on dams or offspring at 1,800 PPM One dam at 3,800 PPM died after exhibiting dyspnea, tremors, hypersalivation, ataxia, and hypothermia. All offspring from the other five surviving animals in 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 fetuses exhibiting exencephaly and rib fusions were recovered from two litters of this exposure group. 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 8,000-PPM fetus exhibited extrathoracic ectopia cordis with accompanying defects in the sternum of the heart. This study identifies a NOAEL and FEL of 1,800 PPM (3,022 mg/m^3) and 3,800 PPM (6,380 mg/m^3) for maternal toxicity, respectively, and a NOAEL and LOAEL of 3,800 PPM (6,380 mg/m^3) and 5,000 PPM (8,395 mg/m^3) for developmental effects.

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

I.B.5. Confidence in the Inhalation RfC

Study — Medium
Database — Medium
RfC — Medium

The NTP (1996) studies were of medium confidence. Although the sample sizes were appropriate, histopathology was extensive, and data were reported in detail, hematology was not measured in mice and only at the 15-month interim evaluation in rats. The confidence in the database for the ACN RfC is rated as medium because of (1) the uncertain role of

inhalation in the development of forestomach lesions in the mouse study; (2) the lack of evaluation of possible effects of ACN on heart rate, ventilatory parameters, and blood pressure; and (3) the absence of two-generation studies. Although acceptable developmental studies involving the F_1 generation were carried out (via inhalation route) in two species (rats and hamsters) with evidence of developmental effects occurring at maternally toxic concentrations, there is a lack of information on reproductive endpoints in animals exposed prior to and during mating through parturition. A medium confidence in the RfC follows.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

I.B.6. EPA Documentation and Review of the Inhalation RfC

Source Document — US EPA. (1999) Toxicological review of acetonitrile in support of summary information on the Integrated Risk Information System (IRIS).

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS Summary. A record of these comments is included as an appendix to the Toxicological Review of Acetonitrile in Support of Summary Information (a PDF document) on the Integrated Risk Information System (IRIS) (US EPA, 1999). ***To review this appendix, exit to the toxicological review, Appendix A, External Peer Review -- Summary of Comments and Disposition (PDF)***.

Other EPA Documentation — US EPA, 1985

Agency Consensus Review Date -- 1/26/1999

A comprehensive review of toxicological studies published through July 2005 was conducted. No new health effects data were identified that would be directly useful in the revision of the existing RfC for Acetonitrile and a change in the RfC is not warranted at this time. For more information, IRIS users may contact the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

I.B.7. EPA Contacts (Inhalation RfC)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (Internet address).

II. Carcinogenicity Assessment for Lifetime Exposure

Acetonitrile (ACN)
CASRN — 75-05-8
Last Revised — 03/03/1999

Section II provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen and quantitative estimates of risk from both oral and inhalation exposure. The 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. The third form in which risk is presented is a concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in the Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. Integrated Risk Information System (IRIS) summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those guidelines where indicated (Federal Register 61 [79]:17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

II.A. Evidence for Human Carcinogenicity

II.A.1. Weight-of-Evidence Characterization

Under the current Risk Assessment Guidelines (US EPA, 1987), ACN is assigned carcinogen class D, not classifiable as to human carcinogenicity. There is an absence of human evidence and the animal evidence is equivocal. Under the Proposed Guidelines for Carcinogen Risk Assessment (US EPA, 1996), the carcinogenic potential of ACN following inhalation, oral, or dermal exposure is best characterized as "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)."

The National Toxicology Program (NTP, 1996) concluded that the evidence for carcinogenicity via inhalation of ACN in the F344/N rat was equivocal based on a positive trend of hepatocellular tumors in male rats. Although there was a statistically significant positive trend in the incidence of hepatocellular adenomas, hepatocellular carcinomas, and hepatocellular adenomas or 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 increased incidence of basophilic foci in liver that was statistically significant in the 200- and 400-PPM groups. Although these foci were notatypical in appearance, as those more closely related to the carcinogenic process (Harada et al., 1989), altered hepatocellular foci are generally considered to be preneoplastic (Williams and Enzmann, 1998; Pitot, 1990). NTP (1996) concluded that "a causal relationship between ACN exposure and liver neoplasia in male rats is uncertain." There was no evidence of carcinogenicity in female rats or in either male or female B6C3F1 mice. The evidence from mutagenicity assays indicates that ACN does not cause point mutations. Acetonitrile was negative in assays with five strains of *S. typhimurium* in the absence of S9 as well as in the presence of rat or hamster S9 induced with Aroclor 1254. However, ACN does have potential to interfere with chromosome segregation, possibly leading to aneuploidy, as evidenced in experiments with *D. melanogaster*.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

II.A.2. Human Carcinogenicity Data

Inadequate; none are available.

II.A.3. Animal Carcinogenicity Data

NTP (1996) conducted a 2-year study with F344/N rats (56/sex/group) exposed to ACN, actual concentrations of 0, 100, 200, or 400 PPM (0, 168, 336, or 672 mg/m^3, respectively), 6 hours/day, 5 days/week, for 103 weeks (duration-adjusted to 30, 60, and 120 mg/m^3, respectively) and with B6C3F1 mice exposed to concentrations of 0, 50, 100, or 200 PPM (0, 84, 168, or 336 mg/m^3, respectively) for 111 weeks (duration-adjusted to 15, 30, and 60 mg/m^3, respectively). 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 examination, there were no neoplastic lesions observed that were attributable to exposure.

Histopathological exam at the 2-year necropsy revealed no neoplastic or nonneoplastic lesions in any organs of exposed females. In male rats, a statistically significant increase in the

incidence of basophilic 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. This suggests that the foci may not be preneoplastic. The incidences of eosinophilic and mixed cell foci were marginally elevated (but not to statistical significance) in 400-ppm males. 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.

At terminal sacrifice of the mice, the incidence of alveolar/bronchiolar adenomas was significantly increased in males 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 also were significantly increased in 200-ppm males ($p = 0.042$) (10/50, 14/50, 14/48, and 21/50). The 100-ppm males also exhibited an 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. However, the incidence of this lesion did not increase with increasing concentration. 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 2/50 in males; 1/49, 0/50, 1/50, and 3/48 in females); however, these increases were not statistically significant and equaled the highest values in historical controls. It is likely that grooming of contaminated fur or mucociliary clearance resulting in oral ingestion of ACN plays a central role.

II.A.4. Supporting Data for Carcinogenicity

The overall data indicate that ACN is not a point mutagen (Mortelmans et al., 1986; NTP, 1996), but does interfere with chromosome segregation (Galloway et al., 1987; NTP, 1996; Schlegelmilch et al., 1988). ACN also induced aneuploidy (chromosome gain and chromosome loss) in the oocytes of *D. melanogaster* females exposed either as larvae or as

adults (Osgood et al., 1991a,b), and in *S. cerevisiae* at 5% in the absence of S9 (Zimmerman et al., 1985). Zimmerman et al. (1985) suggested that the induction of aneuploidy by ACN and other aprotic polar solvents in the absence of point mutations or recombination resulted from interference with tubulin assembly and the formation of microtubules in the spindle apparatus. In this view, aneuploidy would be consistent with a nongenotoxic, nonlinear mechanism of carcinogenicity. Sehgal et al. (1990) obtained in vitro evidence that ACN inhibits microtubule assembly in taxol-purified drosophila or mouse microtubules, further indicating that ACN has potential to induce aneuploidy.

Micronucleated normochromatic erythrocytes 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). Positive micronucleus assays can indicate either clastogenic activity or interference with chromosome segregation. In light of the negative gene mutation assays and the marginal effects in chromosome aberration assays, these data indicate that ACN interferes with chromosome segregation both in vivo and in vitro.

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

Not Applicable

II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

Not Applicable

II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

II.D.1. EPA Documentation

Source Document — U.S. EPA. (1999) Toxicological review of acetonitrile in support of summary information on the Integrated Risk Information System (IRIS).

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS Summary. A record of these comments is included as an appendix to the Toxicological Review of Acetonitrile in Support of Summary Information (a PDF document) on the Integrated Risk Information System (IRIS) (U.S. EPA, 1999). ***To review this appendix, exit to the toxicological review, Appendix A, External Peer Review — Summary of Comments and Disposition (PDF)***.

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Consensus Date — 1/26/1999

A comprehensive review of toxicological studies published through July 2005 was conducted. No new health effects data were identified that would be directly useful in the revision of the existing carcinogenicity assessment for Acetonitrile and a change in the assessment is not warranted at this time. For more information, IRIS users may contact the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

II.D.3. EPA Contacts (Carcinogenicity Assessment)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (Internet address).

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Acetonitrile (ACN)
CASRN — 75-05-8

VI.A. Oral RfD References

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VII. Revision History

Acetonitrile (ACN)
CASRN — 75-05-8

Date	Section	Description
03/03/1999	I., II., VI.	RfD withdrawn, discussion added; new RfC and cancer assessment
12/03/2002	I.A.6., I.B.6., II.D.2.	Screening-Level Literature Review Findings message has been added.
08/15/2005	I.A.6., I.B.6., II.D.2	Screening-Level Literature Review Findings message has been removed and replaced by comprehensive literature review conclusions.

VIII. Synonyms

Acetonotrile (ACN)
CASRN — 75-05-8
Last Revised — 09/30/1987

- 75-05-8
- ACETONITRIL
- Acetonitrile
- CYANOMETHANE
- CYANURE DE METHYL
- ETHANENITRILE
- ETHYL NITRILE
- METHANECARBONITRILE
- METHANE, CYANO-
- METHYL CYANIDE
- NA 1648
- NCI-C60822
- RCRA WASTE NUMBER U003
- UN 1648
- USAF EK-488