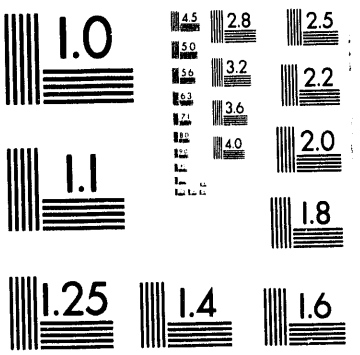
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INHALATION DEVELOPMENTAL TOXICOLOGY STUDIES: ACETONITRILE IN RATS

Final Report
Contract No. NIH-Y01-ES-70153

T.J. Mast
R.J. Weigel
R.B. Westerberg
P.J. Boyd
B.K. Hayden
J.J. Evanoff
R.L. Rommereim

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Pacific Northwerst Laboratory
Richland, Washington 99352

PEER REVIEW STATEMENT

This final technical report has been peer reviewed by two scientists who were members of an ad hoc panel of experts chosen for this purpose as part of the review process by the NTP Board of Scientific Counselors. Reviewers serve as independent scientists, not as representatives of any institution, company, or government agency. In this capacity, these reviewers determine if the design and conduct of the NTP studies were appropriate, ensure that the final report presents the experimental results, and that the conclusions are consistent with the data.

The reviewers of this report on the developmental toxicity of acetonitrile were:

Ms. Jacqueline A. Greene
The Wellcome Research Laboratories
Burroughs Wellcome Co.
Research Triangle Park, NC 27709

Dr. M. Kate Smith
Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

These reviewers agreed that the study design was adequate and that the conclusions were consistent with the data. Suggested corrections and comments related to style and format have been incorporated into the final draft.

Bernard A. Schwetz, D.V.M., Ph.D.
Project Officer

SUMMARY

Acetonitrile is a volatile liquid used primarily as a solvent in extractive distillation and crystallization of pharmaceutical and agricultural products. The potential for acetonitrile to cause developmental toxicity was assessed in Sprague-Dawley rats exposed to 0, 100, 400, or 1200 ppm acetonitrile, 6 hours/day, 7 days/week. Each of the four treatment groups consisted of 10 non-pregnant females (for comparison), 10 positively mated females for a distribution study evaluating maternal blood for acetonitrile and cyanide, and ~33 positively mated females for evaluating developmental toxicity. Rats were exposed for 14 consecutive days (6-19 days of gestation [dg] for pregnant animals). The day of sperm detection was designated as 0 dg. Body weights were obtained throughout the study period, and uterine and fetal body weights were obtained on 20 dg. Implants were enumerated and their status recorded. Live fetuses were sexed and examined for gross, visceral, skeletal, and soft-tissue craniofacial defects. Acetonitrile and cyanide concentrations were determined in the maternal blood of the rats (~6/group) on 8 and 18 dg.

Exposure of rats to these concentrations of acetonitrile resulted in mortality in the 1200 ppm group (2/33 pregnant females; 1/10 non-pregnant females), and the 400 ppm group (1/33 pregnant females). However, there were no treatment-related effects upon body weights or reproduction indices at any exposure level, nor was there a significant increase in the incidence of fetal malformations or variations. The only effect observed in the fetuses was a slight, but not statiscally significant, exposure-correlated increase in the incidence of supernumerary ribs.

Determination of acetonitrile and cyanide concentrations in maternal rat blood showed that acetonitrile concentration in the blood increased with exposure concentration for all exposed maternal rats. Detectable amounts of cyanide in the blood were found only in the rats exposed to 1200 ppm acetonitrile (~2 μg cyanide/g of blood).

In summary, the two highest exposure concentrations were maternally lethal to some rats; however, there was no reduction in body weights, body weight gains, or clinical signs of toxicity in surviving pregnant or non-pregnant rats. The no-observable-adverse-effect-level (NOAEL) for acetonitrile with respect to developmental toxiciyt in this study was the highest exposure concentration, 1200 ppm. The maternal NOAEL was 100 ppm.

TABLE I. Inhalation Developmental Toxicity Study of Acetonitrile: Summary of Results for Sprague-Dawley Rats.

Target Acetonitrile Conc. [ppm]	Maternal Mortality[a] [page 15]	Final Body Weight[b] (Mean[g]±SD) (% Difference from Controls) [page 15]	Clinical Observations (Incidence) [page 15]	Implants per Dam (Mean±SD) (% Difference from Controls) [page 15]	Live Fetuses per Litter (Mean±SD) (% Difference from Controls) [page 15]	Fetal Weight (Mean±SD) (% Difference from Controls) [page 15]	Significant Variations and Malformations [page 16]
0	0/33	413.0 ± 23.5	NS	15.9 ± 3.3	14.9 ± 3.4	3.6 ± 0.2 (M) 3.4 ± 0.2 (F)	NS
100	0/33	408.9 ± 28.5 (-1.0)	NS	15.0 ± 3.5 (-5.7)	14.0 ± 3.4 (-6.0)	3.7 ± 0.3 (M) (+1.5) 3.4 ± 0.2 (F) (+0.2)	↑ Supernumerary ribs
400	1/33	410.2 ± 37.1 (-0.7)	NS	14.7 ± 3.2 (-7.5)	14.0 ± 3.3 (-6.0)	3.6 ± 0.2 (M) (-0.9) 3.4 ± 0.2 (F) (0)	NS
1200	2/33	407.0 ± 43.0 (-1.5)	Hypoactive (14/33) Emaciated (6/33) Dsypnea (1/33) Abnormal posture (2/33) Bloody vaginal discharge (1/33)	16.0 ± 2.8 (+0.6)	14.8 ± 4.1 (-0.7)	3.5 ± 0.2 (M) (-2.6) 3.3 ± 0.2 (F) (-2.3)	NS

[a]Includes all sperm positive developmental toxicity rats.
[b]Includes only pregnant females.
NS = not toxicologically significant.

CONTRIBUTING PERSONNEL

DJ Anderson, BS	Chemistry Specialist
PJ Boyd, BS	Developmental Toxicology Specialist
ML Clark, MS	Health and Safety Officer
JR Decker, BS	Bioengineer
JJ Evanoff, MS	Toxicology Scientist
LJ Florek, AA	Exposure Specialist
LS Gorham, AS	Animal Care Supervisor
BK Hayden	Chemistry Specialist
AE Jarrell, BS	Veterinary Specialist
TJ Mast, PhD, DABT	Principal Investigator /Developmental Toxicology Task Leader
RL Rommereim, BS	Developmental Toxicology Specialist
SE Rowe, DVM, MS, ACLAM	Laboratory Animal Veterinarian
RJ Weigel, PhD	Exposure Task Leader
RB Westerberg, PhD	Chemist/Chemistry Task Leader

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SIGNATURES

Terryl J. Mast, PhD, DABT
Principal Investigator and
Developmental Toxicology Task Leader

2/16/94
Date

R. Bruce Westerberg, PhD
Chemistry Task Leader

2/25/94
Date

Richard J. Weigel, PhD
Exposure Task Leader

2/17/94
Date

Stephen E. Rowe, DVM, ACLAM
Laboratory Animal Medicine Veterinarian

2/25/94
Date

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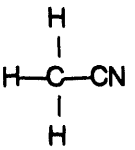
INTRODUCTION



CAS Number: 75-05-8
NTP Number: C60822
Mol. Wt.: 41.05

Acetonitrile (methyl cyanide, cyanomethane, or ethane nitrile) is used primarily as a solvent in extractive distillation and crystallization of pharmaceutical and agricultural products including vitamins, steroids, bactericides, insecticides, plant growth regulators and fungicides. It is used also as a catalyst in chemical reactions. Because acetonitrile is produced commercially as a byproduct in the synthesis of acrylonitrile, its estimated annual production (80 million pounds) is much higher than the total U.S. market (<10 million pounds). The excess portion of acetonitrile is disposed of by the manufacturer. It is estimated that 26,000 workers may be exposed to acetonitrile (NIOSH 1978). Occupational exposure to acetonitrile can occur via inhalation or absorption through the skin (Fassett 1963). The recommended TLV is 40 ppm (ACGIH 1990).

Toxicity

In humans, exposure to acetonitrile vapor produces symptoms ranging from irritation of nose, throat and skin to nausea, headache, extreme weakness, respiratory depression, convulsions, coma and death (Amdur 1959; Hygienic Guide Series 1960).

In animals, administration of acetonitrile by inhalation or injection produces a series of toxicological signs. The lowest published LC_{50} for a single, 8-hour inhalation in rats is reported to be 8000 ppm and the LD_{50} varies widely (0.85 to 5 g/kg) depending on the route of administration and species used (Fassett 1963; NIOSH 1978). Exposure to acetonitrile vapor at 166 or 300 ppm, 7 hours/day for 90 days produced no specific effects in rats (Pozzani et al. 1959); however, at 665 ppm some lesions were observed in the kidney, liver and lung. Rats exposed to acetonitrile vapor at 2800 ppm, 2 hours/day for 5 days, had difficulty in breathing, impaired renal function, and paralysis of the extremities (Haguenoer et al. 1975a). In another study, rats exposed to acetonitrile vapor at 3000 ppm for 4 hours a day showed

marked hepatic toxicity (Drew et al. 1978). Ahmed and Farooqui (1982) compared the toxicities of several aliphatic nitriles in the Sprague-Dawley rat following oral administration and found that all saturated nitriles tested, including acetonitrile, produced signs similar to those resulting from inorganic cyanide poisoning.

A 90-day inhalation study conducted for the NTP (Chou et al. 1986) in Fischer rats (F344/N) exposed to 100, 200, 400, 800, and 1600 ppm acetonitrile, 6 hours/day, 5 days/week, for 13 weeks assessed the prechronic toxicity of acetonitrile. There was significant mortality in the 1600 ppm group (6/10 males; 3/10 females), but only one early death in the 800 ppm group (1/10 males). Histopathological evaluations revealed brain hemorrhage, bone marrow depletion, and thymic atrophy in the 1600 ppm group, and bone marrow depletion and thymic atrophy in one male in the 800 ppm group. The bone marrow depletion and thymic atrophy were found only in early death animals and were thought to result from stress. Histopathological lesions associated with acetonitrile exposure were not observed in surviving rats. The maximum tolerated concentration was 400 ppm in both sexes, although lesions in the 800 ppm group were termed as minimal to mild.

Metabolism

The literature on the metabolism of acetonitrile dates as far back as 1883 when Giacosa postulated that acetonitrile was metabolized to acetic acid and ammonia in the dog. A subsequent study in 1894 correctly concluded that it was converted into formate and cyanide (Lang 1894). Since then many investigators have reported the presence of cyanide and thiocyanate in urine and blood of animals exposed to acetonitrile via inhalation or injection (Pozzani et al. 1959; Williams 1959; Haguenoer et al. 1975a,b).

The toxicity of aliphatic nitriles has been attributed to the metabolic release of cyanide (Willhite and Smith 1981). Cyanide reacts with the trivalent iron of cytochrome oxidase in mitochondria, thereby blocking the reduction of oxygen required for cellular respiration which results in "cytotoxic anoxia". Thus, the inhibition results in impaired oxygen utilization, not impaired oxygen transport.

The detoxification of cyanide is accomplished by the enzyme rhodanese as follows:

$$\underset{\text{Cyanide}}{CN^-} + \underset{\text{Thiosulfate}}{S_2O_3} \xrightarrow{\text{rhodanese}} \underset{\text{Thiocyanate}}{SCN^-} + \underset{\text{Sulfite}}{SO_3^{2-}}$$

Thiosulfate serves as a substrate for rhodanese which mediates the conversion of cyanide to the much less toxic thiocyanate which is excreted in urine. The enzyme serves as an endogenous mechanism for cyanide metabolism; however, the provision of exogenous sulfur greatly accelerates the rate of detoxification. Liver rhodanese is thought to play the major role in cyanide detoxification although

rhodanese is also found in other organs. Biological monitoring of human exposure to cyanide is accomplished by analysis of urine and/or plasma for thiocyanate (Klaasen et al. 1986).

Nasal metabolism of acetonitrile to cyanide, particularly important in inhalation studies, has been shown by Dahl and Waruszewski (1989). They found that aliphatic nitriles were metabolized to cyanide by the ethmoturbinates as well as liver microsomes, although the rate of cyanide formation was lower for acetonitrile than for some other aliphatic nitriles (e.g. propionitrile, butyronitrile, etc.). High concentrations of rhodanese are present in the nasal respiratory and olfactory mucosa of the rat (Dahl 1989). Thus, the net effect of nasal metabolism on cyanogenic compounds such as acetonitrile may not be significant since cyanide may be detoxified as rapidly as it is formed.

Developmental Toxicity

The potential for aliphatic nitriles to cause developmental toxicity in a number of laboratory animal models has been suggested by several studies, although a clear relationship has not been established. A study by Levene (1961) reported that acetonitrile was not a potential teratogenic agent in rodents even though, another lathyrogenic agent, aminoacetonitrile was developmentally toxic (Levene 1961; Steffek et al 1971; Wiley and Joneja 1978). Based on the results of these studies, it was postulated that the amine group was a structural requirement for the teratogenic activity. However, more recent studies (discussed below) indicate that exposure of hamsters to acetonitrile (by inhalation or injection) during gestation produces malformations in the offspring (Willhite et al. 1981a,b; Willhite 1983). Another aliphatic nitrile, acrylonitrile, was assessed for developmental toxicity by Murray et al. (1978) and found to be embryotoxic (and maternally toxic) in Sprague-Dawley rats when administered by oral gavage at 65 mg/kg from 6-15 days of gestation. There was also evidence of teratogenicity (short tail, short runk, missing vertebrae and right-sided aortic arch) at this dose as well as at a lower dose, 25 mg/kg. These investigators also administered acrylonitrile via inhalation at 0, 40 or 80 ppm, 6 hr/day during day 6-15 of gestation, and found evidence of teratogenicity at 80 ppm. Another study in Sprague-Dawley rats produced similar skeletal abnormalities in the offspring of dams orally exposed to 1.23 mmole/kg/day on days 6-15 of gestation (Willhite et al. 1981a).

George et al. (1987) exposed Long-Evans rats to 50, 150, 300 and 500 mg/kg acetonitrile by oral gavage on 7-21 dg. Maternal toxicity, evidenced by a reduction in maternal weight gain and three deaths was present at 500 mg/kg. There were also fewer dams delivering viable litters in the 300 and 500 mg/kg groups. Neither the litter size (number of live pups) or the birth weight of pups from dams producing live litters were affected at any of the dose levels. Other parameters measured, but not affected, included postnatal survival to 4 dpn, and weight gain of pups to 4 dpn.

In a similar study, Sprague-Dawley rats were administered acetonitrile by oral gavage, 0, 125, 190, and 275 mg/kg, on 6-19 dg (Johannsen et al. 1986). There was evidence of maternal toxicity in the 275 mg/kg group including reduced maternal weight gain (18%) with respect to the control group, and 2/25 dams in the group died. There were no significant effects on the number of total implants, resorptions, postimplantation losses, or live fetuses, or on the fetal sex ratio and fetal body weight at 125 and 190 mg/kg acetonitrile. However, the incidence of post-implantation loss was slightly increased and the number of live fetuses slightly decreased in the 275 mg/kg group. A slight increase in the incidence of unossified sternebrae was observed, but was not statistically significant. The authors attributed these developmental effects to maternal toxicity.

Since cyanide levels are known to increase following administration of acetonitrile it is relevant to consider the potential for gestational exposure to cyanide alone to cause developmental toxicity. Pregnant golden Syrian hamsters were exposed to cyanide or cyanide concurrent with thiosulfate by means of Alzet minipumps on days 6-9 of gestation (Doherty et al. 1982). Cyanide administration significantly increased the incidence of resorptions in a dose-related manner, and the number of malformed fetuses was increased relative to controls in the two lower dose groups. Neural tube defects including nonclosure, encephalocoele, and exencephaly accounted for the majority of the abnormalities. Although the incidence of malformations was not increased in the highest exposed group, only 15 fetuses survived the treatment. Concurrent exposure to thiosulfate ameliorated the effects of cyanide with respect to resorptions and malformations.

The results of this study indicate that cyanide may be teratogenic when administered to hamsters by continuous infusion. This conclusion is supported by the fact that concurrent administration of thiosulfate prevented the developmental toxicity as well as lowered cyanide concentrations in the blood. Furthermore, the results substantiate the supposition that cyanide may have been the proximate agent in the developmental toxicity of aliphatic nitriles since malformations produced in this study were similar to those found in hamsters by Willhite et al. (1981b) following administration of either propionitrile or acrylonitrile. Elevated levels of cyanide and the metabolite thiocyanate have been demonstrated in both mice and hamsters following the administration of aliphatic nitriles (Willhite and Smith 1981; Willhite 1981).

Although acetonitrile is a volatile organic solvent, only one inhalation study addressing developmental toxicity has been reported. Willhite (1983) exposed pregnant hamsters via inhalation to 0, 1800, 3800, 5000 or 8000 ppm acetonitrile for 60 min on the morning of 8 dg (first reported by Willhite [1981]). In order to determine if detoxification of the cyanide resulting from acetonitrile exposure would prevent developmental toxicity, a concurrent group was administered thiosulfate, 300 mg/kg, by

intraperitoneal injection (i.p.), 20 min prior to the inhalation exposure. Thiosulfate injections were repeated at 2-hr intervals for the following 10 hr. To compare the inhalation, i.p., and oral routes of administration, a second group was given a single i.p. injection of distilled water, 100, 200, 300 or 400 mg/kg acetonitrile on the morning of 8 dg, and a third group was given the same doses orally. Companion groups were also given the thiosulfate injections as described above.

Dams exposed to 8000 ppm acetonitrile were severely intoxicated and 3/12 died within 90 min. Exposure to 5000 ppm acetonitrile also caused severe maternal toxicity and 1/6 died. No signs of toxicity were observed in the 3800 ppm group although 1/6 in this group also died. The incidence of resorptions was increased in the 5000 and 8000 ppm dams as was the incidence of abnormalities (exencephaly, encephalocoele, and rib defects). The mean fetal weight was reduced in the 8000 ppm group. Concurrent treatment with thiosulfate prevented both maternal toxicity and subsequent developmental toxicity.

Oral administration of acetonitrile resulted in 4/12 deaths in the 400 mg/kg group and 1/6 deaths in the 300 mg/kg group. Like the inhalation group, administration of thiosulfate ameliorated the toxic effects. The incidence of rib defects was increased in the 300 and 400 mg/kg groups, but the incidence of CNS or other major malformations was not increased. Fetal weights were not adversely affected. Parenteral administration did not cause maternal or developmental toxicity.

Cyanide and thiocyanate concentrations were elevated in a dose-related fashion in the blood, liver, brain and kidneys of animals exposed by either the parenteral or oral route. Female hamsters were exposed to equivalent doses by both routes of administration and resulting blood and tissue levels were roughly equivalent. Cyanide concentrations were low in all tissues up to the 400 mg/kg treatment when they increased dramatically. The greatest increase in thiocyanate levels was found in the blood followed by the liver, kidney and brain.

The dose-related increases in fetal abnormalities and the incidence of resorptions implies that acetonitrile is developmentally toxic in hamsters. The fact that these increases are coincidental with increases in blood cyanide levels and that the types of defects seen following inhalation are similar to those produced following cyanide infusion in the hamster (Doherty et al. 1982), suggests that cyanide resulting from acetonitrile metabolism is the causative agent. The complete lack of developmental toxicity following parenteral administration of acetonitrile, especially in light of the blood cyanide levels which were actually greater than those following oral administration of the same doses, is curious and not readily explained from the results of these studies. Since the cyanide and thiocyanate blood levels were

determined on non-pregnant females it is possible that the state of pregnancy itself may alter the metabolism of acetonitrile.

Summary

It can be concluded that acetonitrile is metabolized to cyanide which is subsequently detoxified to thiocyanate in a variety of mammalian species including humans. Furthermore, when administered in sufficient doses acetonitrile and other aliphatic nitriles appear to be developmentally toxic, at least in the hamster. Acetonitrile appears to be more developmentally toxic when administered by inhalation than by other routes. It is possible that the increased toxicity following inhalation is a result of the active metabolism of acetonitrile to cyanide by nasal and pulmonary cytochrome oxidases. The volatile nature of acetonitrile dictates that the inhalation route will be the most likely route of human exposure in the workplace although dermal exposure could also be a significant route. In order to more accurately assess the hazards of gestational exposure to acetonitrile, the following inhalation developmental toxicity study on acetonitrile was conducted.

MATERIALS AND METHODS

The study design employed in assessing the developmental toxicity of acetonitrile in rats is outlined in Table 1. The day of plug or sperm detection was designated as 0 dg. Non-pregnant females were included in the study to serve as internal controls against the pregnant animals, i.e. to determine whether or not the state of pregnancy affected the response of the females rats to the test article. Non-pregnant females were selected from females not found to be sperm-positive during the mating period. They were killed the day after the last day of exposure.

The selection of exposure concentrations is based on results of the acetonitrile subchronic studies conducted at Battelle Northwest Laboratories (Chou 1986). The highest exposure concentration, 1200 ppm, was not expected to cause more than a 10% reduction in adjusted maternal body weight gain with respect to the control group. The lowest exposure concentration, 100 p$_{\text{i}}$ m, was approximately twice the 8-hour TLV (40 ppm) and should provide for an adequate safety margin assuming it is a no observable adverse effect level (NOAEL) for developmental toxicity. The middle dose was chosen to provide a continuum in the dose-response curve.

Exposure System

The animals were exposed and maintained in inhalation exposure chambers developed at BNW (U.S. Patent No. 4,216,741, August 12, 1980; Moss, 1980; Brown and Moss, 1981; Moss *et al.*, 1982) and now commercially produced by the Harford System Division of Lab Products, Inc., Aberdeen, MD. The chamber (Figure 1) facilitated multiple-tier exposures of various laboratory animal species to aerosol- and vapor-laden atmospheres. The total volume of the chamber was 2.3 m^3 with an active mixing volume of 1.7 m^3, the remainder being the inlet and exhaust volumes where animals were not placed. There are three levels of caging, each level split into two tiers which are offset from each other and from the chamber walls. Drawer-like stainless steel cage units composed of individual animal cages are suspended in the space above each tier. Stainless steel catch pans for the collection of urine and feces are suspended below each cage unit. Catch pans were left in position during each exposure period since the chamber was designed to maintain uniform aerosol or vapor concentrations throughout the chamber when the catch pans are left in position. Cageboard was added to catch pans placed under the cage units during non-exposure periods to reduce ammonia concentrations. Tests could be obtained repeatedly showing that uniform aerosol or vapor concentrations within 3 to 8% throughout the chamber, provided the aerosol or vapor was uniformly mixed before passing through the chamber inlet (Moss 1980; Moss *et al.* 1982). These tests were performed at BNW on a dynamically similar model of the chamber, as well as in the full

scale chamber. Work at the Inhalation Toxicology Research Institute of the Lovelace Foundation, Albuquerque, NM has confirmed these findings (Griffis *et al.*, 1981).

The acetonitrile exposures were conducted using an automated data acquisition and control system in an exposure suite (Figure 2) consisting of several exposure rooms and a suite control center room (only one of the exposure rooms was used for acetonitrile exposures). A central computer monitored and controlled the basic chamber functions (i.e., test chemical concentration, airflow, vacuum, temperature, and relative humidity [RH]) in the three exposure rooms.

A schematic diagram of the acetonitrile generation and delivery system is shown in Figure 3. The acetonitrile generator was housed in a vented cabinet located in the suite control room. The acetonitrile to be vaporized was transferred from the original shipping container to a 5.6-liter stainless steel reservoir, which was refilled once each week. A nitrogen cover was maintained at all times while transferring the acetonitrile and in the reservoir. During exposure, acetonitrile was pumped from the reservoir through an eductor tube and delivery tube to a vaporizer located in the fresh air duct leading directly to the vapor distribution manifold.

The vaporizer was a stainless steel cylinder covered with a glass fiber wick from which the liquid was vaporized. The wick was replaced before the start of the study and very little residue was observed during the study. An 80-watt heater and a temperature sensing element was incorporated within the cylinder and was connected to a remotely located temperature controller. A second temperature monitor was incorporated within the vaporizer allowing the operating temperature to be recorded by the automated data acquisition system. The operating temperature of the vaporizer core was maintained below 230°F.

A clear teflon tube of measured volume preceded by a three-way valve was attached just upstream of the reservoir to allow measurement of the pump delivery rate of chemical to the vapor generator. The distribution line concentration was calculated from the flow measurements of liquid and dilution air and agreed with the measured concentration in the distribution line.

The acetonitrile vapor was mixed with charcoal-filtered and HEPA-filtered air from the building air handling system. This mixture was drawn into a stainless-steel distribution manifold by an Air-Vac vacuum pump (Air-Vac Engineering Co, Milford, CT). From the manifold, the appropriate amount of vapor/air mixture needed to reach the target concentration was carried to each exposure chamber by individual delivery lines. Vapor was withdrawn from the manifold by an Air-Vac pump at the chamber end of each delivery line. Chamber concentrations were adjusted by changing the compressed air pressure to the vacuum pumps. At the end of the delivery line, the vapor entered a pneumatic 3-way valve where it was

directed to the exposure chamber or the chamber exhaust system as appropriate. These valves permitted a faster buildup of vapor concentration at start-up as well as a more rapid diversion of chemical flow from chambers at shutdown.

Exposure concentration buildup and decay rates were measured prior to the start of the study without animals and during the study with animals (Figure 4). The time following the start of exposure for the concentration to reach 90% of the final stable concentration in the chamber (T_{90}) and the time following the termination of generation for the vapor concentration to decay to 10% of the stable concentration (T_{10}), were determined from the graphs (Table 2). The value of T_{90} was found to range from approximately 8 to 11 minutes and the value of T_{10} ranged from 9 to 12 minutes. At a chamber airflow rate of 15 air changes per hour, the theoretical value for T_{90} is approximately 12.5 minutes; however, slightly different flow rates in each chamber and variations in the discrete GC sample times relative to the exposure start and stop times generates variability in the data. A T_{90} of 12 minutes was chosen for this study.

Uniformity of vapor concentration in the exposure chambers was measured prior to the start of the study and once during the study (Table 3). Prior to animal loading, 12 chamber positions [six positions in front and six in back] were measured. The vapor concentration measurements with animals were taken from the front and back positions of the chamber only where cage units contained animals. Uniformity in all chambers was found acceptable. Complete data are found in Appendix B.

Prior to the start of the study, before animals were placed in the chambers and again during the study with animals in the chambers, a Gardner Small Particle Detector (Type CN, Gardner Associates, Schenectady, NY) was used to check the room and all chambers for particles during generation. The minimum resolution of the Gardner counter is approximately 200 particles/cm^3. No counts above the minimum resolvable level were measured in any chamber .

In order to determine the persistence of the chemical in the chamber following exposure, the concentration of acetonitrile in the 1200 ppm chamber was monitored following shutoff of the chemical flow to the chamber. Monitoring was performed once during the prestart activities without animals, and again during the study when animals were present (Figure 5). Concentration of acetonitrile in the chamber without animals was below 1% of the initial concentration approximately 21 minutes following shutdown of the vapor generation system compared with approximately 29 minutes when animals were present in the chambers.

The means of concentrations in all chambers for the entire study were between 99 and 101% of the target, with relative standard deviations (%RSD) of 2%. At least 99% of individual concentration

measurements were within ±10% of the target concentrations (Table 4). Summaries of concentration by exposure day are included in Appendix B along with graphic illustrations of the daily mean and standard deviation for each chamber. No concentration excursions or problems occurred during the study.

Chamber and room temperatures (±0.5°F) were measured with calibrated resistance temperature detectors (RTDs) multiplexed to a digital thermometer interfaced to the computer. Chamber temperature was controlled primarily by adjusting the temperature of the exposure room. Percent relative humidity (%RH) was calculated by the executive computer from temperature and dew point measurements. The dew point was determined by pulling an air sample through a polytetraflouroethylene (PTFE) tube into a dew point hygrometer located in the control center. Measurements were taken from different chambers using a valving system which multiplexed the sampling tubes to the hygrometer.

Chamber air flow (±15 l/min) was calculated by measuring the pressure drop across calibrated orifices located at the inlet and exhaust of each chamber. Leaks in the chambers could be detected by comparison of the inlet flow rate with exhaust flow rate. Flow was established by a gate valve in the exhaust line of each chamber. Chamber vacuum, relative to the control center, (±0.2 cm H_2O) was measured using the same pressure transducer system which measured chamber air flows. Chamber vacuum was maintained at approximately -1" H_2O primarily by inlet resistance provided by the HEPA and charcoal filters.

Summations of temperature, relative humidity, and chamber flow data for the entire study are shown in Table 5. All temperature readings were within the specified limits. The mean values of percent relative humidity in all chambers for the entire study were within the specified limits of 40 to 70%. In no case were more than 7% of the individual readings in a single chamber out of the specified range. The mean values of chamber flow in all chambers for the entire study were all within the specified limits of 12 to 18 CFM. A summary of the daily chamber environmental data can be found in Appendix B.

Analytical Chemistry

Acetonitrile test material (J.T. Baker Inc., Phillipsburg, NJ) was stored in its original containers at ~22°C under a nitrogen headspace until use. Approximately 0.73 kg acetonitrile was consumed per exposure day. Five different bottles of acetonitrile were used during the study, four from one batch and one from another. The purity of both batches was ≥99.7% and contained no more than 18.8 ppm acetic acid. (See Appendix A for details.)

Chamber and room concentrations of acetonitrile were determined using an on-line Hewlett-Packard Model 5840 gas chromatograph equipped with a flame ionization detector operated at 250°C. The column was

1/8 inch O.D. x ~1 ft. nickel packed with Porapak Q 80/100 maintained isothermally at 100°C, the carrier gas was nitrogen at ~30 ml/min. Under these conditions, acetonitrile exhibited a retention time of ~0.90 minutes. A 12-port stream select valve mounted in the column oven interfaced the on-line gas chromatograph with the exposure chambers, the control chamber, the exposure room, the on-line standard and a filtered air blank. Each analysis required about 3 minutes. All chamber positions, the room, the blank, and the on-line standard were monitored at ~30 min intervals.

The stability and purity of acetonitrile in the exposure chambers and the distribution line were investigated by analyzing samples collected from the high and low concentration chambers (1200 and 100 ppm) and the vapor distribution line using solvent-filled bubblers that had been cooled in ice. In addition, samples of acetonitrile were obtained from the generator reservoir test material prior to beginning the daily animal exposure and again after the daily animal exposure was terminated. The heated wick was also analyzed during pre-exposure testing since it was a site where impurities could accumulate; however, none were detected.

Samples were analyzed for volatile and moderately volatile contaminants by two different capillary column gas chromatography systems. Propionitrile was present as a minor impurity, <0.1% (w/w) in the 1200 ppm exposure atmosphere and in the distribution line. Any propionitrile present in the 100 ppm chamber was below the limits of detectability. No impurities or degradation products were detected from the analysis of generator reservoir samples. (See Appendix A for details.)

Effluent from the exposure chambers and the exposure generation system was diluted with the exhaust air of the entire LSL-II Building prior to exhausting from the building stack. The expected concentration of acetonitrile in the building exhaust was about 0.5 ppm. The average building exhaust concentration of acetonitrile was determined to be 0.2±0.07 ppm. The ACGIH-TLV for acetonitrile is 40 ppm. The amount of acetonitrile determined in the building exhaust is 20 times below the Battelle action limit of 4 ppm.

Animal Husbandry

Upon receipt, all animals (378 females; 95 males) were housed in quarantine rooms for 3-4 weeks prior to the start of exposure. Males and females were housed separately on stainless steel wire racks equipped with automatic waterers (3-6 rats per cage). During the quarantine period five males and five females were killed and examined for gross and microscopic lesions. Nasopharyngeal washes from these animals were cultured for bacterial pathogens. Serum from each animal was tested for antibodies to selected pathogens (Appendix D). Another check for antibodies to selected pathogens was performed on serum obtained at the final sacrifice from five females in the 0 ppm group and from five females in the highest exposure group. All results were negative for significant pathogens and lesions.

Food, pelleted NIH-07 diet (Ziegler Bros., Inc., Gardner, PA), was provided *ad libitum* during the entire time the animals were in the test facility except during the 6-h exposure period when it was removed to prevent contamination and oral ingestion of the test material. Water was provided *ad libitum* with automatic waterers throughout the study. Room lighting was maintained on a 12-h on-off cycle (0600-1800 h for the light phase). During the quarantine period animal room temperature was maintained at 75±3°F and the percent relative humidity was maintained at 50±15%.

Developmental Toxicity

Female rats were weighed and individually identified by tail tattoos 1-2 days prior to mating. Rats were bred by caging 2-3 females overnight with each male. A positive mating was established on the following morning by the presence of a sperm-positive vaginal lavage. If sperm was detected, this day was designated as 0 days of gestation (dg) and positively mated females were then weighed and randomly assigned to exposure groups using body weight as the blocking variable. Mating was conducted for four consecutive nights to obtain 132 positively mated female rats (33/group). At this time 40 female rats were randomly selected from the females not found to be sperm-positive, and designated as non-pregnant females. Non-pregnant females were also randomly assigned to the four exposure groups, using body weight as the blocking variable. The positively mated females were individually caged on 0 dg (non-pregnant females were individually caged 3 days prior to exposure) in an open exposure chamber in order to acclimate the animals to inhalation chamber housing prior to exposure.

Pregnant rats were exposed from 6-19 dg and sacrificed on 20 dg. Non-pregnant rats were exposed for 14 consecutive days, concurrently with mated animals, and were sacrificed on the day after their last exposure day. Mated rats were weighed on 0, 6, 10, 14, 17 and 20 dg. Non-pregnant rats were weighed 7 days prior to the start of exposure and on exposure days 1, 5, and 10, and at sacrifice. Study animals were observed twice daily for maortality, mobidity, and overt signs of toxicity.

At the time of sacrifice rats were killed with CO_2, weighed and examined grossly for signs of toxicity. Maternal and non-pregnant female liver and kidney weights were obtained. Apparently non-gravid uteri from positively mated females were stained with 10% ammonium sulfide to detect possible implantation sites. The number, position and status of implants were recorded for each gravid uterus. Placentas were examined and discarded unless abnormal. Dams with ≤3 implantation sites were excluded from the study.

Live fetuses were weighed, examined for gross defects, and their sex was determined by internal examination of the gonads after a lethal injection of sodium pentobarbital. Fifty percent of the live fetuses (randomly selected) from each litter and any fetuses with gross external abnormalities were examined for visceral

defects by dissection of fresh tissue (modified from the method of Staples 1977). The heads of one-half of the live fetuses were removed and placed in Bouin's fixative. After fixation the heads were serially sectioned with a razor blade and examined for soft-tissue craniofacial abnormalities. All fetal carcasses, with and without heads, were prepared for skeletal staining. Cartilage, as well as ossified bone, was visualized by double-staining with alcian blue and alizarin red S. The individual identity of each skeletal and head specimen was maintained throughout the study.

Acetonitrile and Cyanide Analysis

Excess sperm-positive females were randomly assigned to the four exposure groups (10/group) to be used for determination of acetonitrile and cyanide concentrations in maternal blood. Animals were exposed concurrently with the developmental toxicity study rats.

Samples were collected and analyzed for acetonitrile and cyanide on 8 and 18 dg (3rd and 13th days of exposure). At the time of collection of maternal blood, rats were anesthetized with CO_2 immediately after exposure shut down. Approximately 2-3 mls of blood was collected by intracardiac puncture from ~5 animals per group. The sampling procedure was completed within 45 min of exposure shutdown. The blood samples were placed on ice until used for analysis (<2 hours later). Following blood sampling rats were killed by inhalation of CO_2 and examined grossly to ascertain pregnancy.

Approximately 0.5 ml from each blood sample was used for analysis of acetonitrile and another ~0.5 ml used for analysis of cyanide. For acetonitrile analysis, the sample was placed into a headspace vial containing 2 ml of 3% NaCl and 1 ml of internal standard solution composed of propionitrile in 3% NaCl solution. The vials were immediately sealed and placed into a Hewlett-Packard 19395 Headspace Sampler.

For the analysis of cyanide concentration, each blood sample was placed in the outer moat of a Conway diffusion cell. NaOH (0.5 ml) was added to the inner moat, and 0.5 ml H_2SO_4 added to the blood sample within the outer moat. The acid was mixed with each sample by gentle agitation, and diffusion between the moats was allowed to proceed for 3 hours at room tempature. Solutions ranging from 0.2-0.4 ml of NaOH were removed from the outer moat and added to 2 ml of NaH_2PO_4 solution. Chloramine T (0.5 ml) was added to this solution and allowed to react for 3 min. Color forming reagents were added and cyanide in the solution quantitated using a Cary 219 spectrophotometer at 585.5 nm.

Cyanide standards ranging from 0.084 to 4.2 µg cyanide/g of blood were analyzed to generate a calibration curve followed by analysis of samples.

Statistical Analyses

All means and standard deviations for animal data were calculated with SAS statistical software on a VAX computer. Mean body weights (as a mean of litter means for fetal data) were analyzed using the SAS General Linear Models (GLM) Procedure (SAS, 1985) with an analysis of variance (ANOVA) model for unbalanced data. Response variables, either body weight or the arc-sin transformations of proportional incidence data, were analyzed against the class variable, "treatment", in a one-way ANOVA model. A Tukey's t-test (two-tailed) was used to assess statistically significant differences between control and exposed groups. If appropriate, the dose-response relationship was determined by means of an orthogonal trend test (Winer, 1971). In the case of proportional data the t-tests and trend analyses were performed on transformed variables. The litter was used as the basis for analysis of fetal variables.

RESULTS

Exposure and Chemistry

Actual mean acetonitrile exposure concentrations were 0 (<1 ppm), 100±2, 397±9, and 1200±28 ppm for the 0, 100, 400, and 1200 ppm groups, respectively. The grand means of chamber concentrations for all exposure levels for the study were within 99% to 100% of the target with relative standard deviations in the range of 2% (Table 4). At least 99% of all individual concentration measurements were within ±10% of the specified operating limits for the exposure target level. There was no measurable concentration of acetonitrile in the control chamber. Chamber air flow, temperature and relative humidity data for exposures were all within specified limits (Appendix B).

Developmental Toxicity

The mean pregnancy rate of the sperm-positive females was 79%. There was mortality in the 1200 ppm group (2/33 pregnant females; 1/10 non-pregnant females), and one early death in the 400 ppm (1/33 pregnant females). Approximately one-half of the pregnant rats (14/33) in the 1200 ppm exposure concentration were hypoactive during some portion of the study and some rats in this group were also reported as emaciated (6/33) in the 1200 ppm exposure concentration. No significant clinical signs were reported in the non-pregnant female rats. There were no significant gross lesions observed at sacrifice in any of the exposed animals. Three females in the non-pregnant group were removed from study when they were found to be pregnant (Table 6).

Mean body weights of non-pregnant females were not significantly affected at any time during the study (Table 7). Similarly there were no statistically significant reductions in maternal body weight (Figure 6), organ weights or adjusted maternal weight gains in pregnant dams (Table 8).

Gestational exposure of rats to acetonitrile vapors on 6-19 dg had no statistically significant effect on the number of implants per dam, the number or percent of live fetuses per litter or on the number or percent of total intrauterine deaths (Table 9). Fetal weights (as means of litter means by sex) were not affected by gestational exposure to acetonitrile vapor (Table 10). The fetal sex ratio was not different between the control and exposed groups.

The incidence of fetal malformations was not significantly increased by gestational exposure of rats to acetonitrile vapors, nor was the percent of live fetuses with malformations per litter[1] affected (Tables 11 and 12). The only variations observed in the fetuses was a slight, but statistically significant exposure-correlated increase in the incidence of supernumerary ribs (Table 13).

Acetonitrile and Cyanide Analysis

Maternal blood concentrations of acetonitrile in rats increased with increasing exposure concentration and were similar for a given exposure group on both 8 and 18 dg (Figure 7). Although blood acetonitrile concentrations were similar between 8 and 18 dg, blood cyanide concentrations appeared to decrease between 8 and 18 dg for the 1200 ppm exposure group.

Unfortunately, cyanide concentrations in the maternal blood were below the limits of detectability for all but the 1200 ppm group and one animal at each time point in the 400 ppm group. The detection limit for the assay was ~0.1 μg cyanide/gram of blood.

[1]The mean percent incidence per litter was calculated based on the number of live fetuses examined for a given malformation or variation per litter, i.e. the incidence of visceral defects was based on the number of fetuses in each litter that received a visceral exam.

DISCUSSION

Exposure of pregnant rats to 0, 100, or 400 ppm acetonitrile did not result in significant maternal toxicity or in developmental toxicity. Although there were no significant treatment-related effects upon maternal body weights or reproductive indices at any exposure level, there were a few deaths in the 1200 ppm exposure concentration and one early death in the 400 ppm exposure concentration. There was no evidence of developmental toxicity at any exposure concentration. Although there was a statistically significant increase in the incidence of supernumerary ribs in the offspring at 100 ppm acetonitrile there was no dose-response relationship and the incidence of this variation at both 400 and 1200 ppm was not different from the control group.

Since the toxicity of aliphatic nitriles, such as acetonitrile, has been attributed to the metabolic release of cyanide resulting in cytotoxic anoxic maternal blood was analyzed for acetonitrile and cyanide on 8 and 18 dg. Analysis results indicated significant and exposure-related concentrations of acetonitrile in the blood of all exposed groups and the presence of cyanide in the blood of 1200 ppm group. (Cyanide was detectable, but not quantifiable in the blood of the 400 ppm group.) In the 1200 ppm group, ~2 mg/ml cyanide was found in maternal blood on 8 dg, however, the concentration declined to ~0.8 mg/ml by 18 dg while acetonitrile concentration remained essentially constant over the exposure period. This decrease in the maternal blood cyanide level may have been due to induction of rhodanase, the enzyme thought to be responsible for the detoxification of cyanide (Klaasen et al. 1986 and others).

Although no developmental toxicity was seen in rats in this study exposed to 1200 ppm acetonitrile, exposure of hamsters, by either intraperitoneal injection or orally, to levels of acetonitrile producing blood cyanide levels much less than found in this study caused significant developmental toxicity (Willhite 1983; Doherty et al. 1982).

In summary, the offspring of Sprague-Dawley rats appeared to be relatively resistant to the toxic effects of acetonitrile at the exposure levels employed in this study. The two highest exposure concentrations were maternally lethal to some rats; however, there was no reduction body weights, body weight gains, or clinical signs of toxicity in surviving pregnant or non-pregnant rats. The no-observable-adverse-effect level (NOAEL) for acetonitrile with respect to developmental toxicity in this study was the highest exposure concentrations, 1200 ppm, approximately 30 times the ACGIH recommended 8-hr TLV. The maternal NOAEL was 100 ppm.

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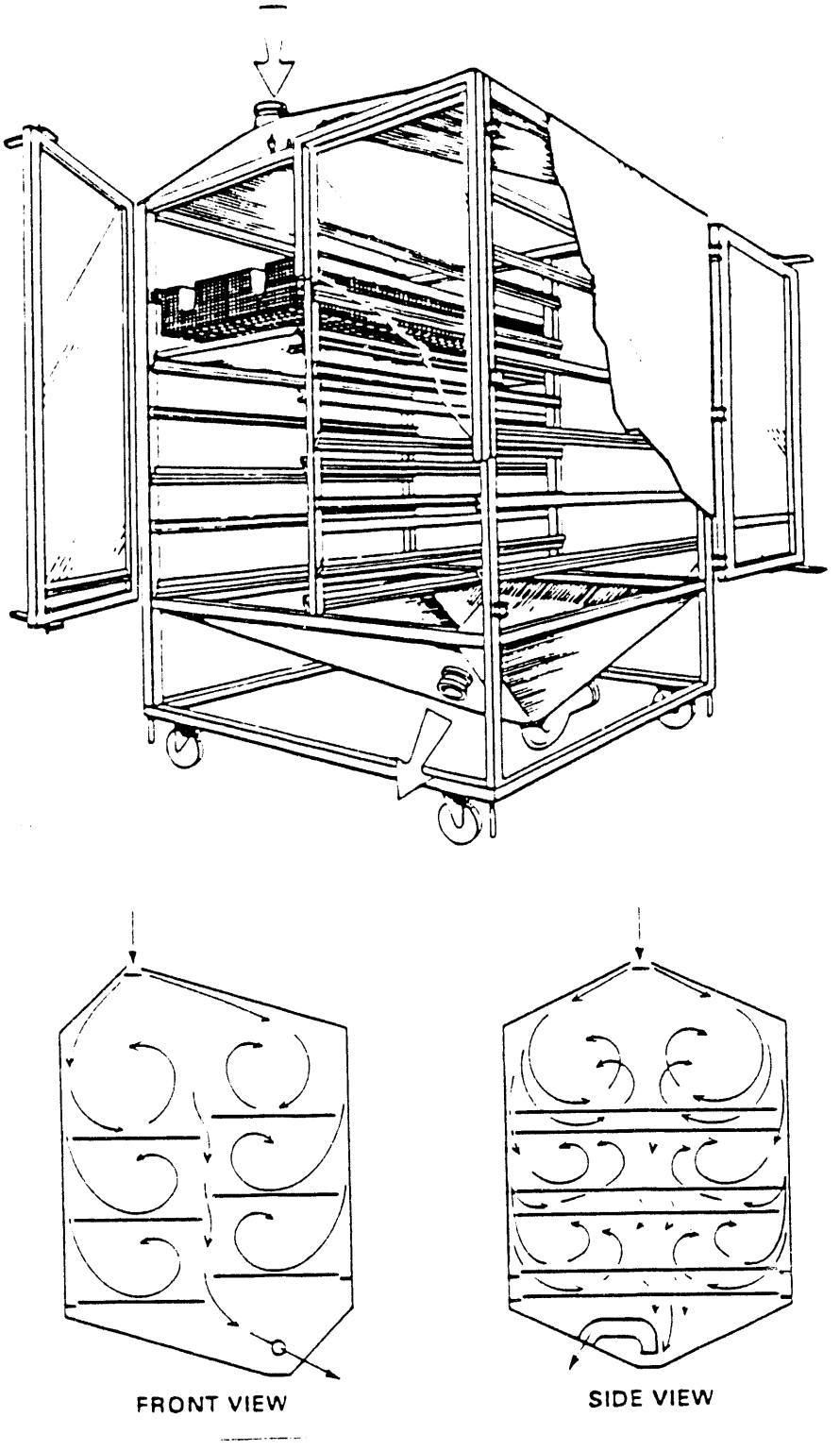

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FIGURE 1. Inhalation Developmental Toxicity of Acetonitrile in Rats: Inhalation Exposure Chamber (Top: Oblique Cutaway View; Bottom: Airflow Patterns).

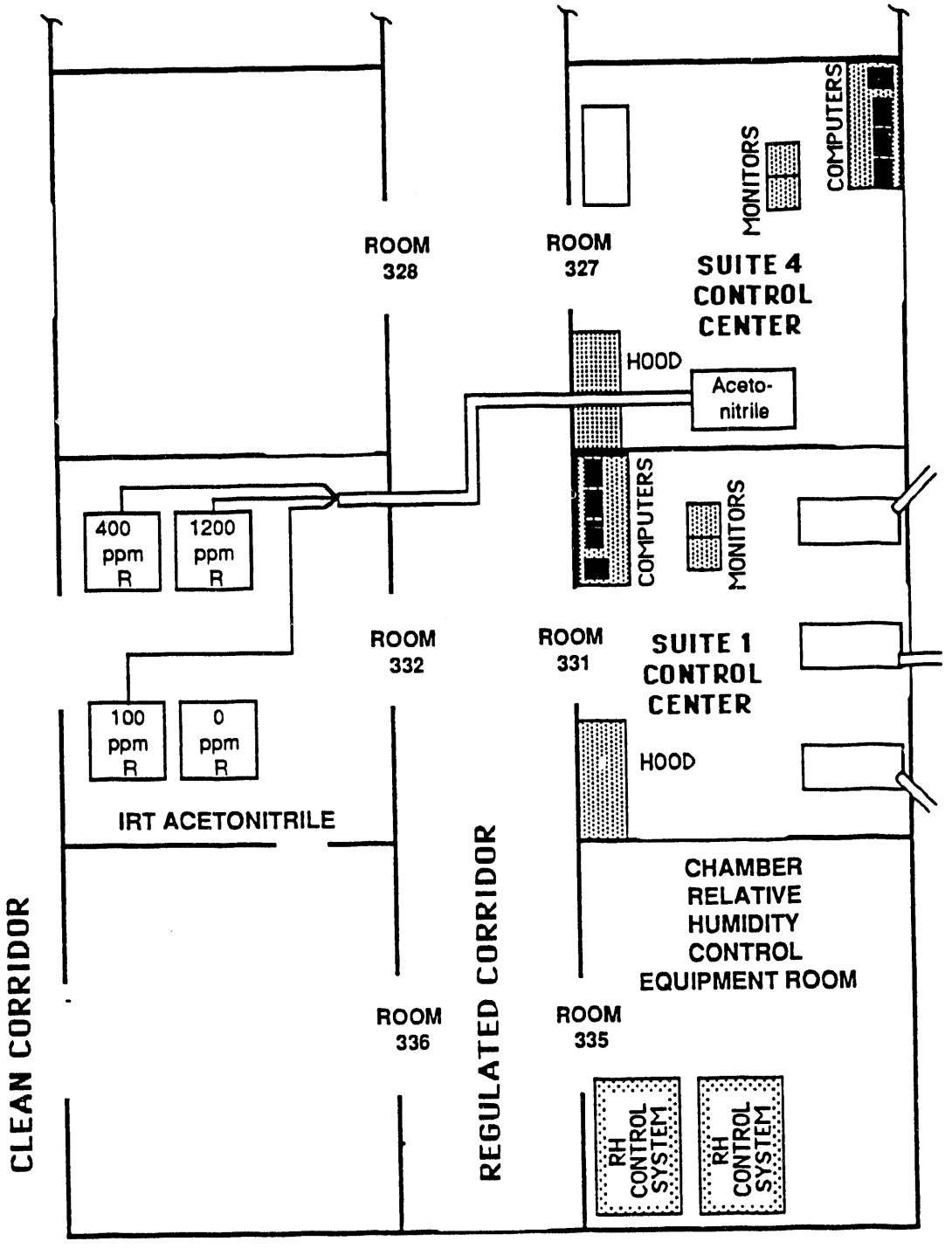


FIGURE 2. Inhalation Developmental Toxicity of Acetonitrile in Rats: Acetonitrile Exposure Suite.

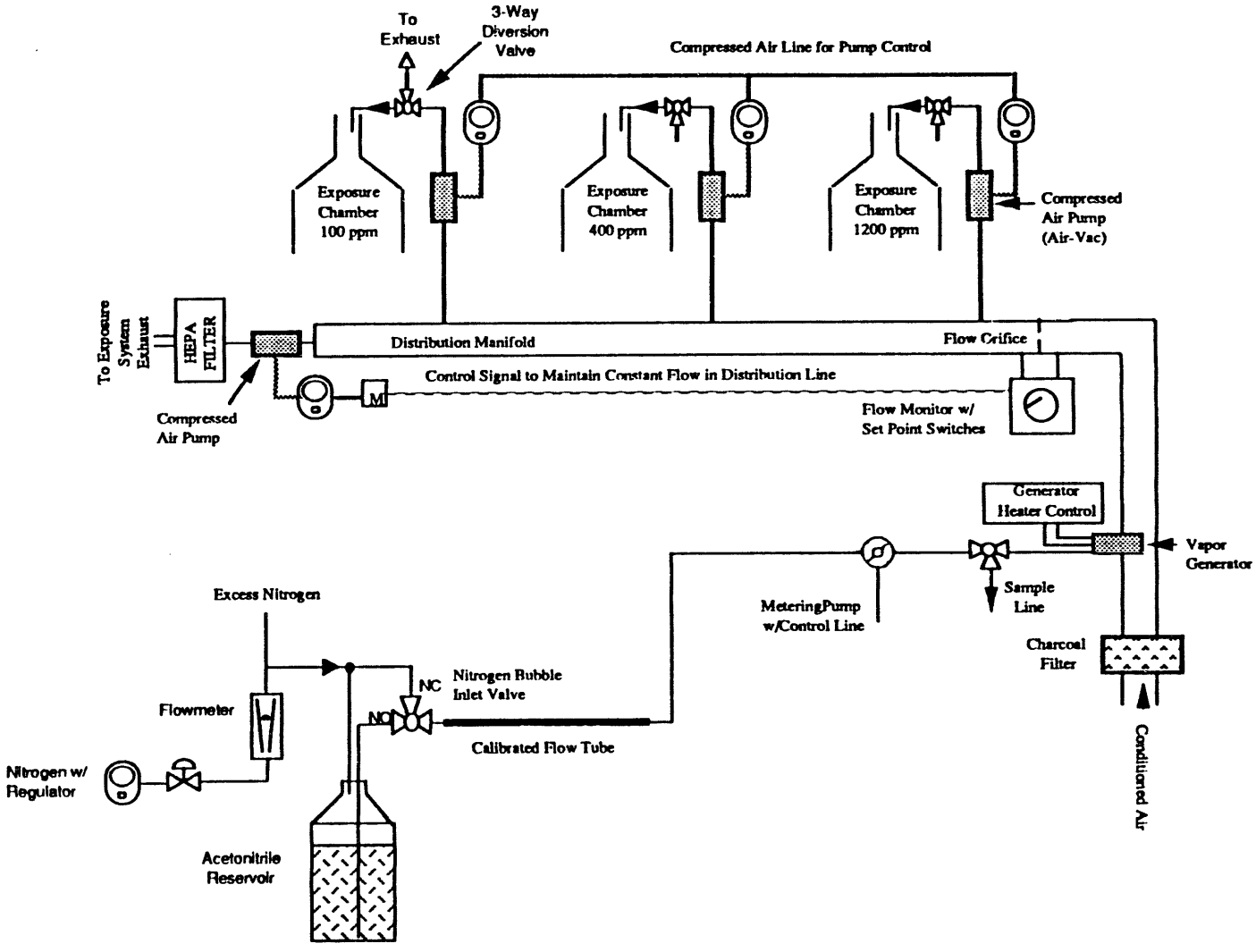


FIGURE 3. Inhalation Developmental Toxicity of Acetonitrile in Rats: Generation and Exposure System

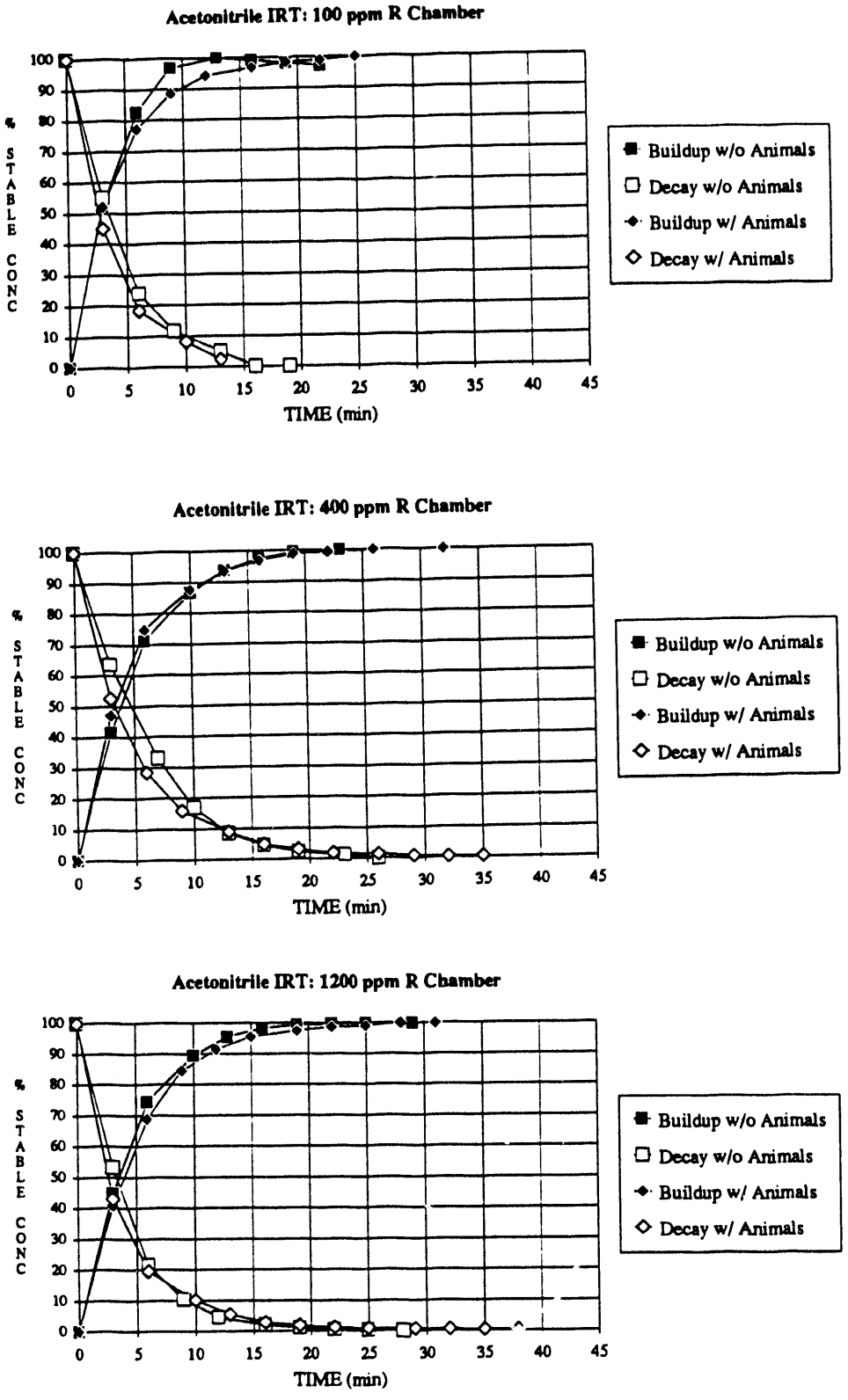


FIGURE 4. Inhalation Developmental Toxicity of Acetonitrile in Rats: Buildup and Decay Curves of Concentration in Animal Chambers.

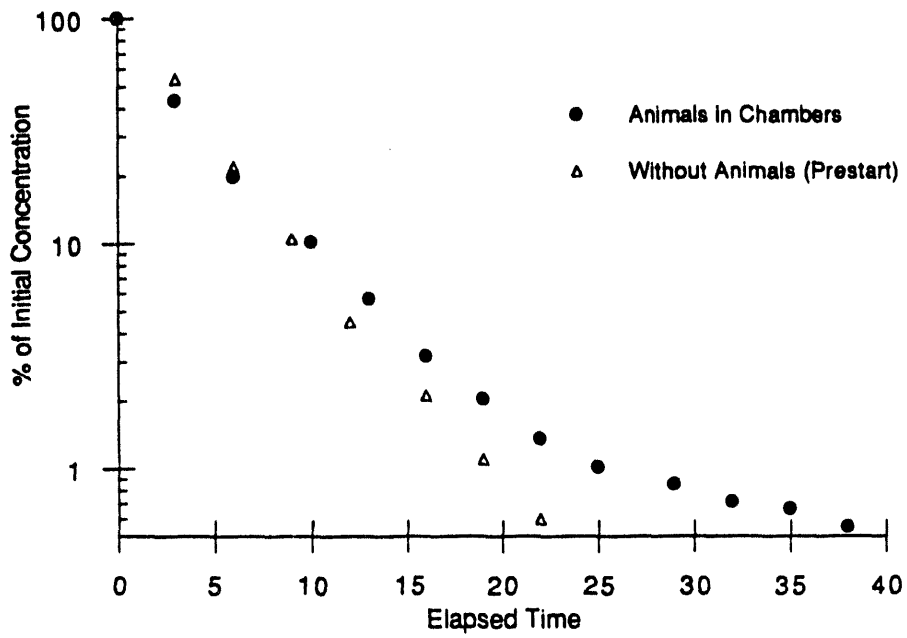


FIGURE 5. Inhalation Developmental Toxicity of Acetonitrile in Rats: Persistence of the Chemical in the Chamber Following Exposure With and Without Animals Present.

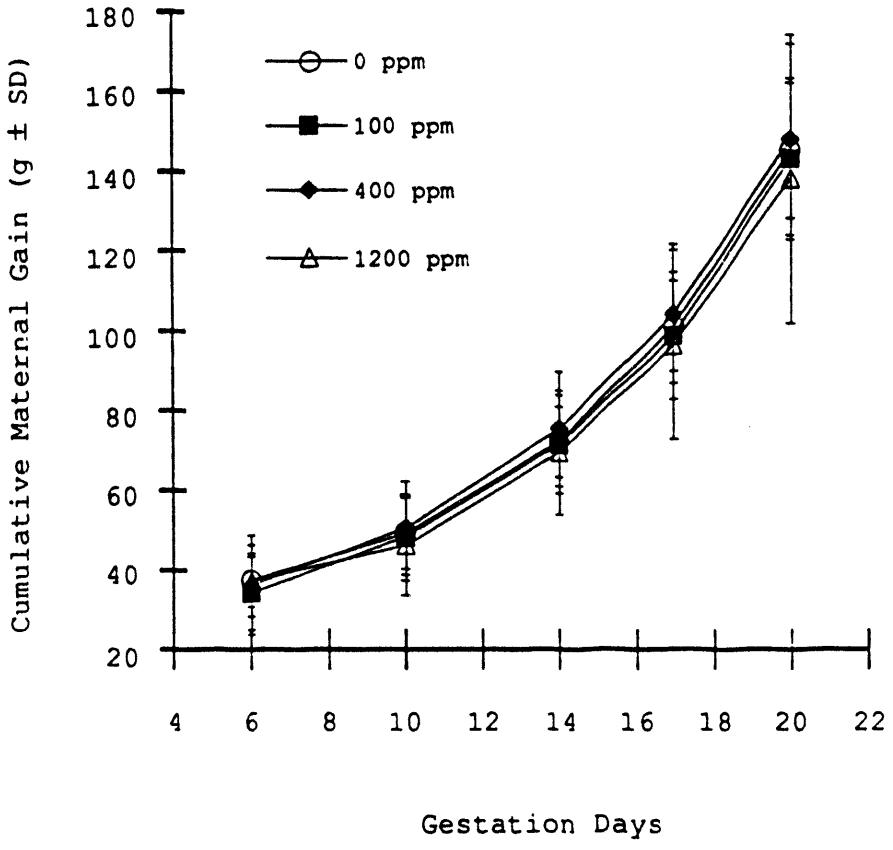


FIGURE 6. Inhalation Developmental Toxicity of Acetonitrile in Rats: Cumulative Maternal Weight Gain.

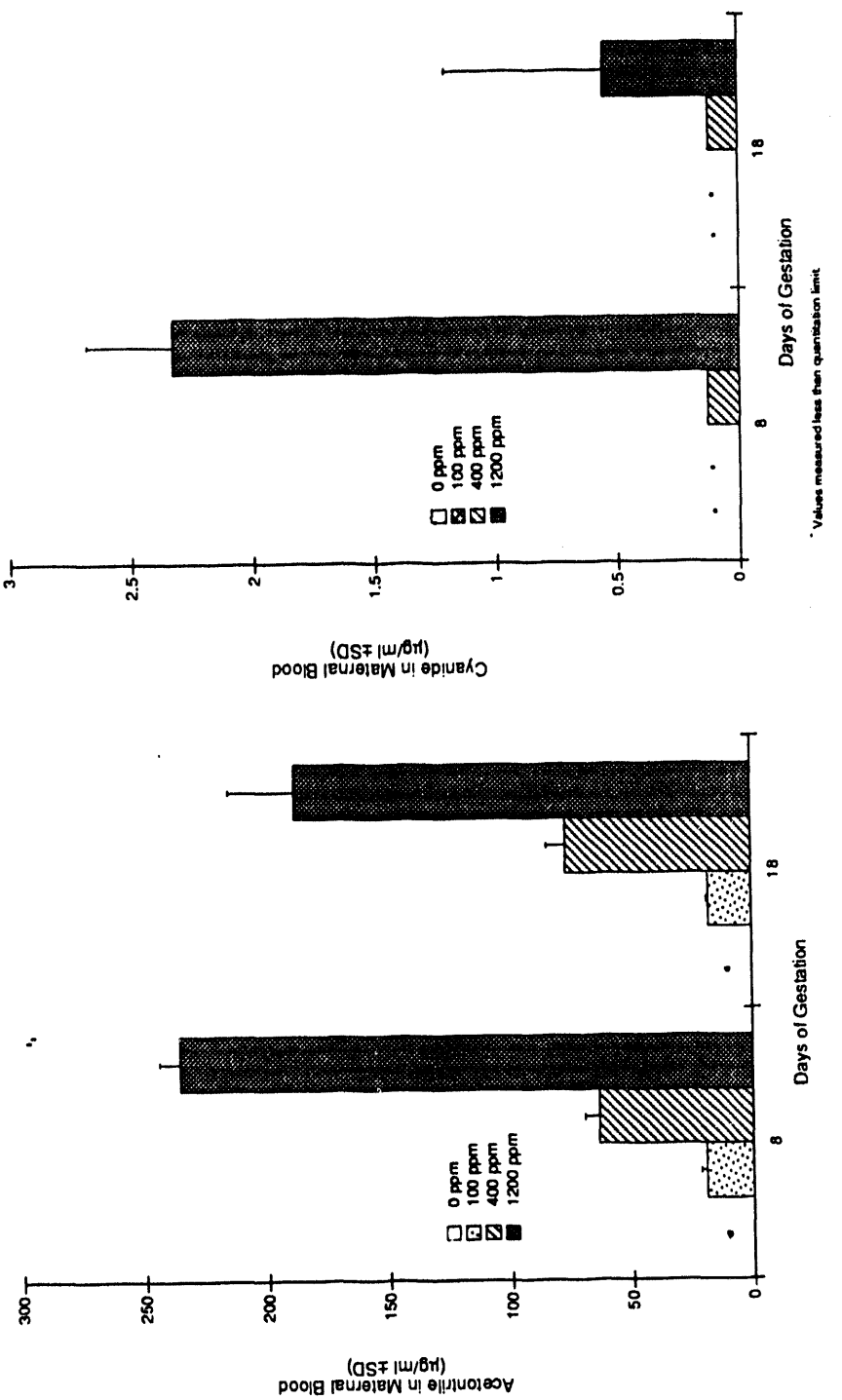

Acetonitrile in Maternal Blood
(µg/ml ±SD)
300
250
200
150
100
50
0
0 ppm
100 ppm
400 ppm
1200 ppm
8
18
Days of Gestation
Cyanide in Maternal Blood
(µg/ml ±SD)
3
2.5
2
1.5
1
0.5
0
0 ppm
100 ppm
400 ppm
1200 ppm
8
18
Days of Gestation
* Values measured less than quantitation limit

TABLE 1. Study Design for Acetonitrile Inhalation Developmental Toxicity Study.

Acetonitrile Exposure Concentrations	0, 100, 400 and 1200 ppm
Species, strain	Sprague-Dawley rat
Supplier	Charles River Laboratories Raleigh, NC.
Number per Group	
Non-pregnant	10 females
Developmental Toxicity	33 sperm-positive females
Distribution Determination acetonitrile and cyanide in blood	10 sperm-positive females
Exposure Duration	6 hr/day; 7 days/week; 6-19 days of gestation; 14 consecutive days for non-pregnant females
Fetal Evaluations	20 dg; gross, visceral, skeletal, and cranial

TABLE 2. Inhalation Developmental Toxicity Study of Acetonitrile in Rats: Summary of T_{90} and T_{10} Data in Each Chamber With and Without Animals.

Target Concentration (ppm)	T_{90} [min][a]		T_{10} [min]	
	Without Animals	With Animals	Without Animals	With Animals
100	8	10	10	9
400	11	11	12	12
1200	10	11	9	10

[a] A value of 12 minutes was used for T_{90} for the study.

TABLE 3. Inhalation Developmental Toxicity Study of Acetonitrile in Rats: Summary of Chamber Uniformity Data Obtained Before Exposure (Prestart) and During Exposure (Poststart).

Target Concentraton (ppm)	TPV [%RSD]		WPV [%RSD]		BPV [%RSD]	
	Prestart	Poststart	Prestart	Poststart	Prestart	Poststart
100	0.2	0.3	0.6	0.2	--[a]	0.2
400	0.7	0.2	1.2	0.5	--[a]	--[a]
1200	0.9	0.6	2.2	0.5	--[a]	0.3

[a] When the WPV is greater than the TPV, the BPV is very small and it cannot be resolved from the WPV.

TPV = Total Port Variation. Acceptable limit ≤ 7% RSD.
WPV = Within Port Variation. Acceptable limit ≤ 5% RSD.
BPV = Between Port Variation. Acceptable limit ≤ 5% RSD.

TABLE 4. Inhalation Developmental Toxicity Study of Acetonitrile in Rats: Summation of Exposure Concentration Data.

Target Conc. (ppm)[a]	Mean±SD (ppm)	Percent of Target ±%RSD	Maximum (ppm)	Minimum (ppm)	Number of Samples	Number of Samples In Range	Percent of Samples In Range
Room	<MDL[b]	———	<MDL	<MDL	253	———	———
0	<MDL	———	<MDL	<MDL	255	———	———
100	100±2	100±2%	107	89	188	187	>99
400	397±9	99±2%	437	341	190	189	>99
1200	1200±28	100±2%	1420	1130	190	188	99
Std Gas[c]	701±7	101±1%	721	684	196	196	100

[a] Acceptable Range = Target ± 10%.
[b] MDL (Minimum Detectable Limit = 1.1 ppm); QL (Quantifiable Limit = 8.3 ppm)
[c] Standard Gas Target Concentration = 695 ppm.

TABLE 5. Inhalation Developmental Toxicity Study of Acetonitrile in Rats: Summation of Chamber Environmental Data.

TEMPERATURE (°F)[a]

Target Concentration (ppm)	Mean±SD	Percent of Target±%RSD	Maximum	Minimum	Number of Samples	Number Samples In Range	Percent of Samples In Range
Room	73.0±1.4	—	77.8	69.7	532	—[b]	—[b]
0	75.4±1.1	101±1%	77.6	72.8	122	122	100
100	74.3±1.0	99±1%	76.5	72.0	122	122	100
400	74.9±1.0	100±1%	77.5	72.7	121	121	100
1200	74.7±1.1	100±1%	77.8	72.4	123	123	100

[a] Acceptable Range = 75±3°F for the exposure chambers.

RELATIVE HUMIDITY (%RH)[c]

Target Concentration (ppm)	Mean±SD	Percent of Target±%RSD	Maximum	Minimum	Number of Samples	Number Samples In Range	Percent of Samples In Range
0	59.8±5.1	109±9%	72	49	117	115	98
100	57.0±5.4	104±10%	69	46	119	119	100
400	56.1±6.5	102±12%	70	44	120	120	100
1200	57.5±7.4	105±13%	75	42	121	112	93

[c] Acceptable Range = 55±15%.

AIRFLOW (CFM)[d]

Target Conc. (ppm)	Mean±SD (cfm)	Percent of Target±%RSD	Maximum (cfm)	Minimum (cfm)	Number of Samples	Number of Samples In Range	Percent of Samples In Range
0	15.2±0.3	102±2%	15.8	14.7	125	125	100
100	15.0±0.4	100±3%	15.9	14.4	125	125	100
400	15.0±0.4	100±3%	15.8	14.3	125	125	100
1200	15.0±0.3	100±2%	15.8	14.5	126	126	100

[d] Acceptable Range = 12 to 18 CFM.

TABLE 6. Inhalation Developmental Toxicity of Acetonitrile in Rats: Disposition of Study Animals.

Target Acetonitrile Concentration (ppm)	Treatment Group	Sperm-Negative Females	Sperm-Positive Females	Early Deaths: Sperm-Negative Females	Early Deaths: Sperm-Positive Females	Sperm-negative Females Removed	Pregnant	Removed
0	1	10	33	0	0	2[a]	26	0
100	2	10	33	0	0	0	30	0
400	3	10	33	0	1[b]	0	26	0
1200	4	10	33	1[c]	2[d]	1[a]	26	0

[a]Negative sperm smear, but were pregnant.
[b]Mated dam found dead on 14 dg; cause of death was possible spontaneous cerebral hemorrhage.
[c]Mated female, moribund sacrifice on exposure day 8 (hypoactive, emaciated).
[d]One pregnant dam, moribund sacrifice on 15 dg (emaciated); another pregnant dam found dead on 19 dg (previously noted as hypoactive and emaciated).

TABLE 7. Inhalation Developmental Toxicity Study of Acetonitrile: Mean Body and Organ Weights of Nonpregnant Rats (g ± SD).

Target Acetonitrile Concentration (ppm)	0	100	400	1200
N	8	10	10	8
Body Weight				
Exposure Day 1	273.2 ± 17.7	273.6 ± 17.7	274.4 ± 18.8	276.0 ± 13.4
Exposure Day 5	292.6 ± 25.0	301.2 ± 29.7	302.2 ± 21.7	302.4 ± 24.0
Exposure Day 10	293.5 ± 22.5	306.5 ± 34.5	309.7 ± 21.0	308.6 ± 31.4
Terminal	295.7 ± 18.1	307.4 ± 30.2	316.6 ± 23.2	314.0 ± 23.1
Liver	11.4 ± 1.3	11.8 ± 1.8	12.4 ± 1.6	13.5 ± 1.2
Percent LBWR[a]	3.9 ± 0.3	3.8 ± 0.3	3.9 ± 0.4	4.3 ± 0.4
Kidney	2.2 ± 0.3	2.2 ± 0.3	2.1 ± 0.2	2.1 ± 0.2
Percent KBWR[b]	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.1
Adrenal	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.01
Percent ABWR[c]	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00

[a] LBWR = liver to body weight ratio x 100.
[b] KBWR = kidney to body weight ratio x 100.
[c] ABWR = adrenal to body weight ratio x 100.

TABLE 8. Inhalation Developmental Toxicity Study of Acetonitrile in Rats: Mean Body, Uterine, Adjusted Maternal Gain[a] and Organ Weights of Pregnant Rats (g ± SD).

Target Acetonitrile Concentration (ppm)	0	100	400	1200
N	26	30	23[a]	26
Body Weight				
0 dg	268.1 ± 16.6	266.0 ± 15.7	262.4 ± 16.9	269.1 ± 18.3
6 dg	305.7 ± 18.0	300.3 ± 17.2	298.7 ± 23.9	306.5 ± 23.7
10 dg	317.5 ± 17.6	314.4 ± 18.1	313.1 ± 25.6	315.3 ± 24.4
14 dg	340.3 ± 17.4	337.6 ± 20.2	337.8 ± 28.1	338.6 ± 26.7
17 dg	369.2 ± 19.9	364.6 ± 21.9	366.6 ± 31.7	365.3 ± 32.6
20 dg	413.0 ± 23.5	408.9 ± 28.5	410.2 ± 37.1	407.0 ± 43.0
Adjusted Maternal Weight Gain[b]	64.9 ± 12.5	65.9 ± 11.7	74.4 ± 24.0	61.9 ± 27.7
Uterine	80.0 ± 17.7	77.0 ± 18.2	73.4 ± 16.8	76.0 ± 24.6
Liver	16.2 ± 1.4	16.5 ± 1.8	16.8 ± 2.1	16.7 ± 2.2
Percent LBWR[c]	3.9 ± 0.3	4.0 ± 0.4	4.1 ± 0.3	4.1 ± 0.4
Kidney	2.1 ± 0.2	2.1 ± 0.2	2.0 ± 0.3	2.1 ± 0.2
Percent KBWR[d]	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Adrenal	0.075 ± 0.007	0.073 ± 0.014	0.080 ± 0.015	0.075 ± 0.011
Percent ABWR[e]	0.018 ± 0.002	0.018 ± 0.003	0.019 ± 0.003	0.019 ± 0.004

[a] Data from three dams was not used because the number of implant sites was ≤3.
[b] Adjusted Maternal Body Weight Change = body weight(20 dg) - body weight (0 dg) - uterine weight.
[c] LBWR = liver to body weight ratio x 100.
[d] KBWR = kidney to body weight ratio x 100.
[e] ABWR = adrenal to body weight ratio x 100.

TABLE 9. Inhalation Developmental Toxicity Study of Acetonitrile: Reproductive Measures (Mean ± SD) in Rats.

Target Acetonitrile Concentration (ppm)	0	100	400	1200
NUMBER OF:				
Sperm-Positive Females	33	33	33	33
Number Pregnant	26	30	26	26
Pregnancies Examined	26	30	23[a]	26
Implantations/Dam	15.9 ± 3.3	15.0 ± 3.5	14.7 ± 3.2	16.0 ± 2.8
Live Fetuses/Litter	14.9 ± 3.4	14.0 ± 3.4	14.0 ± 3.3	14.8 ± 4.1
Resorptions/Litter:	1.0 ± 1.0	1.0 ± 1.2	0.8 ± 1.1	1.2 ± 2.8
Early	0.9 ± 1.0	0.8 ± 1.0	0.7 ± 1.0	0.5 ± 0.8
Late	0.1 ± 0.3	0.2 ± 0.5	0.1 ± 0.3	0.7 ± 2.7
Dead Fetuses/Litter	0	0	0	0
Litters with Resorptions	16	17	11	12
Litters with ≥2 Resorptions	8	7	4	6
PERCENTAGE OF:				
Pregnant Females	79	91	70	76
Live Fetuses/Litter	93.2 ± 6.6	93.6 ± 7.5	94.9 ± 6.8	92.0 ± 19.8
Resorptions/Litter:	6.8 ± 6.6	6.4 ± 7.5	5.4 ± 7.6	8.1 ± 19.8
Early	6.1 ± 6.5	5.1 ± 6.6	4.6 ± 6.7	3.1 ± 5.6
Late	0.7 ± 2.1	1.3 ± 3.1	0.8 ± 2.7	4.9 ± 19.5
Dead Fetuses/Litter	0	0	0	0
Litters with Resorptions	62	57	48	48
Litters with ≥2 Resorptions	31	23	17	24

[a] Data from three dams was not used because the number of implant sites was ≤3 .

TABLE 10. Inhalation Developmental Toxicity Study of Acetonitrile: Average Fetal Weights (g±SD) and Fetal Sex Ratio (mean of litter means; % ± SD).

Target Acetonitrile Concentration (ppm)	0	100	400	1200
Litters Examined with Live Fetuses	26	30	23	25
Fetal Weight	3.5 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.4 ± 0.2
Male	3.6 ± 0.2	3.7 ± 0.3	3.6 ± 0.2	3.5 ± 0.2
Female	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.3 ± 0.2
Percent Male Fetuses	52 ± 13	49 ± 14	41 ± 15	50 ± 13

TABLE 11. Inhalation Developmental Toxicity Study of Acetonitrile: Malformations Observed in Live Rat Fetuses.

Target Acetonitrile Concentration (ppm)		Fetuses[a] 0	100	400	1200	Litters[a] 0	100	400	1200
Total Examined[b]		387	421	321	384	26	30	23	25
Heads examined[c]		195	210	158	193	26	30	23	25
Skulls examined[d]		192	211	163	191	26	30	23	25
Viscera examined[e]		192	211	163	191	26	30	23	25
MALFORMATIONS:									
Fused Ribs	No.	—	1	-	-	—	1	-	-
	(%)		(0.2)				(3.3)		
Fused Vertebral Arches	No.	-	—	1	-	-	—	1	-
	(%)			(0.3)				(4.3)	
Missing Rib	No.	—	1	-	-	—	1	-	-
	(%)		(0.2)				(3.3)		
Missing Vertebral Arches	No.	-	1	-	-	-	1	-	-
	(%)		(0.2)				(3.3)		
Anury	No.	—	1	-	-	—	1	-	-
	(%)		(0.2)				(3.3)		
Fused Adrenals	No.	-	1	-	-	-	1	-	-
	(%)		(0.5)				(3.3)		
Fused Kidneys	No.	—	1	-	-	—	1	-	-
	(%)		(0.5)				(3.3)		
Hemorrhagic Adrenals	No.	-	—	1	-	-	—	1	-
	(%)			(0.6)				(4.3)	
Edema	No.	—	-	1	-	—	-	1	-
	(%)			(0.3)				(4.3)	
Microophthalmia	No.	-	—	1	-	-	—	1	-
	(%)			(0.3)				(4.3)	
Ectopic Ovaries	No.	—	1	1	-	—	1	1	-
	(%)		(0.5)	(0.6)			(3.3)	(4.3)	
Major Vessel Malformation	No.	-	—	1	-	-	—	1	-
	(%)			(0.6)				(4.3)	
TOTAL:									
Malformations[f]	No.	0	7	6	0	—	—	—	—
Fetuses (Litters) with Malformations	No.	0	2	1	0	0	2	1	0
	(%)	(0.0)	(0.5)	(0.3)	(0.0)	(0.0)	(6.7)	(4.3)	(0.0)

[a] A single fetus or litter may be represented more than once in this table.
[b] All fetuses examined for external and skeletal defects. One-half of the fetuseshad heads removed prior to skeletal staining.
[c] Heads fixed in Bouin's solution for evaluation of soft-tissue craniofacial evaluations.
[d] Heads remained on the fetuses for skeletal examination; see (b).
[e] Visceral examinations performed on 50% of live fetuses.
[f] There may be >1 malformation per fetus.

TABLE 12. Inhalation Developmental Toxicity Study of Acetonitrile. Mean Percent of Live Rat Fetuses Affected per Litter (Mean Percent ± SD).

Target Acetonitrile Concentration (ppm)	0	100	400	1200
Litters Examined	26	30	23	25
MALFORMATIONS:				
Fused Ribs	-[a]	0.2 ± 1.3	-	-
Fused Vertebral Arches	-	-	0.3 ± 1.4	-
Missing Rib(s)	-	0.2 ± 1.2	-	-
Missing Vertebral Arches	-	0.2 ± 1.2	-	-
Anury	-	0.2 ± 1.2	-	-
Fused Adrenals	-	0.4 ± 2.3	-	-
Fused Kidneys	-	0.4 ± 2.3	-	-
Hemorrhagic Adrenals	-	-	0.5 ± 2.6	-
Edema	-	-	0.3 ± 1.4	-
Microophthalmia	-	-	0.3 ± 1.4	-
Ectopic Ovaries	-	0.4 ± 2.3	0.5 ± 2.6	-
Major Vessel Malformation	-	-	0.5 ± 2.6	-
Total Malformations	0.0 ± 0.0	1.6 ± 7.4	1.7 ± 8.3	0.0 ± 0.0
VARIATIONS:				
Supernumerary Rib	2.6 ± 5.7	10.6 ± 15.5[b]	6.4 ± 11.9	4.1 ± 5.4
Dilated Ureter	5.9 ± 13.4	5.3 ± 9.9	1.0 ± 4.6	10.3 ± 19.0
Renal Pelvic Cavitation	0.5 ± 2.8	1.0 ± 3.7	-	-
Misaligned Sternebra	0.3 ± 1.5	0.2 ± 1.3	1.1 ± 3.5	0.9 ± 2.7
Bent Rib	-	-	-	0.2 ± 1.1
Rudimentary Rib	-	0.2 ± 1.1	0.5 ± 2.5	0.6 ± 2.9
REDUCED-OSSIFICATION:				
Pelvis	3.3 ± 10.4	4.5 ± 9.1	1.3 ± 3.8	4.8 ± 8.0
Phalanges	1.3 ± 5.5	1.9 ± 3.9	-	0.9 ± 2.7
Skull	17.0 ± 20.0	10.7 ± 19.2	9.8 ± 16.8	13.0 ± 16.4
Sternebra	6.9 ± 7.2	8.8 ± 10.5	8.9 ± 10.9	9.3 ± 11.5
Vertebral Centra	5.1 ± 6.8	9.3 ± 11.9	3.8 ± 5.4	4.8 ± 7.5
TOTAL VARIATIONS	31.2 ± 26.1	44.2 ± 31.3	27.4 ± 20.4	37.5 ± 23.6

[a]Mean percent affected equals zero.
[b]Significantly different than control, $p<0.05$.

TABLE 13. Inhalation Developmental Toxicity Study of Acetonitrile: Variations and Reduced Ossifications Observed in Live Rat Fetuses.

Target Acetonitrile Concentration (ppm)		Fetuses[a] 0	100	400	1200	Litters[a] 0	100	400	1200
Total Examined[b]		387	421	321	384	26	30	23	25
Heads examined[c]		195	210	158	193	26	30	23	25
Skulls examined[d]		192	211	163	191	26	30	23	25
Viscera examined[e]		192	211	163	191	26	30	23	25
VARIATIONS:									
Supernumerary Rib	No.	10	38	21	17	6	16	8	11
	(%)	(2.6)	(9.0)	(6.5)	(4.4)	(23.1)	(53.3)	(35.8)	(44.0)
Dilated Uretur	No.	12	12	2	20	7	9	1	9
	(%)	(6.3)	(5.7)	(1.2)	(10.5)	(27.0)	(30.0)	(4.3)	(36.0)
Renal Pelvic Cavitation	No.	1	2	-	-	1	2	-	-
	(%)	(0.5)	(0.9)			(3.8)	(6.7)		
Misaligned Sternebra	No.	1	1	3	4	1	1	2	3
	(%)	(0.3)	(0.2)	(0.9)	(1.0)	(3.8)	(3.3)	(8.7)	(12.0)
Bent Rib	No.	-	-	-	1	-	-	-	1
	(%)				(0.3)				(4.0)
Rudimentary Rib	No.	-	1	2	2	-	1	1	1
	(%)		(0.2)	(0.6)	(0.5)		(3.3)	(4.3)	(4.0)
REDUCED OSSIFICATIONS:									
Pelvis	No.	15	17	4	18	4	8	3	10
	(%)	(3.9)	(4.0)	(1.2)	(4.7)	(15.4)	(26.7)	(13.0)	(40.0)
Phalanges	No.	6	9	-	4	2	7	-	3
	(%)	(1.6)	(2.1)		(1.0)	(7.7)	(23.3)		(12.0)
Skull	No.	30	23	17	26	13	11	8	11
	(%)	(15.6)	(10.9)	(10.4)	(13.6)	(50.0)	(36.7)	(35.8)	(44.0)
Sternebra	No.	30	37	29	38	16	17	15	12
	(%)	(7.8)	(8.8)	(9.0)	(9.9)	(61.5)	(56.7)	(65.2)	(48.0)
Vertebral Centra	No.	20	43	11	19	12	18	9	12
	(%)	(5.2)	(10.2)	(3.4)	(4.9)	(46.2)	(60.0)	(39.1)	(48.0)
TOTAL: Reduced Ossifications and Variations[f]	No.	125	183	89	150	-	-	-	-
Fetuses (Litters) with Variations	No.	102	133	75	119	26	29	21	23
	(%)	(26.4)	(31.6)	(23.4)	(31.0)	(100)	(96.7)	(91.3)	(92.0)

[a] A single fetus or litter may be represented more than once in this table.
[b] All fetuses examined for external and skeletal defects. One-half had heads removed prior to skeletal staining.
[c] Heads fixed in Bouin's solution for evaluation of soft-tissue craniofacial evaluations.
[d] Heads remained on the fetuses for skeletal examination; see (b).
[e] Visceral examinations performed on 50% of live fetuses.
[f] There may be >1 variation per fetus.

APPENDIX A

CHEMISTRY MONITORING NARRATIVE AND DATA

Test Chemical Analysis, Storage and Disposition
Test Chemical Stability Studies
Test Chemical Monitoring
Determination of Cyanide and Acetonitrile in Blood of Exposed Animals

Test Chemical Analysis, Storage and Disposition

Test Material Receipt, Storage and Usage

Receipt

Acetonitrile test material was received from J.T. Baker Inc, Phillipsburg, NJ 08865. Sixteen, four-liter bottles containing a total of ~50 kg of Acetonitrile [BNW Lot No. 53438-3, bottles 1 through 16] were received 10/1/90.

Storage Conditions

The bulk chemical was stored in its original containers at ~22°C under a nitrogen headspace in the Chemical Storage and Transfer Facility adjacent to the LSL-II laboratory.

Usage

An average of 0.73 kg acetonitrile was consumed per exposure day. The study required 12.34 kg for animal exposure.

Transfer Procedures

Exposure material was transferred from the original containers by using a vacuum system to draw the acetonitrile into the 5.6 liter, stainless steel, exposure reservoir. A nitrogen head space was maintained at all times while the acetonitrile was being transferred. The stainless steel reservoir was filled every three to four exposure days.

Waste Disposal

Excess used test material was stored at LSL-II until disposed of by the BNW Waste Management and Environmental Compliance Group.

Surplus Disposal

Surplus test material (e.g., unused bulk material) will be disposed of by the BNW Waste Management and Environmental Compliance Group at the end of all studies currently planned unless otherwise instructed.

Chemical Analysis

Manufacturers Analysis

The test material consisted of three different manufacture lots of acetonitrile. The identification of BNW assigned lot numbers and the manufactures lot analyses are provided below. BNW lots 53438-5 through 9 were used for animal exposures.

Manufacturer Lot# BNW Assigned Lot#	D01103 53438-3-1 to 4	D14109 53438-3-5 to 8	D14082 53438-3-9 to 16
Manufacturer Analysis			
Assay Purity % (by GC)	100.0	100.0	100.0
Ultraviolet Absorbance (1.00 cm path vs Water)			
200 nm	0.01	0.03	0.04
220 nm	0.007	0.009	0.010
254 nm	<0.002	<0.002	<0.002
280 nm	<0.002	<0.002	<0.002
UV Cutoff, nm	189	189	189
Fluorescent Trace Impurities (as quinine base in ppb)			
Measured at 450 nm	0.1	0.1	0.1
Measured at Emission Maximum for Solvent Impurities	0.2	0.2	0.2
Titratable Acid (meq/g)	0.0003	0.0002	0.000003
Titratable Base (meq/g)	<0.00006	<0.0001	0.0002
Residue after Evaporation, ppm	0.2	0.3	0.8
Water (by Coulometry) , %	<0.003	0.01	0.01
Refractive Index	1.3435	1.3435	1.3434

Analysis at Battelle Pacific Northwest Laboratories

The MRI recommended procedure for acetonitrile purity analysis, based on the June 9, 1981, MRI Report for NCI Contract No. N01-CP-95615, was implemented as BNW SOP# ØB-AC-3A11. The identity and purity, as well as the acid content, of each lot of test material was determined upon receipt. Two additional purity analyses were performed before animal exposures. This included an analysis within 30 days prior to animal exposures.

Identity of each bottle of bulk chemical BNW Lot No 53438-3 was confirmed during initial bulk analysis by infrared spectroscopy. Gas chromatography showed BNW Lot No. 53438-3 (bottle #1) to be 100.0% pure by area percent compared to reference acetonitrile. The amount of titratable acid (expressed as acetic acid) was determined to be 15.8 ppm. Gas chromatography showed BNW Lot No. 53438-3 (bottle #5) to be 100.2% pure by area percent compared to reference acetonitrile. The amount of acidic components was determined to be 16.6 ppm. Gas chromatography showed BNW Lot No. 53438-3 (bottle #9) to be 99.7% pure by area percent compared to reference acetonitrile. The amount of titratable acid was determined to be 17.9 ppm.

Subsequent purity analyses included gas chromatographic analysis for bulk purity and titration for the analysis of acidic components. A summary of the purity results can be found in Table A.I.1.

Table A.I.1. Acetonitrile Purity Analyses Summary

Analysis Date	Test Material	Status	Relative %Purity	Acid Content ppm as Acetic Acid
10/3/90	BNW53438-3-1[a]	initial	100.0	15.8
10/3/90	BNW53438-3-5[b]	initial	99.7	16.6
10/3/90	BNW53438-3-9[c]	initial	100.2	17.9
11/13/90	BNW53438-3-5[b]	~6 weeks after initial	100.2	12.8
11/13/90	BNW53438-3-9[c]	~6 weeks after initial	100.3	17.2
1/21/91	BNW53438-3-5[b]	~17 weeks after initial	100.2	17.5
1/21/91	BNW53438-3-9[c]	~17 weeks after initial	100.3	18.8

a Vendor Lot No. D01103
b Vendor Lot No. D14109
c Vendor Lot No. D14082

Test Chemical Stability Studies

Introduction

In general, nitriles undergo a variety of reactions that produce many different classes of compounds, including aldehydes, amines, amides, amidines, immines, carboxylic acids, esters, and ketones. However, acetonitrile is very stable near ambient temperature and pressure, and this stability is demonstrated by its widespread acceptance as a solvent.

In this study, acetonitrile was generated by pumping liquid test chemical onto a heated stainless steel cylinder covered with a fiberglass wick. The liquid acetonitrile was vaporized and swept into the vapor delivery line by the constant air flow through the distribution system. Thus, if acetonitrile decomposes as a result of the generation system employed, thermally catalyzed oxidation was one possible mechanism. Decomposition by this route could result in formation of various oxidation products of acetonitrile, including acids, aldehydes and ketones. However, the stability of acetonitrile makes thermally catalyzed oxidation unlikely.

Acetonitrile commercially produced in the United States is typically isolated as a by-product from the production of acrylonitrile. Thus, acrylonitrile is typically present as an impurity in commercially available acetonitrile. Other major impurities in commercially available acetonitrile are propionitrile and allyl alcohol (Kirk-Othmer, 1981). Analysis for acrylonitrile, propionitrile, and allyl alcohol impurities were included as part of the test chemical stability studies discussed below.

The stability and purity of acetonitrile in the exposure chambers and the distribution line were investigated by analyzing samples collected from the high and low concentration chambers (1200 and 100 ppm) and the vapor distribution line using solvent-filled bubblers that had been cooled in ice. Sample bubblers were cooled in ice to increase sample collection efficiency by minimizing sample breakthrough. In addition, samples of acetonitrile were obtained from the generator reservoir test material prior to beginning the daily animal exposure and again after the daily animal exposure was terminated.

Also, given the design of the generation system, the heated wick represented a site where trace, less volatile impurities, such as polymers of acetonitrile could accumulate. However, analysis of methanol extracts of the generator wick obtained during study prestart work did not detect any impurities present in the wick extract that were not present in methanol blank samples. Therefore, during the animal exposure portion of this study additional wick analysis was not performed.

Experimental Methodology and Results

Determination of Recovery and Detection Limits for Allyl Alcohol, Acrylonitrile and Propionitrile.

Two chromatographic systems and solvents were used for this study to allow for analysis of volatile and moderately volatile degradation products or contaminants. Samples analyzed for moderately volatile contaminants and degradation products were collected in methanol filled bubblers chilled in ice and were analyzed using a HP 5890 gas chromatograph with a FID and a 30 m x 0.53 mm, 1.0 µm film, DB-Wax capillary column (J&W). On-column injections were made using a HP 7673A autosampler. Samples analyzed for volatile contaminants and degradation products were collected in DMF filled bubblers chilled in ice and were analyzed using a HP 5890 gas chromatograph with an FID and a 30 meter x 0.53 mm GS-Q capillary column (J&W). Injections were made on-column using an HP 7673A autosampler. A full list of parameters for each system is given in Table A.II.1.

Gravimetric standards were prepared in methanol and DMF using acrylonitrile, propionitrile and allyl alcohol. A constant amount of internal standard (nitromethane) was added to each standard and sample to allow for variations in injection volume, sample volume and drift in detector response. Each standard was analyzed by gas chromatography using the appropriate solvent parameters as listed in Table A.II.1.

Excellent resolution of acetonitrile, acrylonitrile, propionitrile and nitromethane was achieved using chromatographic system A (Table A.II.1) but allyl alcohol was not well resolved from the solvent (DMF). Allyl alcohol and nitromethane were well resolved from the solvent (methanol) using chromatographic system B (Table A.II.1) however acrylonitrile and acetonitrile coeluted with the solvent. Propionitrile was slightly resolved from the solvent using chromatographic system B.

Sample size for both collection solvents was approximately 1 liter from the distribution line, 10 liters from the 1,200 ppm chamber, and 20 liters from the 100 ppm chamber. Collection efficiency, determined by breakthrough, influences the validity of the chamber samples. Bubblers were prepared using both solvents and chilled in ice followed by the addition of known concentrations of the suspect degradation products and impurities acrylonitrile, propionitrile and allyl alcohol. The amount added to each bubbler was less than 1% (w/w) of the expected amount of acetonitrile collected from the distribution line, 1200 ppm exposure chamber and 100 ppm exposure chamber. A calibrated volume of room air was drawn through each of the bubblers to closely approximate the sample volume taken for the distribution line, 1200 ppm exposure chamber and the 100 ppm exposure chamber. A constant amount of internal standard was then added to each bubbler and the percent recovery of each degradation product and impurity was determined for each bubbler by comparison to gravimetrically prepared standards. The spiking procedure exposed the quantity of the impurities and degradation products contained in each bubbler to the entire sample volume for the distribution line, 1200 ppm exposure chamber and 100 ppm exposure chamber samples. Thus, all of the acrylonitrile, propionitrile and allyl alcohol contained in each spiked bubbler were available to partition from the solvent back into the gas phase for the entire sample volume. However, during normal sample collection, any acrylonitrile, propionitrile or allyl alcohol present in the chamber atmosphere is accumulated in the liquid phase throughout the sampling interval. Thus the spiking procedure tends to over estimate the losses and under estimate recovery.

The recovery of acrylonitrile, propionitrile and allyl alcohol was greater than 90% for each of the sample volumes. Thus, collection efficiency for each of these compounds during normal sample collection from the distribution line, 1200 ppm exposure chamber and 100 ppm exposure chamber is expected to meet or exceed 90% recovery. Table A.II.2 summarizes the recovery of acrylonitrile, propionitrile and allyl alcohol in each of the spiked bubblers.

The detection limit assigned to acrylonitrile, propionitrile and allyl alcohol was based on the concentration of each that was present in the low bubbler standards. Acrylonitrile, propionitrile and allyl alcohol were easily detected in each of the low bubbler standards, the concentration of each represented less than 1% (w/w) of the total amount of acetonitrile sampled from the distribution line, 1200 ppm exposure chamber and the 100 ppm exposure chamber. In instances when acrylonitrile, propionitrile or allyl alcohol were not detected in samples taken from the distribution line, 1200 ppm exposure chamber, 100 ppm exposure chamber or generator reservoir a "less than" value was assigned to each based upon the amount of acetonitrile sampled at each location and on the detection limits of acrylonitrile, propionitrile and allyl alcohol. Table A.II.2 lists detection limits for each degradation product and impurity.

Determination of Trace Contaminants or Degradation Product in the Chamber Atmosphere, Distribution Line and the Generator Reservoir

The possible presence of trace amounts of contaminants or degradation products was investigated in samples collected from various points in the exposure generation system. Analysis was by gas chromatography using a flame ionization detector. Samples were collected using ice cooled solvent-filled bubblers from the 1200 and 100 ppm exposure chambers, the control chamber and the distribution line within the first and last hours of generation on a normal six hour generation day. Gravimetrically prepared generator reservoir samples taken prior to the start of the daily exposure and after the termination of the daily exposure were also prepared and analyzed for volatile and less volatile degradation products and trace contaminants. Duplicate samples were collected from each sampling location using both DMF and methanol filled bubblers. All bubblers were packed in ice when samples were taken to prevent solvent loss and to retard breakthrough of acetonitrile and suspected degradation products and impurities. Gravimetrically prepared standard solutions of the suspected degradation product and impurities were analyzed with the samples (see Figure A.II.1 for standard chromatograms). These degradation products and impurities include allyl alcohol, propionitrile and acrylonitrile. Chromatograms of samples were also screened for the presence of any unidentified degradation products or impurities.

Analysis of samples collected in DMF were screened for the presence of acrylonitrile, propionitrile and any volatile degradation products or impurities. Samples collected in DMF during the first and last hour of generation from the 100 ppm exposure chamber indicated no impurities or degradation products. Samples collected from the distribution line and 1200 ppm exposure chamber during the first hour of generation detected propionitrile at a concentration of 0.011% (w/w) and 0.013% (w/w), respectively. Samples collected from the distribution line and 1200 ppm exposure chamber detected propionitrile at a concentration of 0.013% (w/w) and 0.011% (w/w), respectively. No impurities or degradation products were detected from the analysis of generator reservoir samples prepared in DMF. Acetonitrile was not detected in any of the control chamber samples.

Analysis of samples collected in methanol were screened for the presence of allyl alcohol and any semi-volatile degradation products or impurities. Samples collected in methanol during the first hour of generation from the distribution line, 1200 ppm chamber, 100 ppm chamber and control chamber indicated no allyl alcohol. In the 100 ppm chamber, 1200 ppm chamber and control chamber however, two unknown impurities were detected with retention times of 7.4 and 8.6 minutes. In the distribution line only the unknown impurity with a retention time of 8.6 minutes was detected. Both of these impurities were also detected in the exposure room bubbler sample and in an unrelated standard prepared in the laboratory. Therefore, it is likely these unknown impurities are the result of contaminated glassware resulting from improper handling or cleaning techniques. Samples collected in methanol from the last hour of exposure from these locations also failed to indicate the presence of allyl alcohol, however the two unknown impurities present at 7.4 and 8.6 minutes were present at about the same concentration as was observed in the samples collected during the first hour of generation.

One unknown impurity was detected from the analysis of generator reservoir samples (prepared in methanol) which were taken prior to the beginning of exposure and after the end of the exposure day. The unknown impurity corresponded to the one detected at 8.6 minutes in the chamber samples, room and laboratory flask. The concentration at the end of the day was about one-third that found at the beginning of the exposure day, approximately 0.17% (w/w) and 0.06% (w/w), respectively

Table A.II.3 summarizes the results from each sample location. Sample chromatograms from the distribution line and low exposure chamber can be found in Figure A.II.2 and A.II.3.

Discussion

The studies described above indicated that propionitrile was present as a minor impurity in the exposure atmosphere and distribution line at concentrations <0.1%(w/w). About 20 mg of acetonitrile was sampled from the distribution line and the 1200 ppm exposure chamber. Based upon the estimated detection limit of acrylonitrile and allyl alcohol these compounds were not present in the distribution line or 1200 ppm exposure chamber at concentrations >0.14%(w/w), with respect to acetonitrile. About 3.4 mg of acetonitrile was sampled from the 100 ppm chamber and no acrylonitrile, propionitrile or allyl alcohol were detected in these samples. Based upon the estimated limit of detection for acrylonitrile, propionitrile and allyl alcohol for samples taken from the 100 ppm chamber, neither acrylonitrile, propionitrile or allyl alcohol were present at concentrations of >0.7%(w/w). Two unknown impurities were detected in samples taken in methanol from each of the exposure chambers (also in a room sample and laboratory flask sample) and one unknown impurity was detected in the distribution line. These are believed to be impurities associated with contaminated glassware and were not present in the test material. This conclusion is supported the observation of these impurities in laboratory spikes. In any event the concentrations were low, the highest observed being 1% (w/w) relative to acetonitrile in the 100 ppm exposure chamber.

One unknown impurity was detected (RT of 8.6 minutes) in the generator reservoir sample taken before the beginning of the exposure day and at the end of the exposure day and was estimated to be present at 0.17% (w/w) and 0.06% (w/w), respectively. However, this unknown impurity is believed to have originated from contaminated glassware. No other impurities were detected in the generator reservoir samples.

The failure to detect propionitrile in the 100 ppm exposure chamber samples and in the gravimetrically prepared generator reservoir samples was due to an insufficient amount of acetonitrile sampled. However, since propionitrile was detected in the distribution line and 1200 ppm exposure chamber (0.01% (w/w)) and since the test material is derived from a common source the amount of propionitrile in the 100 ppm exposure chamber was expected to also be 0.01% (w/w). Based upon the test material generation process and the similar volatilities of propionitrile and acetonitrile (97°C and 82°C, respectively) the estimated concentration of propionitrile in the reservoir material is expected to be the same as was found in the distribution line and the 1200 ppm exposure chamber (0.01% (w/w)). Therefore, based on these chromatographic results, degradation product and impurity detection limits and the excellent recovery observed for degradation products and impurities, test chemical stability for this study was considered acceptable.

Table A.II.1. Gas Chromatographic Parameters Used to Analyze Acetonitrile Exposure Chamber Samples (with Animals) and Generator Reservoir Samples Collected in DMF and Methanol for the Presence of Possible Degradation Products and Impurities.

Solvent: DMF	
Gas Chromatograph:	Hewlett Packard 5890
Detector:	Flame Ionization
Analytical Column:	30 meter x 0.53 mm ID Porous Layer Open Tubular Fused Silica Column with Porapak Material. Manufactured by J&W Scientific
Injection:	3 µl On-column
Carrier Gas:	Helium
Head Pressure:	~15 psi using Packed Jet
Initial Temperature:	110°C
Initial Time:	4.0 minutes
Rate A:	10°C/minute
Final Temperature A:	120°C
Final Time A:	0.00 minutes
Rate B:	30°C/minute
Final Temperature B:	225°C
Final Time B:	2.00 minutes
Solvent: Methanol	
Gas Chromatograph:	Hewlett Packard 5890
Detector:	Flame Ionization
Analytical Column:	30 meter x 0.53 mm ID DB-Wax with 1µ film Manufactured by J&W Scientific
Injection:	3 µl On-column
Carrier Gas:	Helium
Head Pressure:	~15 psi using Packed Jet
Initial Temperature:	50°C
Initial Time:	1.0 minutes
Rate A:	10°C/minute
Final Temperature A:	70°C
Final Time A:	1.00 minutes
Rate B:	15°C/minute
Final Temperature B:	250°C
Final Time B:	2.50 minutes

Table A.II.2. Recovery and Detection Limits of Potential Acetonitrile Degradation Products and Impurities Acrylonitrile, Allyl Alcohol and Propionitrile.

Degradation Product or Impurity	Sample Volume in Liters	% Recovery	Minimum Detectable Amount (µg)[a]	Minimum Detectable Concentration Wt. %[b]
Acrylonitrile	1	103	24	0.12
Propionitrile	1	99	23	0.12
Allyl Alcohol	1	110	26	0.13
Acrylonitrile	10	99	24	0.12
Propionitrile	10	95	23	0.12
Allyl Alcohol	10	107	26	0.13
Acrylonitrile	20	96	24	0.70
Propionitrile	20	91	23	0.70
Allyl Alcohol	20	105	26	0.79

[a]Determined from GC analysis of bubbler samples that had a metered amount of room air pulled through the bubbler after spiking the bubbler with the indicated amount of either acrylonitrile, allyl alcohol or propionitrile.

[b]This value was determined by dividing the minimum detectable amount of allyl alcohol, propionitrile and acrylonitrile by the amount of acetonitrile sampled from the distribution line, 1200 ppm exposure chamber and the 100 ppm exposure chamber (20,000 µg, 20,000 µg and 3,300 µg respectively).

Table A.II.3. Summary of Acetonitrile Degradation (with Animals) Exposure Chamber Samples Taken Within the First (BOD) and Last Hour (EOD) of Exposure and Generator Reservoir Samples Taken Prior to Exposure Start and After Exposure Termination.

Sample Description	Sample Number	Approximate RT (minutes)	Compound	Weight %[a]
100 ppm BOD	1	7.4	Unknown	0.1
100 ppm BOD	1	8.6	Unknown	0.7
100 ppm BOD	2	7.4	Unknown	0.1
100 ppm BOD	2	8.6	Unknown	0.6
100 ppm EOD	1	7.4	Unknown	0.092
100 ppm EOD	1	8.6	Unknown	0.626
100 ppm EOD	2	7.4	Unknown	0.105
100 ppm EOD	2	8.6	Unknown	1.06
1200 ppm BOD	1	5.2	Propionitrile	0.009
1200 ppm BOD	1	7.4	Unknown	0.046
1200 ppm BOD	1	8.6	Unknown	0.093
1200 ppm BOD	2	5.2	Propionitrile	0.017
1200 ppm BOD	2	7.4	Unknown	0.008
1200 ppm BOD	2	8.6	Unknown	0.089
1200 ppm EOD	1	5.2	Propionitrile	0.017
1200 ppm EOD	1	7.4	Unknown	0.007
1200 ppm EOD	1	8.6	Unknown	0.083
1200 ppm EOD	2	5.2	Propionitrile	0.009
1200 ppm EOD	2	7.4	Unknown	0.005
1200 ppm EOD	2	8.6	Unknown	0.090
Dist. Line BOD	1	5.2	Propionitrile	0.011
Dist. Line BOD	1	8.6	Unknown	0.144
Dist. Line BOD	2	5.2	Propionitrile	0.011
Dist. Line BOD	2	8.6	Unknown	0.143
Dist. Line EOD	1	5.2	Propionitrile	0.015
Dist. Line EOD	1	8.6	Unknown	0.094
Dist. Line EOD	2	5.2	Propionitrile	0.015
Dist. Line EOD	2	8.6	Unknown	0.068
Reservoir BOD	1	8.6	Unknown	0.171
Reservoir EOD	1	8.6	Unknown	0.060

[a]Calculations for propionitrile were made from a propionitrile standard curve. Calculations for unknown compounds were made using the allyl alcohol standard curve. Unknown weight % values were made assuming these compounds had a detector response similar to that of allyl alcohol.

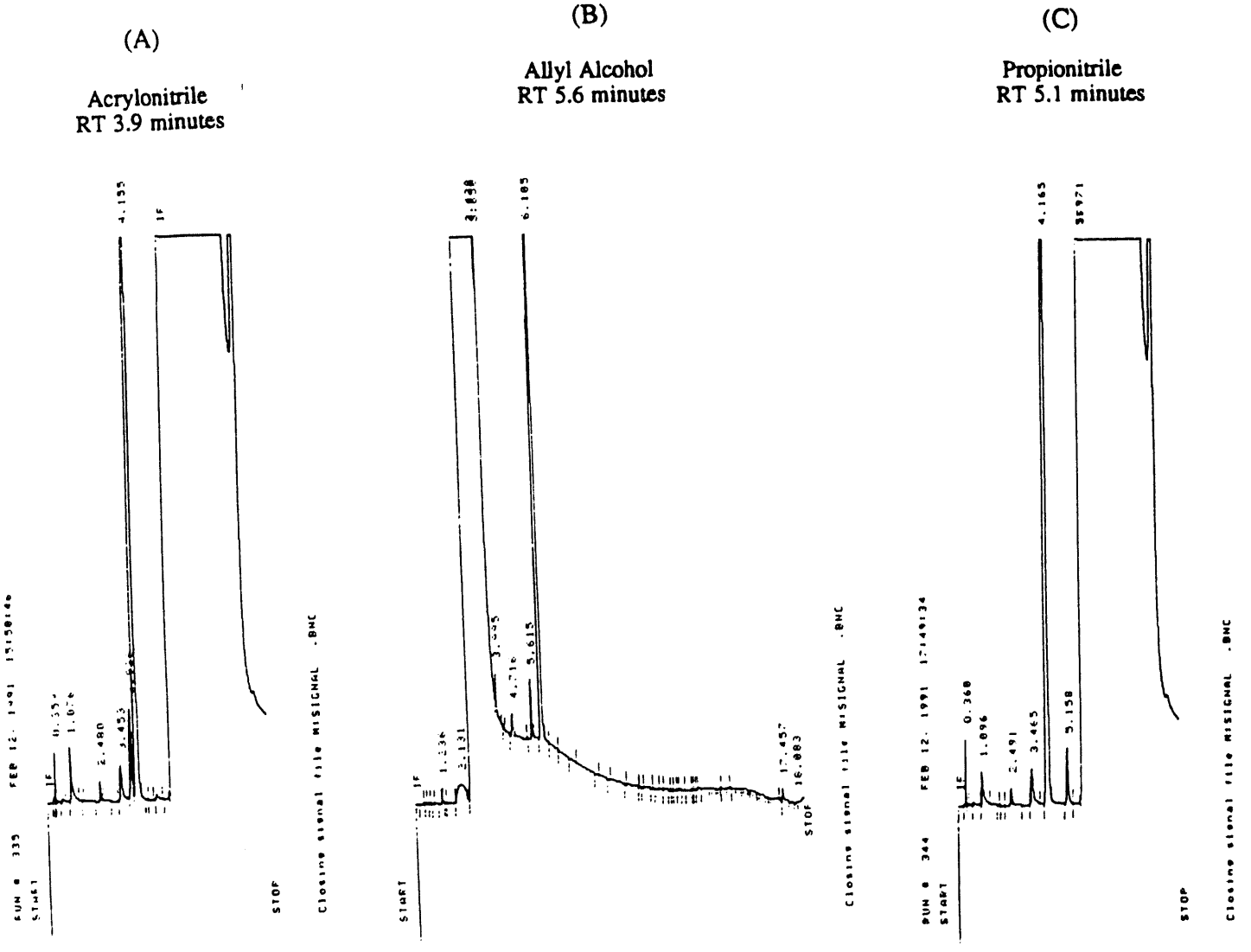
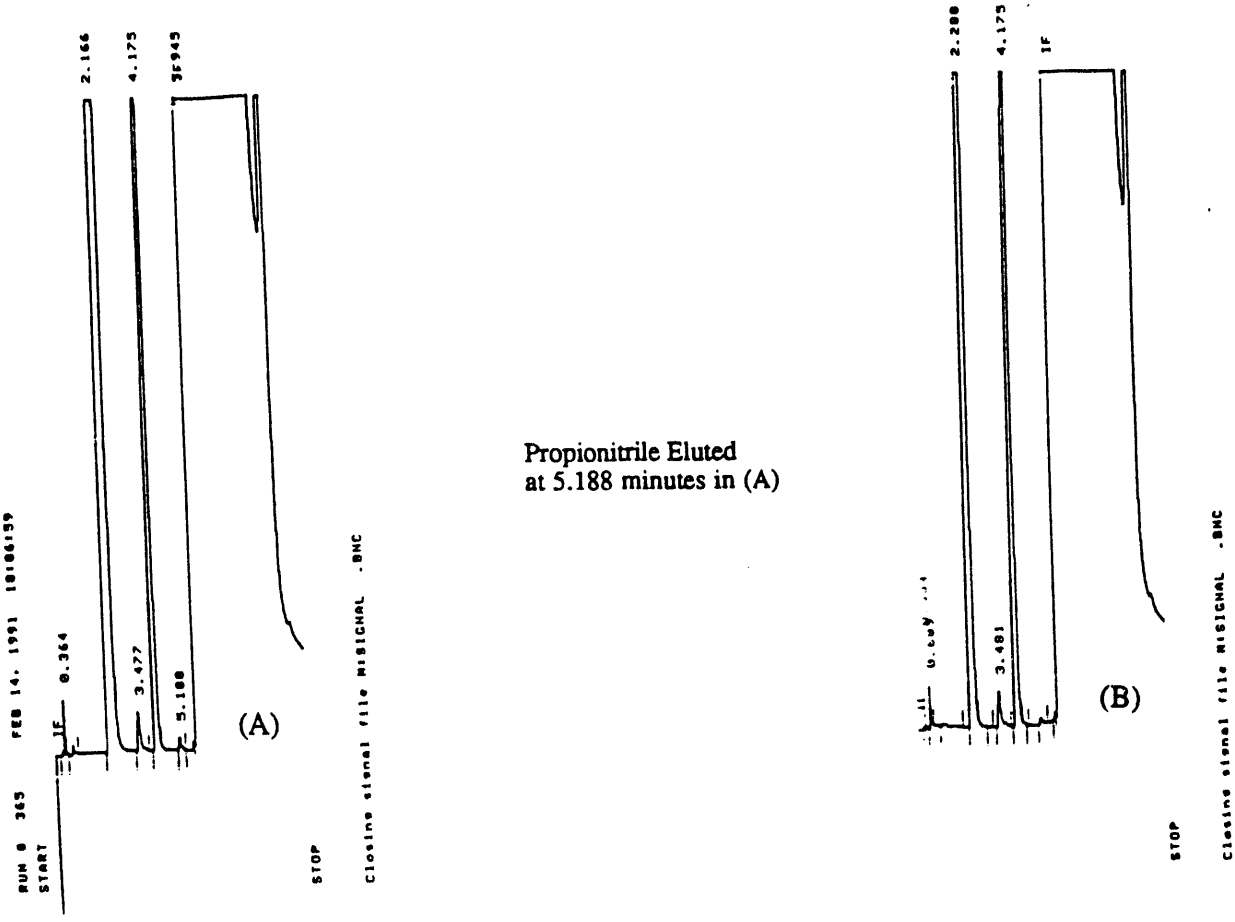


Figure A.II.1. Representative Chromatograms of Acetonitrile Degradation Product and Impurity Standards. (A) Acrylonitrile, (B) Allyl Alcohol and (C) Propionitrile.



Propionitrile Eluted
at 5.188 minutes in (A)

Figure A.II.2. Representative Chromatograms of Acetonitrile Degradation Samples Taken in DMF from the (A) Distribution Line and (B) Low (100 ppm) Exposure Chamber.

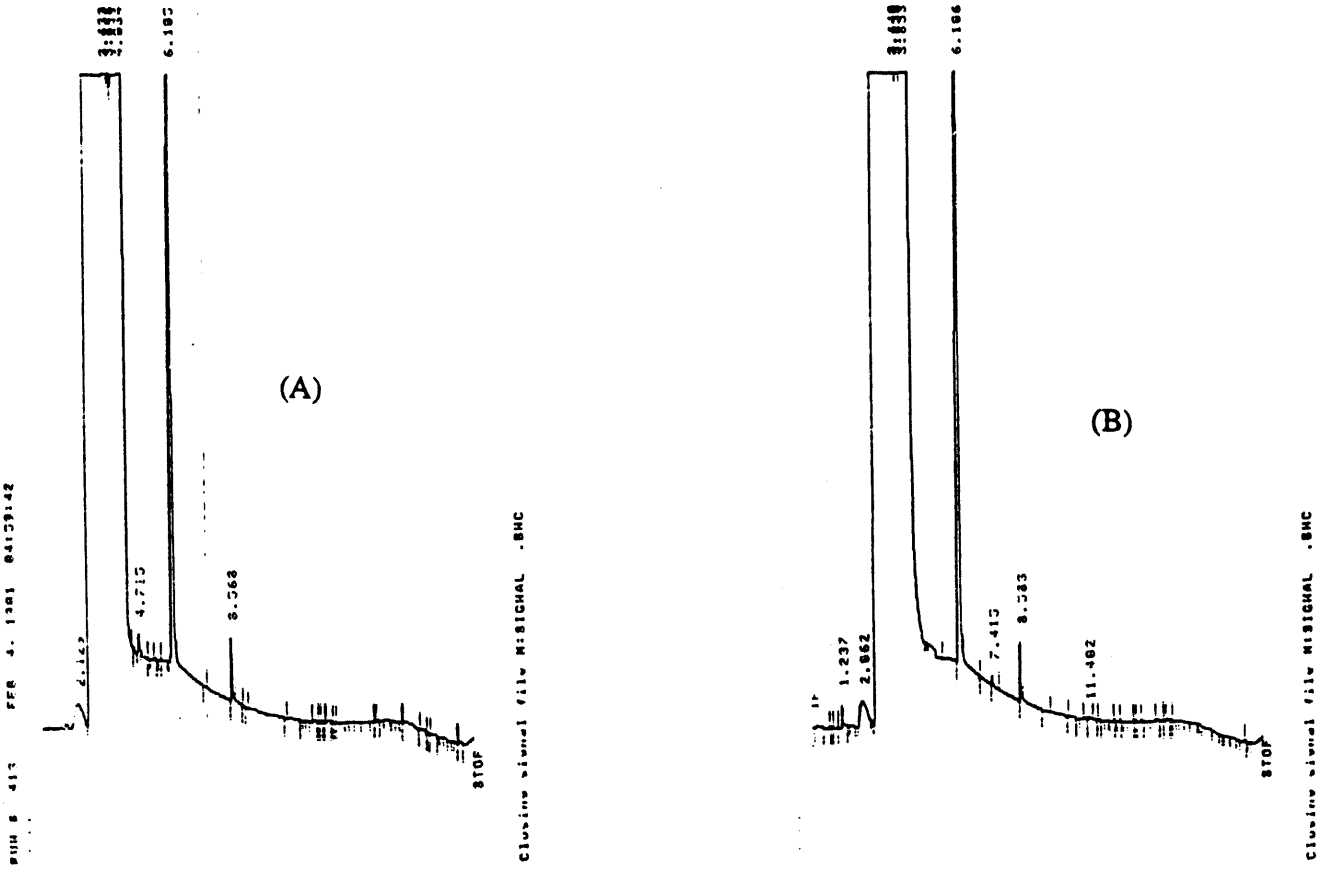


Figure A.II.3 Representative Chromatograms of Acetonitrile Degradation Samples Taken in Methanol from the (A) Distribution Line and (B) Low (100 ppm) Exposure Chamber.

Test Chemical Monitoring

On-Line Chamber Monitoring System Description

Chamber and room concentrations of acetonitrile were determined using an on-line Hewlett-Packard Model 5840 gas chromatograph equipped with a flame ionization detector operated at 250°C. The column was 1/8 inch O.D. x ~1 ft. nickel packed with Porapak Q 80/100 maintained isothermally at 100°C, the carrier gas was nitrogen at ~30 ml/min. Under these conditions, acetonitrile exhibited a retention time of ~0.90 minutes.

A 12-port stream select valve constructed of Hastelloy-C, mounted in the column oven interfaced the on-line gas chromatograph with the exposure chambers, the control chamber, the exposure room, the on-line standard and a filtered air blank. This valve directed a continuous stream of the sampled atmosphere to an oven-mounted sampling valve (Hastelloy-C) equipped with a 1.0 ml nickel sample loop. Automatic switching of the stream select valve allowed access to all test chambers, the room, a blank and the on-line standard. A schematic diagram of the chamber concentration monitoring system is shown in Figure A.III.1. Individual 1/4-inch Teflon sample lines (also used for monitoring relative humidity) lead from each chamber to a position close to the on-line monitor. These sample lines had a flow of 2 to 2.5 l/min. Teed from each of these lines were 1/8-inch Teflon lines, ~4 feet in length, which lead to each port of the stream select valve. These lines were continuously purged at >30 ml/min. The contents of the sample line was directed through the sample loop at ~30 ml/min for ~2.6 min prior to sample injection. Each analysis required about 3 minutes. All chamber positions, the room, the blank, and the on-line standard were monitored approximately every 27 minutes.

Data was transferred from the HP5840 chromatographic integrator to an HP85B computer. The HP85B computer remotely controlled the selection of the correct sample stream and the operation of the monitor. The equation of the calibration curve was contained in the HP85B and was applied to the data transmitted by the on-line GC monitor. The HP85B also accumulated and printed the sample concentration data until the 12th port of the stream select valve was measured. Chamber concentration data were then sent to the executive computer for printing and storage. Each concentration accumulated by the HP85B was compared with limit concentrations for that particular location. If the concentration was beyond the control limits, the HP85B would immediately send the information to the executive computer which would take the appropriate action.

As described in the section that follows, the chamber monitor was calibrated against gravimetrically prepared standards. The gravimetric standards were related to the on-line chamber monitor response through quantitative analysis of bubbler samples taken from exposure chambers simultaneously sampled by the on-line GC. The normal operating cycle of the on-line GC was not interrupted during the bubbler sampling procedure.

Additionally, the operation of the chamber monitor was checked throughout the day against an on-line standard. This check provided a measure of day-to-day instrument detector drift. The chamber monitor detector response exhibited excellent stability throughout the course of the study, requiring no unscheduled recalib..ations.

Daily operating procedures for the concentration monitoring system were contained in SOP#'s ØB-AC-3B1K and ØB-BE-3B4B.

Calibration of the On-Line Monitor

The relationship between the on-line GC response and the concentration of acetonitrile in the chamber was established from independent analysis of chamber bubbler samples taken directly from the exposure chambers during a routine exposure period. The calibration of the on-line monitor was established by correlation of the on-line monitor peak area (at the time of sampling) against independent measurement of chamber concentration (analysis of chamber bubbler samples). The normal dynamic operation of the monitor was not disrupted during collection of these bubbler samples.

Calibration samples were obtained by collecting acetonitrile in bubblers containing 25 ml of dimethylformamide (DMF). Known volumes of chamber atmosphere from each chamber were collected using a calibrated critical-orifice-controlled sampler at a constant flow rate of approximately 0.5 l/min. Internal standard solution was added to each bubbler after sample collection to correct for any volume change in the DMF, variations in injection volume, and instrument detector drift. During the study prestart activity, breakthrough of acetonitrile was determined with a back-up bubbler linked in series to the primary bubbler used to sample the exposure chambers. The breakthrough was found to be less than 1%, therefore, the total mass of acetonitrile collected in the primary bubblers was not corrected for breakthrough during the study.

Bubbler samples and standards were analyzed on an off-line Hewlett-Packard gas chromatograph equipped with a flame ionization detector operated at 270°C and a 30 meter GS-Q capillary column (J&W Scientific, porous-polymer-coated, open tubular, fused silica, 0.53 mm ID). One microliter sample volumes were injected using an on-column configuration and an automatic liquid sampler. The initial oven temperature was 50°C with a 0.5 minute initial isothermal hold time. The temperature was then ramped at 10°C/minute to 155°C to elute the acetonitrile and the internal standard. The final oven temperature program was a 30°C/minute ramp to 230°C for 3.5 minutes to rapidly elute the DMF. Acetonitrile eluted at ~6 minutes and the nitromethane internal standard at ~8 minutes using these parameters.

Gravimetrically prepared standard solutions of acetonitrile in DMF were used to calibrate the off-line GC used to quantitate acetonitrile collected in the bubbler samples. A set of five standards was run for each analysis session. The concentration range of the standards bracketed the expected concentration range of the chamber bubbler samples. Two independently weighed stock solutions were used each time the standard series was prepared. The preparation of dual stock solutions helped detect any weighing errors.

A single calibration equation was used for the on-line monitor determination of acetonitrile concentrations in the exposure chambers and the on-line standard. This equation was linear over the range of interest (See Figure A.III.2). A separate equation, based upon the monitor response for the 100 ppm chamber, with a zero intercept was used for the room, air blank and control chamber.

During animal exposures two sets of chamber bubbler samples were taken. On each occasion analysis of the duplicate chamber bubbler samples using the off-line calibration method showed excellent agreement between the on–line monitor predicted chamber concentration and the concentration determined from the bubbler samples. Table A.III.1 summarizes the results from each set of calibration bubblers.

On-Line Standard

An on-line standard of acetonitrile was provided from a compressed gas cylinder (Byrne Specialty Gases) containing about 700 ppm acetonitrile in nitrogen. Nitrogen was employed to ensure stability of the acetonitrile. The on-line standard was used to check on-line monitor drift throughout each exposure day. The on-line standard showed excellent precision, reproducible to about ±1%, within a single day and from day-to-day as shown in Table A.III.2. The data used to determine the reproducibility of the on-line standard was taken from the first three days of exposure, each having 6 hours of continuous generation.

The standard was checked before the start of each exposure day. The following on-line standard criteria were established for use during the exposure. The measured concentration for the standard was required to be within ±10% of the assigned target value before any exposure could begin without consultation with the exposure control task leader. During the course of the exposure, if the on-line standard was within ±5% of the target value, no change in calibration was required. If the on-line standard was beyond ±5% of its assigned target, the responsible chemist was to be informed immediately by an exposure specialist. Additional grab sampling was to be initiated by the chemist on a case by case basis when drift in the on-line standard was between ±5-10%. A drift of 10% or more would require high priority recalibration of the on-line monitor by grab sampling.

During the course of the study a cumulative drift of less than ±5% of the on-line standard concentration was observed. Before each exposure day the on-line standard was within ±5% of the assigned target value and during each exposure day the on-line standard remained within ±5% of the assigned target value.

Detection Limits

Definition of MDL, MQL, MLQ and QL

The Minimum Detection Limit (MDL) is defined as the lowest concentration of analyte that can be reliably detected. The Minimum Quantitation Limit (MQL) is defined as that concentration of analyte sufficiently in excess of MDL such that a reliable quantitative value can be assigned to the analyte concentration. When a blank value is observed, MDL and MQL are established as 3 and 10 times the standard deviation of the blank value. However, for the acetonitrile prestart study a zero value was obtained for blank measurements. In the absence of a finite blank value the lowest concentration of acetonitrile that could be practically maintained in an exposure chamber was used to determine MDL and MQL values. The generation of low concentrations of acetonitrile was accomplished as described in the section on determination of MLQ.

The Minimum Limit of Quantitation (MLQ) was defined as the lowest concentration of analyte that could reliably be assigned a numerical value with an RSD of ≤10% and a relative error of ≤10%. Therefore, to determine MLQ, samples were taken using solvent filled bubblers to allow a comparison between the on-line monitor determination and a reference method assumed to yield the true value for acetonitrile concentration.

The Quantitation Limit (QL) was defined as the concentration below which acceptable quantitation could not be achieved. The quantitation limit is usually defined the same as the MLQ. However, in some cases where MLQ is near the lowest exposure concentration, the QL may be assigned the same value as MQL. Measured values below the QL are not normally reported in daily or study summaries; they are however included in computation of all exposure concentration related calculations.

Determination of MDL and MQL

Analysis of the charcoal-filtered air blank by the on-line monitor while the stream select valve was in normal rotation failed to detect acetonitrile. Since no blank value was observed to allow a determination of MDL and MQL, the precision measurements were performed by monitoring a chamber with a low acetonitrile concentration. A standard deviation of 47 area counts was found for an acetonitrile concentration measured at 0.82 ppm by the on-line monitor. MDL and MQL are normally set at three and ten times the standard deviation expressed in concentration units. On this basis MDL and MQL would be 0.3 and 1 ppm, respectively.

However, it was evident that there was a minimum peak area (threshold area value required for peak detection) that the integrator could detect (using the integrator parameters chosen for this study) and that any peak with an area less than the threshold would not be detected. The on-line monitor average threshold area value was found to be 335 area counts with a standard deviation of ±47 (11/8/90, data from HP runs 1696 - 1700). The on-line monitor calibration equation for non-exposed locations (low exposure chamber through the origin) was used to determine alternate values for MDL and MQL. MDL and MQL values of 1.1 and 1.9 ppm, respectively, where MDL and MQL were taken as the threshold plus three times and ten times the standard deviation of the lowest measurable value. These values were believed to be more realistic values for MDL and MQL and were used for the study.

Determination of MLQ

Experimental

To determine MLQ, samples were taken using DMF filled bubblers. The bubbler samples were analyzed on a second off-line GC calibrated using gravimetrically prepared acetonitrile standards. Each standard and bubbler had a constant amount of internal standard added to compensate for any fluctuations in sample volume, injection volume and detector drift. Grab samples were taken from the low concentration chamber, and the vapor delivery rate into the exposure chamber was reduced so that concentrations of about 50, 25, 12, 8 and 5 ppm were achieved in addition to the exposure concentration of 100 ppm.

Gravimetric standards were prepared that bracketed the expected concentration of acetonitrile to be sampled at each chamber concentration level. The standards and samples were analyzed by GC using a procedure similar to that used to analyze grab samples acquired from the chamber for purposes of on-line monitor calibration.

Results

The off-line GC showed acceptable sensitivity and precision for the lowest gravimetric standard. Based upon the criteria that MLQ was the concentration of analyte for which the precision and the relative error were ≤10%, MLQ was set to 8.3 ppm using the data summarized below. The established MLQ was ~10% of the low exposure chamber concentration. The values for monitor response represent the average of 5 concurrent readings.

Amnt Acetonitrile from Grab (ppm)	Amnt Acetonitrile from Monitor Response (ppm)	Absolute Error (ppm)	Relative Error (%)	Avg Monitor Response	Precision (% RSD)
93.1	97.5	4.4	4.7	40966	0.21
51.9	53.6	1.7	3.3	22096	0.17
29.3	28.0	1.3	4.4	11116	0.52
15.9	14.5	1.4	8.8	5350	0.66
8.3	7.6	0.7	8.4	2373	1.7
5.1	4.0	1.1	22	810	6.4

Determination of QL

QL was set to 8.3 ppm. This value represented the more conservative value of the two determinations, MQL, which was 1.9 ppm, and MLQ, which was determined to be 8.3 ppm. The value set for QL was ~10% of the concentration of the low chamber.

Precision, Linearity and Absolute Recovery of the On-Line Monitor

Agreement between the various sampling ports for the on-line monitor (important in evaluating monitor performance i.e. valve or valve plumbing leaks) was estimated from measurements of the on-line standard sampled from the ports that were used during the study (10 out of a total of 12 sample valve ports). These measurements had a relative standard deviation of 0.4% (N=10). In addition, in order to estimate precision for the on-line monitor at each concentration level, repeated measurements were made from each exposure chamber. The following values were obtained:

Location	Number of Measurements (N)	Mean (ppm)	Standard Deviation (SD)	Relative Standard Deviation (RSD)
1200 ppm chamber	13	1202	14	1.16%
400 ppm chamber	12	398.1	3.5	0.88%
100 ppm chamber	12	99.87	0.59	0.59%
on-line standard	10	119.8	0.76	0.63%

The precision results reported above for the measurements made on the chamber concentrations were affected by both variations in test material concentration in the chamber and instrument imprecision of the on-line monitor. However, the overall precision was sufficient to demonstrate adequate instrument precision at each sampling point. The excellent precision seen for the on-line standard was relatively free of variations accompanying the generation process and indicated good reproducibility for the on-line monitor.

Linearity of the on-line monitor was determined by calibrating the on-line monitor against a gravimetrically calibrated GC (see "Calibration of the On-Line Monitor" section). The on-line monitor calibration was linear through the range of interest (Fig III-2.

Absolute recovery of the bubbler grab sampling method was confirmed during prestart work by obtaining grab samples with backup bubblers from each of the exposure chambers. An average breakthrough of <1% was detected. The determination of chamber concentration by analysis of grab samples was not corrected to account for breakthrough during the study.

Monitoring for Acetonitrile in Building Exhaust

Effluent from the exposure chambers and the exposure generation system was diluted with the exhaust air of the entire LSL-II Building prior to exhausting from the building stack. The expected concentration of acetonitrile in the building exhaust was about 0.5 ppm.

The acetonitrile concentration in the exhaust of the building was determined once during the study using grab sampling employing DMF filled bubblers. Good trapping efficiency of acetonitrile into DMF has been previously demonstrated. Gravimetric standards were prepared that bracketed the expected grab concentration of acetonitrile in the building exhaust samples. The gravimetric standards showed good linearity. The equation from the gravimetric standard curve was used to determine the grab concentration of acetonitrile in the bubbler samples.

Three samples were collected from different locations within the exhaust stream. The average building exhaust concentration of acetonitrile was determined to be 0.2 ppm with a standard deviation of 0.07 ppm for the three samples. The ACGIH-TLV for acetonitrile is 40 ppm. The amount of acetonitrile determined in the building exhaust is 20 times below the Battelle action limit of 4 ppm.

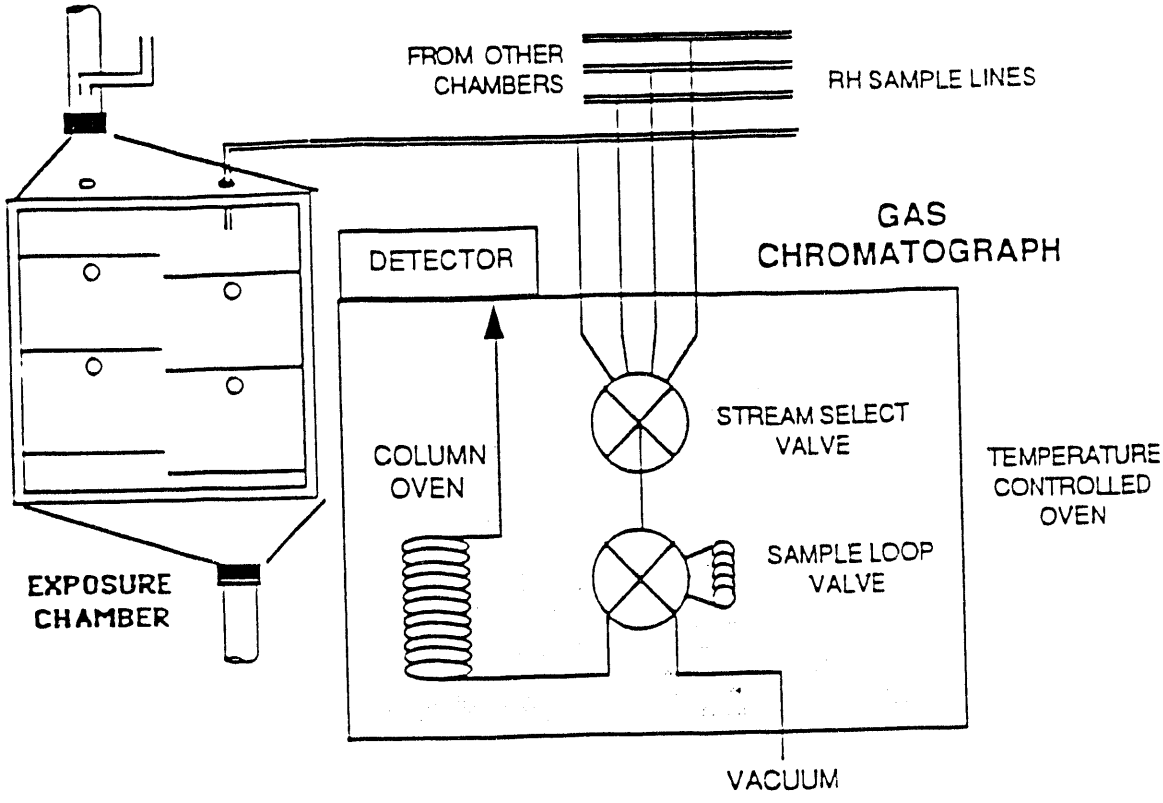
Table A.III.1. Summary of Acetonitrile-IRT On-Line Monitor Calibrations. A Comparison of the Chamber Concentration as Predicted by the On-Line Monitor to the Gravimetrically Determined Chamber Grab Concentration.

Sample Location	Conc. Acetonitrile Determined Gravimetrically, ppm	On-Line Monitor Conc. Acetonitrile, ppm	% Relative Error	Absolute Error (ppm)
Sample Date: 2/6/91				
1,200 ppm #1	1,211	1,207	0.33	4
1,200 ppm #2	1,232	1,209	1.9	23
500 ppm #1	417	405	2.9	12
500 ppm #2	415	407	1.9	8
100 ppm #1	109	103	5.5	6
100 ppm #2	112	103	8.0	9
Sample Date: 2/15/91				
1,200 ppm #1	1,176	1,183	-0.60	7
1,200 ppm #2	1,159	1,185	-2.2	26
500 ppm #1	386	395	-2.3	9
500 ppm #2	396	395	0.25	1
100 ppm #1	103	98	4.9	5
100 ppm #2	104	98	5.8	6

Table A.III.2. Acetonitrile-IRT On-Line Standard Precision. Each Day Represents 6 Hours of Continuous Generation.

Individual Exposure Day Standard Precision	February 5th	February 6th	February 7th
n=	12	12	13
Average Standard Area:	284,483	286,308	287,030
Standard Deviation:	3,004	2,122	2,025
% Relative Standard Deviation:	1.1	0.74	0.71

Combined Three Day Standard Precision	
n=	37
Average Standard Area:	284,713
Standard Deviation:	3,408
% Relative Standard Deviation:	1.2



Port #	Sampling Point Description
1	1200 ppm Chamber
2	400 ppm Chamber
3	100 ppm Chamber
4	Not Used
5	Not Used
6	Not Used
7	Filtered Air Blank
8	Exposure Room
9	0 ppm Chamber
10	Filtered Air Blank
11	Filtered Air Blank
12	On-Line Standard

Figure A.III.1. Schematic Diagram of Acetonitrile Exposure Chamber and Monitoring System.

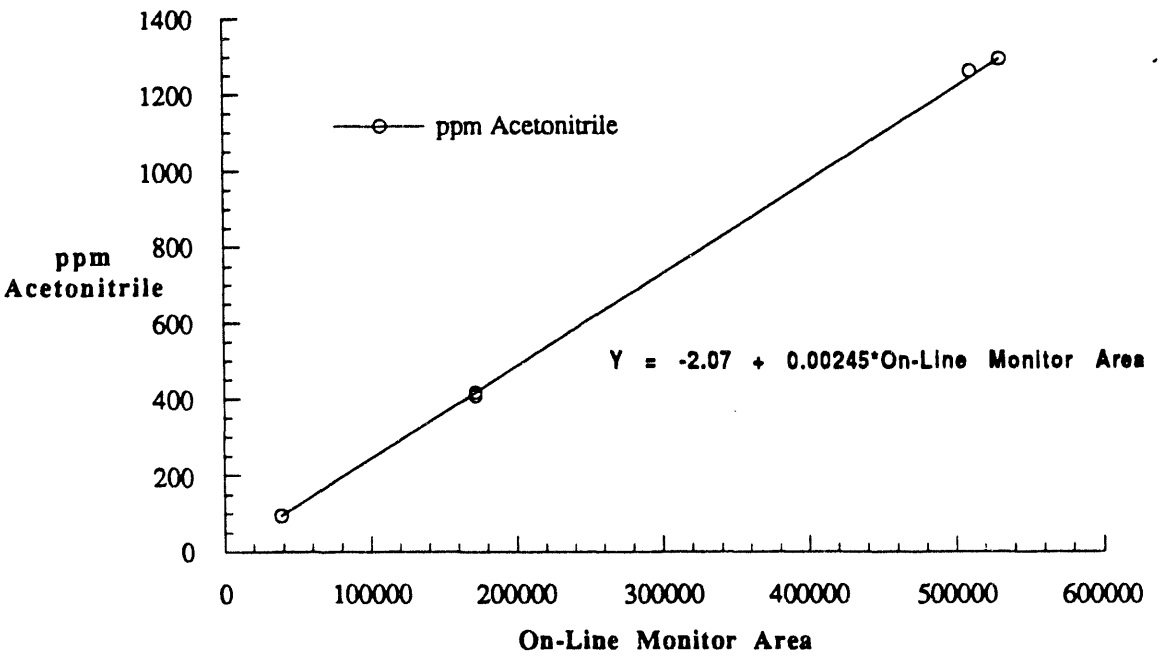


Figure A.III.2. Acetonitrile On-Line Monitor Calibration Curve Used for Exposed Locations. Curve Exhibits Excellent Linearity Over the Entire Exposed Concentration Range.

Determination of Cyanide and Acetonitrile in Blood of Exposed Animals

Introduction

The amount of cyanide (CN), an intermediate metabolite of acetonitrile (ACN), was determined in the blood of Sprague-Dawley rats exposed by inhalation to 1200, 400, and 100 ppm ACN. The study was designed as an inhalation, reproductive toxicology exposure, and the study animals were pregnant females. The blood level concentrations of both ACN and CN were investigated in selected animals at 8 and 18 days of gestation.

A variety of methods for determination of CN blood concentration are listed in the literature. In most of the methods hydrogen cyanide (HCN) is evolved from blood by addition of acid. The evolved HCN is either trapped in a sodium hydroxide solution for later derivitization and/or detection (1-3) or introduced directly into a detection and quantitation system such as a gas chromatograph with a specific detector (4-6).

Initial method development efforts focused on the use of gas chromatographic, head space analysis using specific detectors. In these assays cyanide was evolved in headspace sample vials by the addition of acid. The headspace was then introduced to the gas chromatograph via an automated headspace sampler (HP-19395 A). Detection was by either a nitrogen/phosphorous detector or by electron capture after reaction with Chloramine T to form cyanogen chloride. While these procedures offered the hope of rapid sample analysis with a minimal amount of sample work up, significant sample loss and carryover was experienced.

Following failure of the headspace method, microdistillation was also evaluated. In this assay the cyanide solution for analysis was added to the bottom portion of a disposable microdistillation column (Lachet Instruments, Milwaukee, WI). An acid solution was combined with the cyanide solution and the microdistillation column was then fully assembled. Evolved cyanide was passed through a NaOH solution trap by active transport with the resulting steam caused by heating the bottom portion of the distillation tube to ~150°C. The cyanide content of the NaOH solution was then determined by either ion chromatography with electroconductivity detection, or reaction with a color forming reagent and detection by spectrophotometry. This procedure showed promise using both methods of detection during validation using aqueous cyanide solutions. However, blood solutions were found to be too viscous to be used with the microdistillation tubes. Bumping of the blood solutions caused mechanical breakdown of the frits used to separate the sample compartment from the NaOH traps.

Finally, a diffusion cell assay with spectrophotometric detection was evaluated. In this procedure a NaOH solution was added to the inner well of a Conway Diffusion Cell. The blood sample and an acid solution were added to different portions of the outer well. The diffusion cell was then capped and the blood and acid mixed together. The cell was allowed to sit for several hours, allowing the evolved HCN to diffuse into the NaOH solution. After removal from the cell, the cyanide trapped in the NaOH solution was added to a NaH_2PO_4 buffer to maintain pH at ~8. The cyanide was then reacted with Chloramine T to form cyanogen chloride which subsequently reacted with a pyridinebarbaturic acid reagent to form a red/blue complex. The resulting absorbance, proportional to cyanide concentration, was measured using a UV-Vis spectrophotometer.

A gas chromatographic headspace analysis was used for the determining the ACN concentrations in whole blood. For this procedure samples of blood were added to headspace vials along with an internal standard in a solution of dilute NaCl. An automated headspace sampler (HP 19395 A) was used to heat the vials and introduce the headspace to the gas chromatograph. Flame ionization was used to detect ACN after elution from the chromatographic column.

Cyanide Analysis

Experimental Method

Samples of blood were obtained from exposed animals by cardiac puncture using syringes washed with an EDTA solution. The animals were anesthetized with CO_2. The blood was immediately transferred to EDTA, Vacuutainer Tubes (Benton Dickinson, Rutherford, New Jersey). The tubes were kept on ice until used for analysis (<2 hours). The sampling procedure began immediately after exposure shut down and was completed within 45 min.

Duplicate analyses were performed on each sample by weighing ~0.5 ml of blood to the outer moat of a Conway Diffusion Cell. The inner moat of the diffusion cell was filled with 0.5 mls of 0.1N NaOH. One half ml of 3.6N H_2SO_4 was added to the outer moat of the cell in a position away from the blood sample. The rim of the lid was coated with a thin bead of silicon grease to produce an air-tight seal then placed on the diffusion cell. The acid was mixed with the blood by gentle agitation. Diffusion was allowed to proceed for three hours at room temperature. A schematic representation of the diffusion cell and the solutions prior to mixing is presented in Figure A.IV.1.

For samples from animals exposed to 1200 ppm ACN, 0.2 ml of the NaOH solution was removed from the inner well of the diffusion cell for analysis. For animals exposed to 400, 100, and Ø ppm ACN, 0.4 ml of NaOH was removed. The NaOH solution was added to a test tube containing 2.0 ml of a NaH_2PO_4 solution, prepared by diluting 14 g NaH_2PO_4 to 100 ml with deionized water. To the test tube was added 0.5 ml of a Chloramine T solution, prepared by diluting 0.125 g Chloramine T to 50 ml with deionized water. The solutions were mixed and allowed to react for 3.0 min. After 3.0 min, 3.75 ml of a color reagent was added to the test tube. The color reagent was prepared by combining 7.2 g of barbituric acid with 28.8 ml pyridine and 7.2 ml concentrated HCL with a final dilution to 150 ml with deionized water. The solutions were mixed and allowed to react for 12.0 min.

The contents of the test tube were then transferred to a 5.0 cm path length cuvette. The absorbance of the mixture was measured at 585.5 nm using a Cary 219 spectrophotometer (Varian) in the autogain mode with a 1.0 nm spectral band width.

The cyanide concentration was determined from a calibration curve established from standards as described below. Standards were prepared by adding varying volumes (50-200µl) of cyanide solutions to 0.5 ml aliquots of blood from unexposed animals. The cyanide solutions were prepared by diluting KCN with 0.1N NaOH. The prepared standards ranged from ~4.2 to ~0.084µg cyanide per gram of blood. As with the samples, the standards were handled by processing in the Conway Diffusion Cells and subsequent color forming reagents prior to analysis by UV-Vis spectrophotometry.

Validation

As detailed above, the standards for quantitation were matrix matched by using blood from animals which had not been exposed to ACN. This was assumed to represent 'blank' blood for matrix matched standards. A comparison was made of response from standards prepared with the blood matrix and standards prepared in water.

For this analysis standards were prepared and treated as detailed in the experimental method section above. Standards were prepared from 4.1 to 0.081µg cyanide per gram of blood and duplicated using water.

The results of this analysis, graphically represented in Figure A.IV.2, show close agreement for the matrix matched standards as compared to standards prepared in water.

Solutions of ACN and sodium thiocyanate (NaCNS) were analyzed to assess possible interferences for the cyanide assay. It was expected the blood from exposed animals would contain significant amounts of ACN (approaching 300 µg ACN per gram of blood). In addition, thiocyanate is the principle metabolism product of cyanide and could possibly interfere with the cyanide assay.

Standard solutions of ACN were prepared in water to represent blood concentrations of ~1500µg ACN per gram of blood. Thiocyanate solutions were prepared in water to represent concentrations of ~11µg CN per gram of blood. These samples were processed according to the procedure detailed in the experimental method section above.

The average absorbance of the ACN samples and the thiocyanate samples was essentially the same as seen for blanks. No evidence of interference was observed from ACN or thiocyanate.

The cyanide assay was performed twice for exposed animals, once at 8 days of gestation (DG) and again at 18 DG. Cyanide content of the blood was compared to cyanide solutions prepared in blank blood. Separate suites of calibration standards were prepared for each assay. In addition to the six calibration standards, four additional spikes of intermediate concentration were prepare by adding varying amounts of cyanide to blank blood. The spikes were used as an internal check of the calibration.

The cyanide standards and spikes were prepared using solutions of potassium cyanide diluted in 0.1N NaOH. For the standards, appropriate amounts of cyanide were added to blank blood to represent cyanide levels of ~4 to 0.08µg CN per gram of blood. The spikes were prepared to represent blood cyanide levels of 3 to 0.16µg CN per gram of blood. The standards and spikes were submitted to the same regime of sample treatment (diffusion assay and color forming reactions) as the samples. The suite of calibration standards was analyzed immediately before the samples. The spikes were intermingled with the sample analysis.

A linear fit was calculated from the cyanide concentrations and response of the calibration standards. The quality of fit can be assessed by examination of residuals. Tables of these values along with similar measurements for the spikes are shown in Table A.IV.1. Graphical representations of the calibrations are shown in Figure A.IV.3. As can be seen by comparing the calibration information for the two analysis sets, the assay performed for the 18 DG samples was much better controlled. This was due to a change in the procedure for filling the spectrophotometer cuvettes which resulted in better reproducibility for the 18 DG sample set.

Acetonitrile Analysis

Experimental Method

The same blood samples obtained from exposed animals for the cyanide assay were also used for blood level ACN determinations.

Analyses were performed for each sample by weighing ~0.5 ml of blood to 20 ml headspace vials containing 2.0 ml of 3% NaCl. Next, 1.0 ml of an internal standard solution was added. The internal standard solution was comprised of propionitrile at 2300 µg per ml prepared in 3% NaCl. The vials were immediately capped in preparation for chromatographic analysis.

A Hewlett-Packard 19395 A Headspace Sampler was used to introduce the sample headspace to the chromatographic system. The sample vials were loaded into the automated headspace sampler

and maintained at 60°C for at least 20 min before sampling. The headspace sampler processed the samples in the following manner: The septum cap of the sample vial was pierced with a sample needle. The pressure in the sample vial was then brought to ~2 atmospheres and held for 10 seconds. The headspace of the vial was then allowed to vent for 5 seconds through a 1.0 ml sample loop. Through use of a sample valve, the sample loop was then brought in line with the carrier gas for the chromatograph for 10 seconds. The carrier gas was nitrogen and ran at 20 ml per min. The initial temperature of the HP 5890 gas chromatograph was maintained at 140°C for 0.2 min. The GC oven was then ramped to 190°C at 15°C per minute and held for 1.0 min. Under these conditions acetonitrile was found to elute at ~1.3 min and the propionitrile internal standard at ~2.0 min. Flame ionization was used for detection. The transfer line from the headspace analyzer to the GC was heated to 70°C and directly coupled to the chromatographic column. The analytical chromatography column was a 30 meter x 0.53 mmeter i.d., porous layer open tubular capillary column, coated with Porapak Q (J&W Scientific, GS-Q). An example chromatogram is shown in Figure A.IV.4.

The relative response of the samples was related to the relative response of ACN standards to calculate quantitative values of ACN content. The standards were prepared by adding 2.0 ml of varying concentrations of ACN solutions to 0.5 ml aliquots of blood from unexposed animals in 20 ml headspace vials. The ACN standard solutions were prepared in 3% NaCl. One ml of the internal standard solution (2300 µg per ml propionitrile in 3% NaCl) was added to the headspace vials before capping. The prepared standards ranged from ~460 to ~9 µg ACN per gram of blood. As with the samples, the standards were processed with the headspace sampler with subsequent chromatographic analysis and flame ionization detection.

Validation

As detailed above, the standards for quantitation were prepared by combining ACN solutions with blood from animals which had not been exposed to ACN. This was assumed to represent 'blank' blood for matrix matched standards. A comparison was made of response from standards prepared with the blood matrix to standards prepared in water.

For this analysis standards were prepared and treated as detailed in the experimental method section above. Standards were prepared to represent from ~31 to 0.4 mg ACN per gram of blood and duplicated using water.

The results of this analysis, graphically represented in Figure A.IV.5, show excellent agreement for the matrix matched standards as compared to standards prepared without the blood matrix.

Solutions of ACN in blood were analyzed to assess the time needed for equilibration in the heated headspace bath as well as to asses the sample to sample carryover.

Standard solutions of ACN at ~16 mg ACN per standard (delivered in 2.0 ml 3% NaCl solution) were combined with 0.5 ml of blank blood in 20 ml headspace vials. One ml of internal standard solution was added to the vials prior to capping. In addition blanks were made by combining 2.0 ml of 3% NaCl with 0.5 ml of blank blood and 1.0 ml of internal standard. The ACN standards were analyzed one after another immediately after being placed in the 60°C headspace bath. The approximate time for each analysis was 5.5 min. The blank solutions were analyzed at the end of the analysis set.

The ACN and the propionitrile internal standard were found to yield a constant response after ~20 min in the heated bath. However, the area ratio between ACN and the internal standard were found to be constant from the very first analysis. The approximate yield and area ratio for the ACN and the internal standard were found to remain constant throughout the analysis time period

of one and a half hours. A blank solution analyzed immediately after the last standard showed less than 0.1% carryover from the previous sample.

The acetonitrile assay was performed twice for exposed animals. Once at 8 days of gestation (DG) and again at 18 DG. ACN content of the blood was compared to ACN solutions prepared in blank blood. Separate suites of calibration standards were prepared for each assay. Five levels of calibration standards were used to bracket the response of the samples from the exposed animals.

The ACN standards were prepared using varying concentrations of ACN diluted with 3% NaCl. The ACN solutions were added in 2.0 ml aliquots to 20 ml heads space vials containing 0.5 ml blank blood. One ml of propionitrile internal standard (2300µg per ml in 3% NaCl) was added to each standard solution before capping the headspace vial. The standards were prepared to represent ~460 to 8µg ACN per gram of blood. The standards were submitted to the same regime of sample treatment as the samples (headspace sampler and chromatographic analysis). The suite of calibration standards was intermingled with the samples during sample analysis.

A linear fit was calculated from the ACN concentrations and the area ratio of ACN as compared to the internal standard. The quality of fit can be assessed by the reverse calculated standard points as compared to the nominal values. Tables of these values are shown in Table A.IV.2. Graphical representations of the calibrations are shown in Figure A.IV.6. Both analysis sets showed excellent linearity.

Results

Blood samples for cyanide determinations were collected from 3 animals per exposure dose from animals exposed to 1200, 400, 100, and Ø ppm acetonitrile. The analysis was performed twice, once for animals at 8 days of gestation and on a different group of animals at 18 days. The animals had been exposed to acetonitrile for 3 and 15 days respectively. Details of actual animal exposure can be found elsewhere in the main body of this report. The results for the cyanide determinations are shown in Table A.IV.3. For the most part, detectable quantities of cyanide were found only in animals exposed to 1200 ppm acetonitrile. The detection limit for the assay was ~0.1 µg cyanide per gram of blood.

The same blood samples were also used for acetonitrile determinations. Acetonitrile was detected in all samples collected from exposed animals. The time elapsed between exposure end and sample collection was recorded for samples taken from the animals at 18 days of gestation. The results of the analyses are summarized in Table A.IV.3. As can be seen in the table of results, the concentration of acetonitrile in the animal blood was proportional to exposure concentration.

Table A.IV.1 Quality of Fit for Standards Used for Cyanide Determinations

Sample Date	Standard and Spike ID Number	ug Cyanide per Standard	Reverse Calculated ug Cyanide per Standard	Percent Difference
8 DG	53438-115-6	0.816	0.668	-18%
	53438-115-7	0.408	0.286	-30%
	53438-115-8	0.408	0.263	-36%
	53438-115-9	0.131	0.146	12%
	53438-115-10	0.066	0.069	4%
	53438-115-11	0.033	0.035	7%
	53438-115-13	0.612	0.681	11%
	53438-115-14	0.408	0.327	-20%
	53438-115-15	0.204	0.238	17%
	53438-115-16	0.066	0.076	15%
18 DG	53438-142-6	0.840	0.904	8%
	53438-142-7	0.4	0.438	4%
	53438-142-8	0.	0.397	-5%
	53438-142-9	0.134	0.136	2%
	53438-142-10	0.067	0.068	2%
	53438-142-11	0.034	0.030	-13%
	53438-142-13	0.432	0.618	-2%
	53438-142-14	0.420	0.407	-3%
	53438-142-15	0.210	0.208	-1%
	53438-142-16	0.067	0.081	21%

Table A.IV.2 Quality of Fit for Standards Used for Acetonitrile Determinations

Sample Date	Standard ID Number	ug Acetonitrile per Standard	Reverse Calculated ug Acetonitrile per Standard	Percent Difference
8 DG	53438-118-8	192	197	2.8%
	53438-119-1	38.5	37.8	-1.7%
	53438-119-2	15.4	15.0	-2.9%
	53438-119-3	7.70	7.82	1.5%
	53438-119-4	3.85	3.85	0.1%
18 DG	53438-146-1	228	223	-2.1%
	53438-146-2	45.6	46.1	1.2%
	53438-146-3	18.3	18.5	0.9%
	53438-146-4	9.13	9.15	0.3%
	53438-146-5	9.13	9.15	0.3%
	53438-146-6	4.57	4.55	-0.4%

Table A.IV.3. Analysis of Blood for Cyanide and Acetonitrile

Acetonitrile IRT: Analysis of Blood for Cyanide and Acetonitrile

Sampling Date (DG)	Exposure Concentration (ppm)	Animal Number	Time Elapsed Between Exposure End and Sampling (min)	Acetonitrile Amount (ugACN/gBlood)	Avg. Cyanide Amount (ugCN/gBlood)	RSD Cyanide Amount (N=2)
8	1200	172		234	2.0	4%
		322		245	2.7	5%
		268		229	2.3	2%
	400	287		63.9	*0.10*	*34%*
		120		69.3	*0.09*	*1%*
		124		58.1	0.13	9%
	100	183		22.0	*<0.05*	
		19		18.7	*<0.05*	
		207		18.1	*<0.05*	
	Ø	26		*<8.2*	*<0.05*	
		2		*<7.9*	*<0.05*	
		22		*<7.9*	*<0.05*	
18	1200	179	31	215	1.3	3%
		41	10	161	0.19	11%
		206	26	207	*<0.16*	
		68	17	170		
	400	298	10	68.5	0.12	43%
		50	29	83.1	*<0.07*	
		10	22	79.3	*<0.07*	
	100	305	29	18.1	*<0.08*	
		193	7	17.2	*<0.07*	
		246	20	18.5	*<0.07*	
	Ø	345		*<9.2*	*<0.07*	
		62		*<9.3*	*<0.07*	
		304		*<9.2*	*<0.07*	

Values shown in italics exhibited analysis response lower than the least concentrated calibration standards.

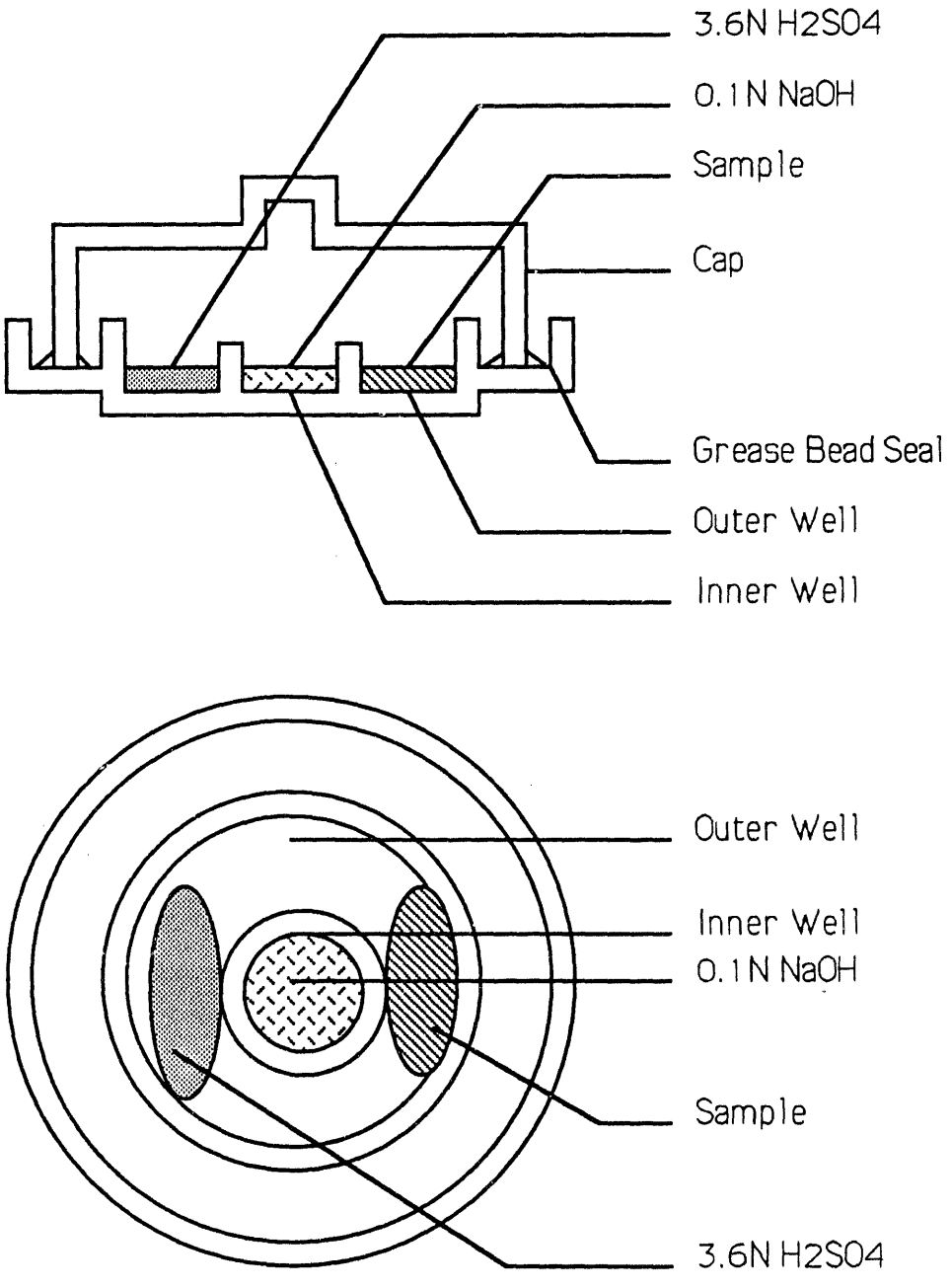


Figure A.IV.1. Conway Diffusion Cell

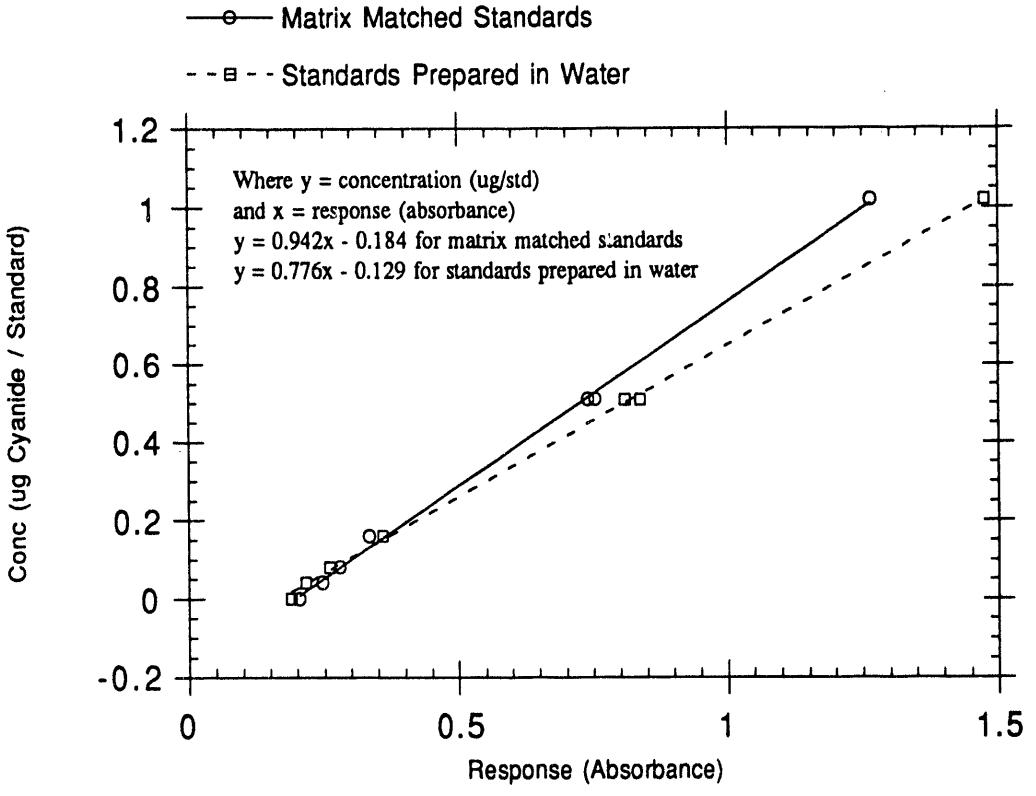
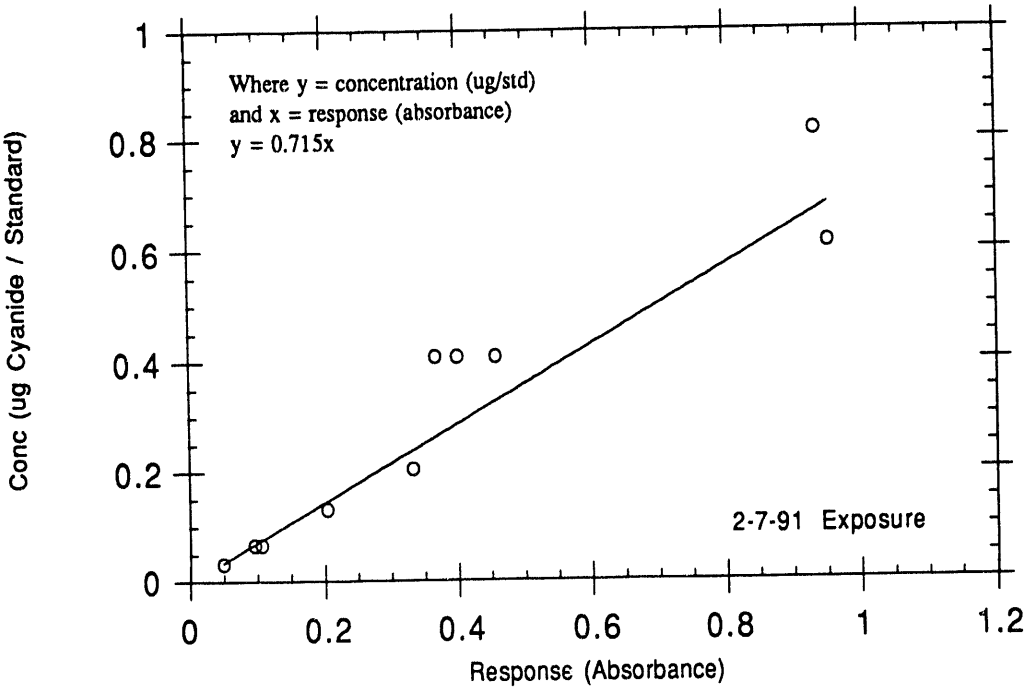


Figure A.IV.2. Comparison of Cyanide Standards Prepared in Water or Blood



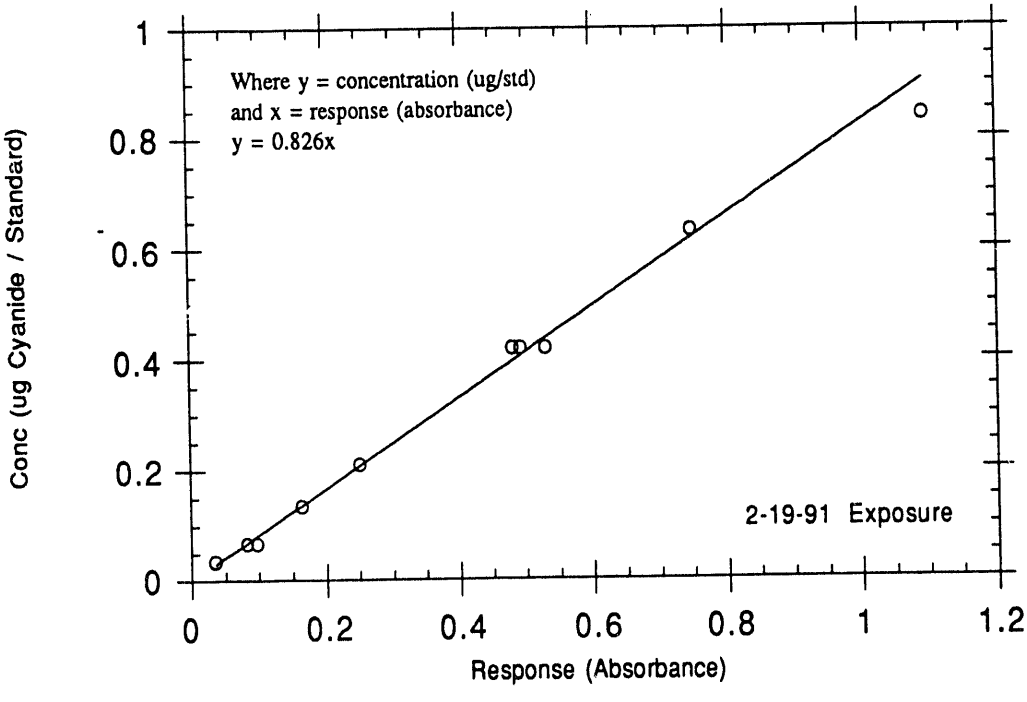
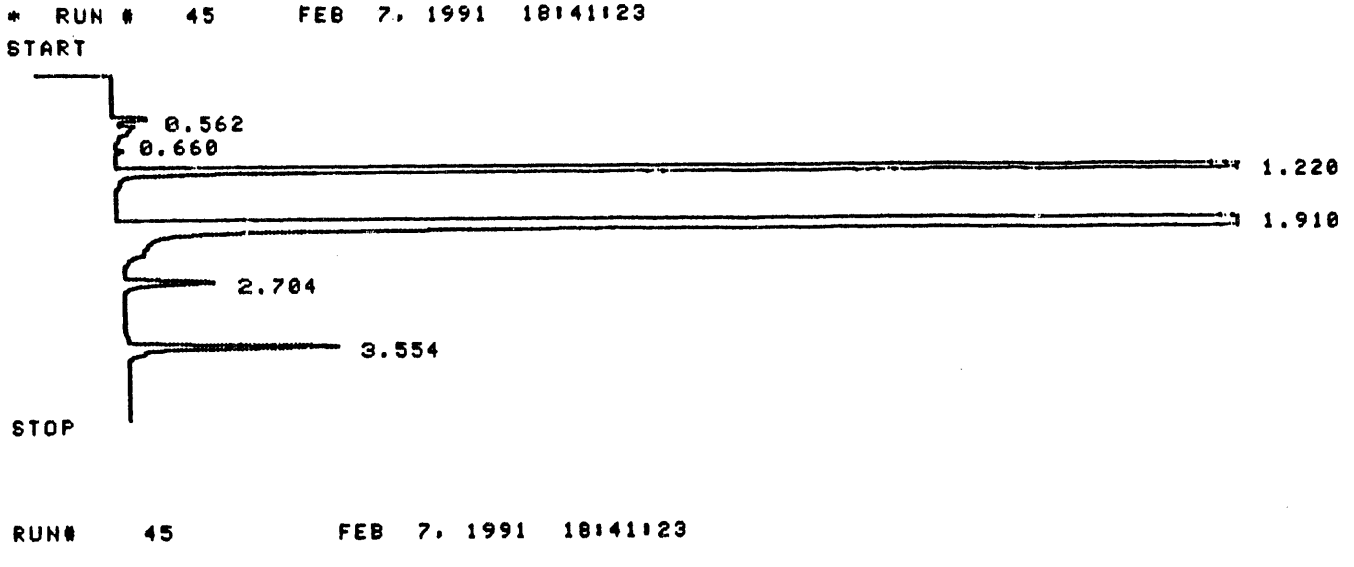


Figure A.IV.3. Calibration Curves Used for Cyanide Determinations



In this trace Acetonitrile elutes at 1.220 min and the propionitrile internal standard elutes at 1.1910 min.

Figure A.IV.4. Representative GC Trace for Acetonitrile Determinations

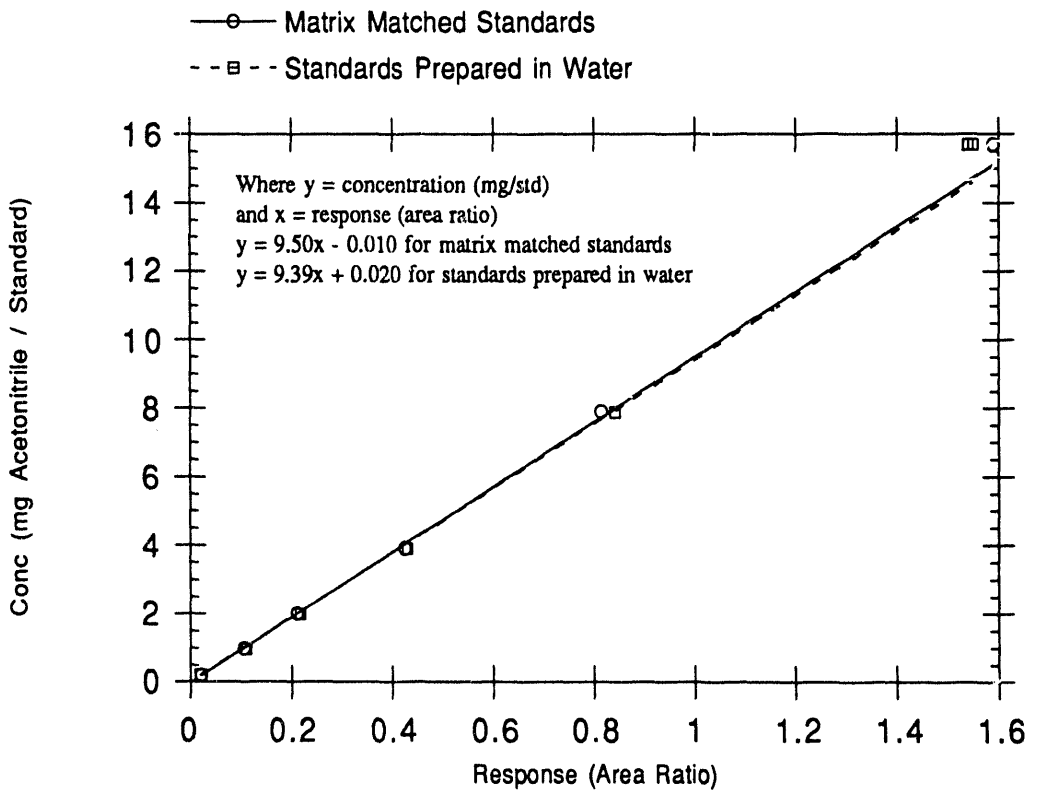
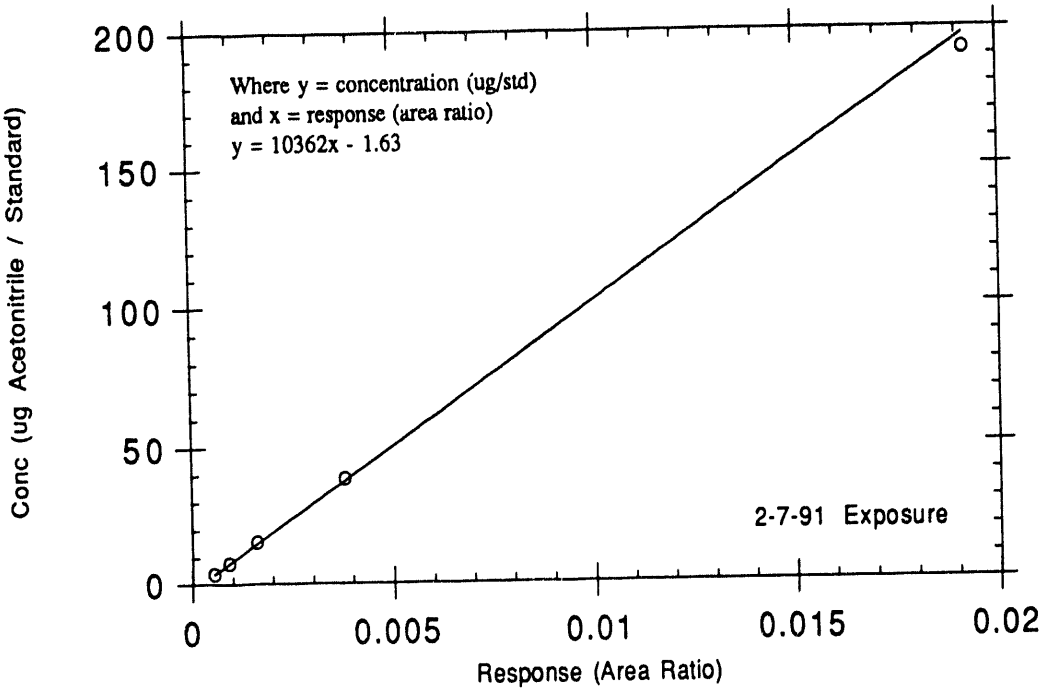


Figure A.IV.5. Comparison of Acetonitrile Standards Prepared in Water or Blood



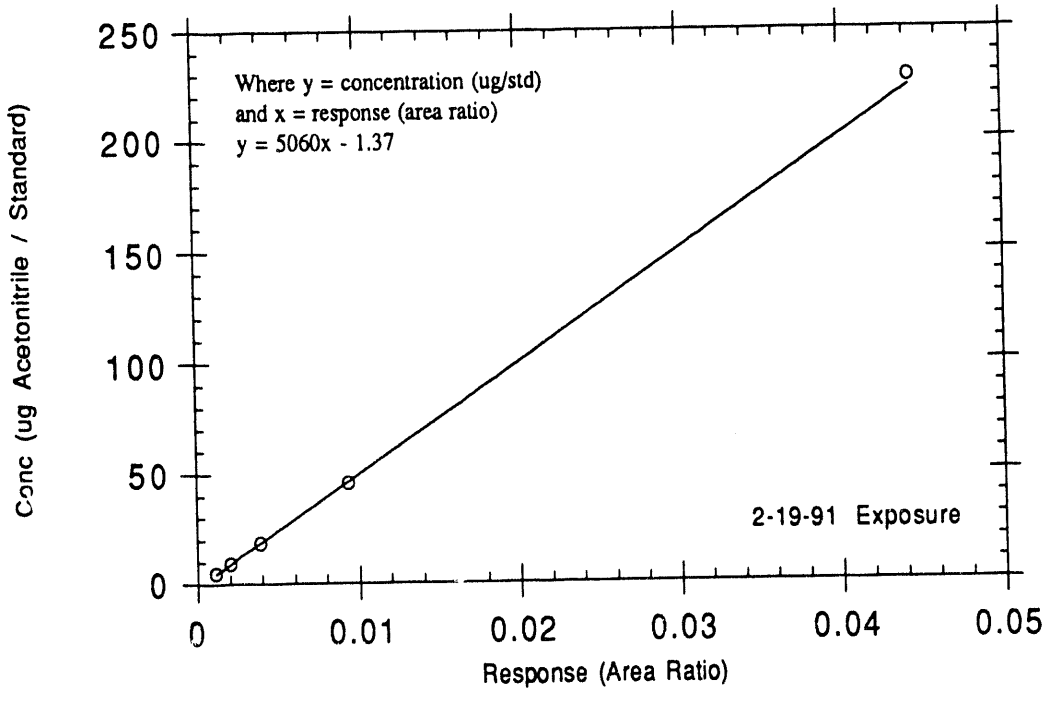


Figure A.IV.6. Calibration Curves Used for Acetonitrile Determinations

APPENDIX B

EXPOSURE DATA

Summation Equations
Concentration Data
Temperature Data
Relative Humidity Data
Exhaust Airflow Data
Exposure Discussion Sheets
Chamber Uniformity Data

Summation Equations

SUMMATION EQUATIONS

Mean: $$\bar{X} = \frac{1}{n} \sum_{i=1}^{n} X_i$$

Standard Deviation:

$$S = \sqrt{\frac{\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2 / n}{n-1}}$$

where:

X_i = individual reading of concentration, temperature or relative humidity

n = number of individual readings

The weekly and study means and standard deviations for concentration were derived from the daily means and standard deviations using the following equation.

Mean: $$\bar{X} = \frac{\sum_{j=1}^{K} (n_j)(\bar{X}_j)}{\sum_{j=1}^{K} n_j}$$

Standard Deviation:

$$S = \sqrt{\frac{\sum_{j=1}^{K} (n_j - 1)\ (S_j^2)}{\sum_{j=1}^{K} n_j - 1}}$$

where:

n_j = number of daily readings

$\bar{X}_j$ = daily mean

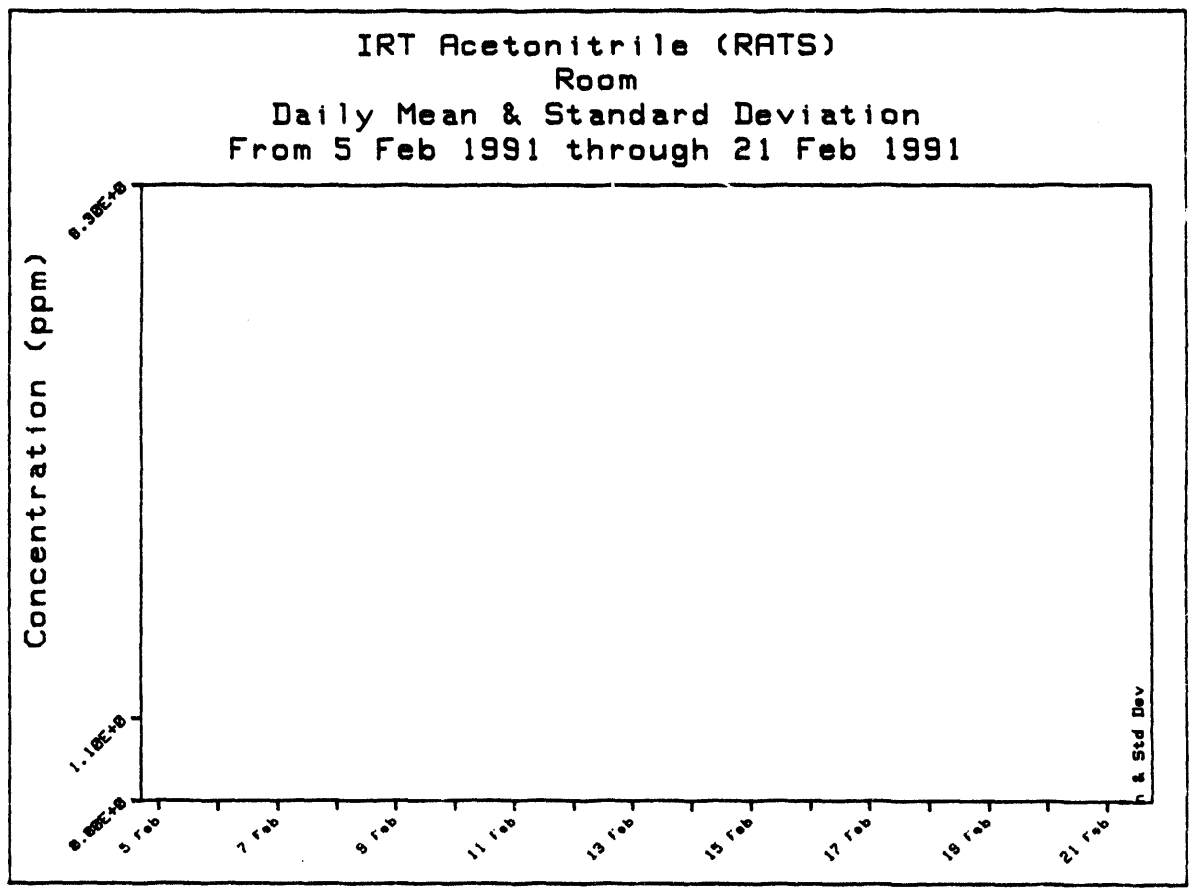
S_j = daily standard deviation

K = number of days included in summations

Concentration Data

Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991
Summary Data for: Room/Concentration Range=0.00E+0 to 1.10E+0
Minimum Detectable Limit: 1.100E+00 Quantifiable Limit: 8.300E+00

Date	Mean	Std Dev	Maximum	Minimum	N	N in	% N in
5 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
6 Feb 1991	<MDL		<MDL	<MDL	15	15	100.0%
7 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
8 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
9 Feb 1991	<MDL		<MDL	<MDL	14	14	100.0%
10 Feb 1991	<MDL		<MDL	<MDL	11	11	100.0%
11 Feb 1991	<MDL		<MDL	<MDL	14	14	100.0%
12 Feb 1991	<MDL		<MDL	<MDL	11	11	100.0%
13 Feb 1991	<MDL		<MDL	<MDL	11	11	100.0%
14 Feb 1991	<MDL		<MDL	<MDL	17	17	100.0%
15 Feb 1991	<MDL		<MDL	<MDL	18	18	100.0%
16 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
17 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
18 Feb 1991	<MDL		<MDL	<MDL	17	17	100.0%
19 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
20 Feb 1991	<MDL		<MDL	<MDL	14	14	100.0%
21 Feb 1991	<MDL		<MDL	<MDL	15	15	100.0%
Summary	<MDL		<MDL	<MDL	253	253	100.0%

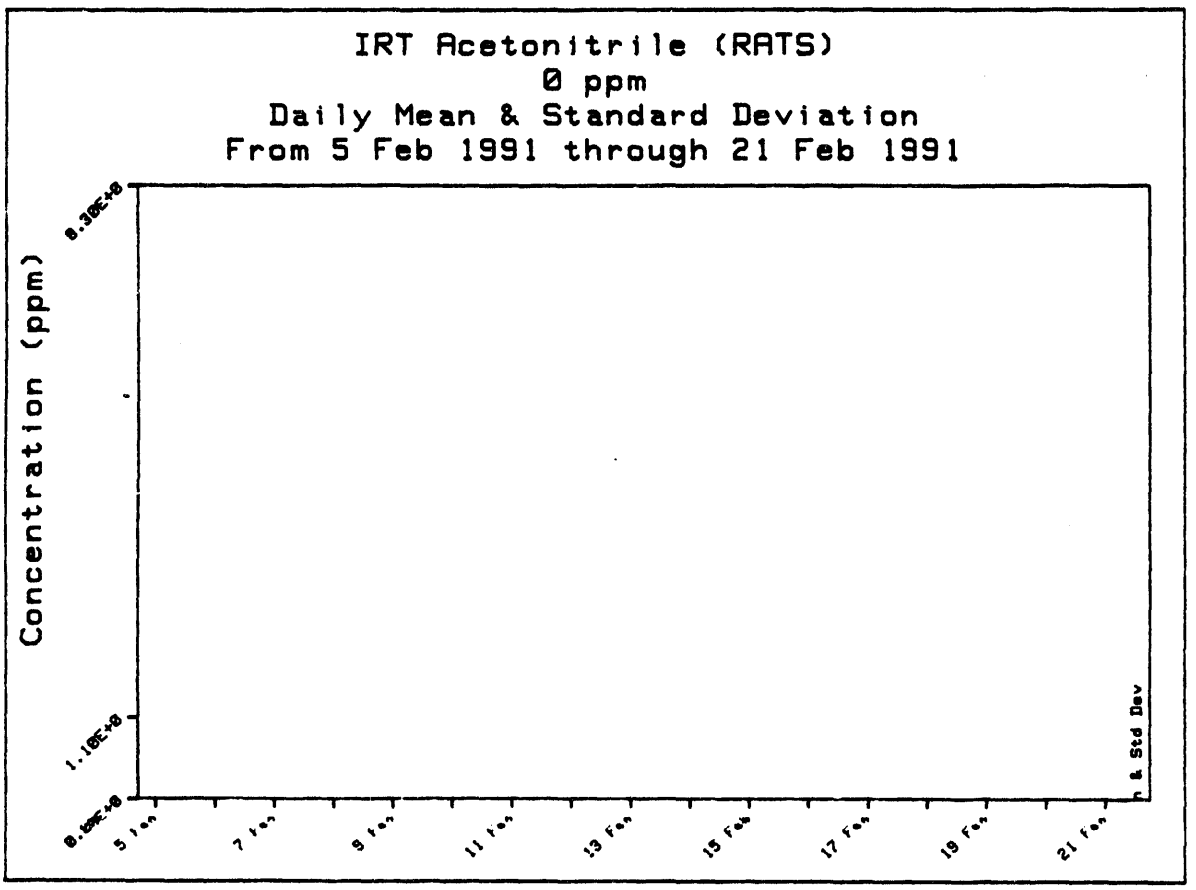


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6

2 of 3

Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991
Summary Data for: 0 ppm/Concentration Range=0.00E+0 to 1.10E+0
Minimum Detectable Limit: 1.100E+00 Quantifiable Limit: 8.300E+00

Date	Mean	Std Dev	Maximum	Minimum	N	N in	% N in
5 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
6 Feb 1991	<MDL		<MDL	<MDL	15	15	100.0%
7 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
8 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
9 Feb 1991	<MDL		<MDL	<MDL	14	14	100.0%
10 Feb 1991	<MDL		<MDL	<MDL	11	11	100.0%
11 Feb 1991	<MDL		<MDL	<MDL	14	14	100.0%
12 Feb 1991	<MDL		<MDL	<MDL	12	12	100.0%
13 Feb 1991	<MDL		<MDL	<MDL	11	11	100.0%
14 Feb 1991	<MDL		<MDL	<MDL	17	17	100.0%
15 Feb 1991	<MDL		<MDL	<MDL	18	18	100.0%
16 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
17 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
18 Feb 1991	<MDL		<MDL	<MDL	17	17	100.0%
19 Feb 1991	<MDL		<MDL	<MDL	16	16	100.0%
20 Feb 1991	<MDL		<MDL	<MDL	15	15	100.0%
21 Feb 1991	<MDL		<MDL	<MDL	15	15	100.0%
Summary	<MDL		<MDL	<MDL	255	255	100.0%

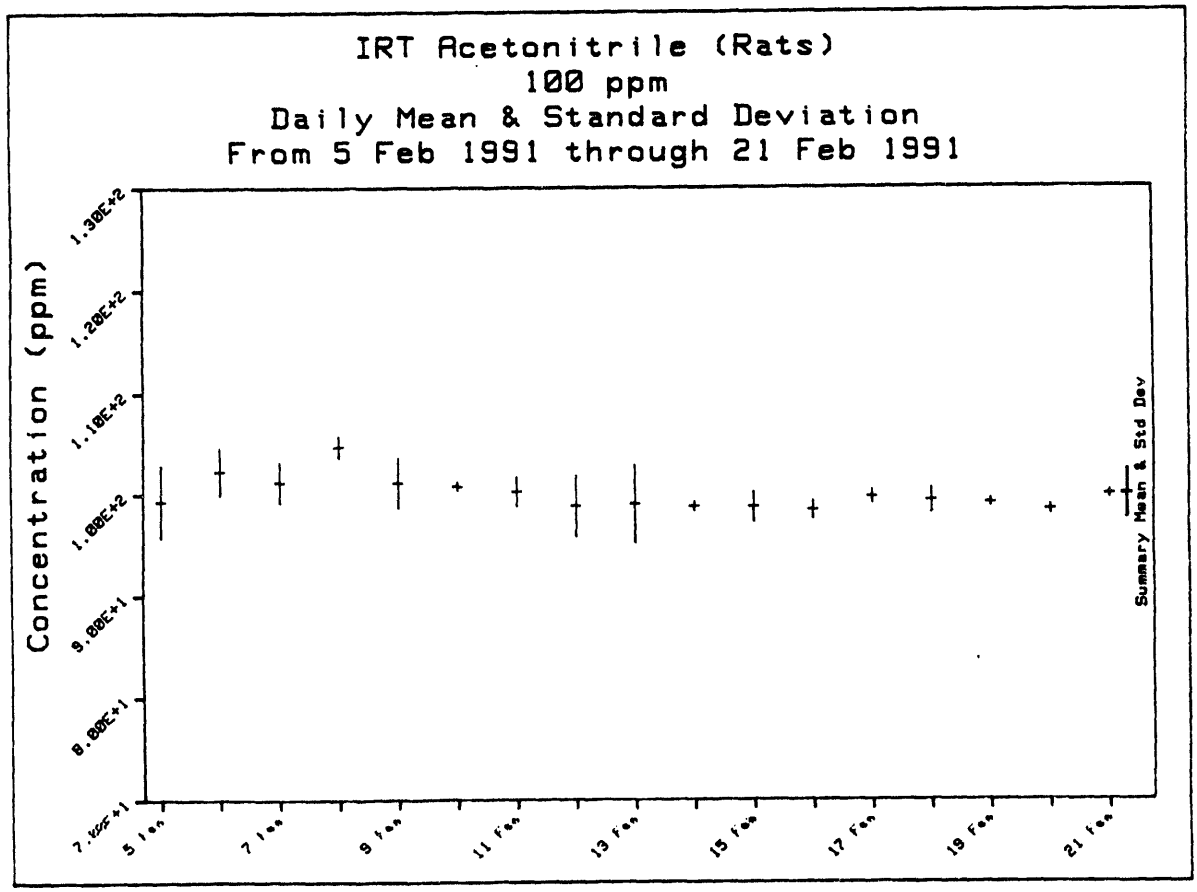


Daily Summation for IRT Acetonitrile (Rats) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 100 ppm/Concentration Range=9.00E+1 to 1.10E+2

Minimum Detectable Limit: 1.100E+00 Quantifiable Limit: 8.300E+00

Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	9.93E+01	99.3%	3.536E+00	3.6%	1.07E+02	9.38E+01	12	12	100.0%
6 Feb 1991	1.02E+02	102.2%	2.314E+00	2.3%	1.07E+02	9.83E+01	12	12	100.0%
7 Feb 1991	1.01E+02	101.1%	1.360E+00	1.9%	1.04E+02	9.78E+01	12	12	100.0%
8 Feb 1991	1.05E+02	104.6%	1.050E+00	1.0%	1.07E+02	1.03E+02	11	11	100.0%
9 Feb 1991	1.01E+02	101.1%	2.442E+00	2.4%	1.05E+02	9.70E+01	11	11	100.0%
10 Feb 1991	1.01E+02	100.7%	3.325E-01	.4%	1.01E+02	1.00E+02	11	11	100.0%
11 Feb 1991	1.00E+02	100.2%	1.440E+00	1.4%	1.02E+02	9.80E+01	9	9	100.0%
12 Feb 1991	9.88E+01	98.8%	2.387E+00	3.0%	1.02E+02	9.45E+01	9	9	100.0%
13 Feb 1991	9.90E+01	99.0%	3.812E+00	3.9%	1.03E+02	8.93E+01	9	8	88.9%
14 Feb 1991	9.87E+01	98.7%	4.439E-01	.4%	9.94E+01	9.80E+01	12	12	100.0%
15 Feb 1991	9.87E+01	98.7%	1.445E+00	1.5%	1.02E+02	9.75E+01	12	12	100.0%
16 Feb 1991	9.84E+01	98.4%	8.579E-01	.9%	9.99E+01	9.71E+01	12	12	100.0%
17 Feb 1991	9.97E+01	99.7%	6.260E-01	.6%	1.01E+02	9.87E+01	11	11	100.0%
18 Feb 1991	9.93E+01	99.3%	1.226E+00	1.2%	1.00E+02	9.64E+01	12	12	100.0%
19 Feb 1991	9.91E+01	99.1%	3.595E-01	.4%	9.96E+01	9.86E+01	10	10	100.0%
20 Feb 1991	9.84E+01	98.4%	4.280E-01	.4%	9.90E+01	9.78E+01	11	11	100.0%
21 Feb 1991	9.99E+01	99.9%	3.718E-01	.4%	1.00E+02	9.93E+01	12	12	100.0%
Summary	1.00E+02	100.0%	2.364E+00	2.4%	1.07E+02	8.93E+01	188	187	99.5%

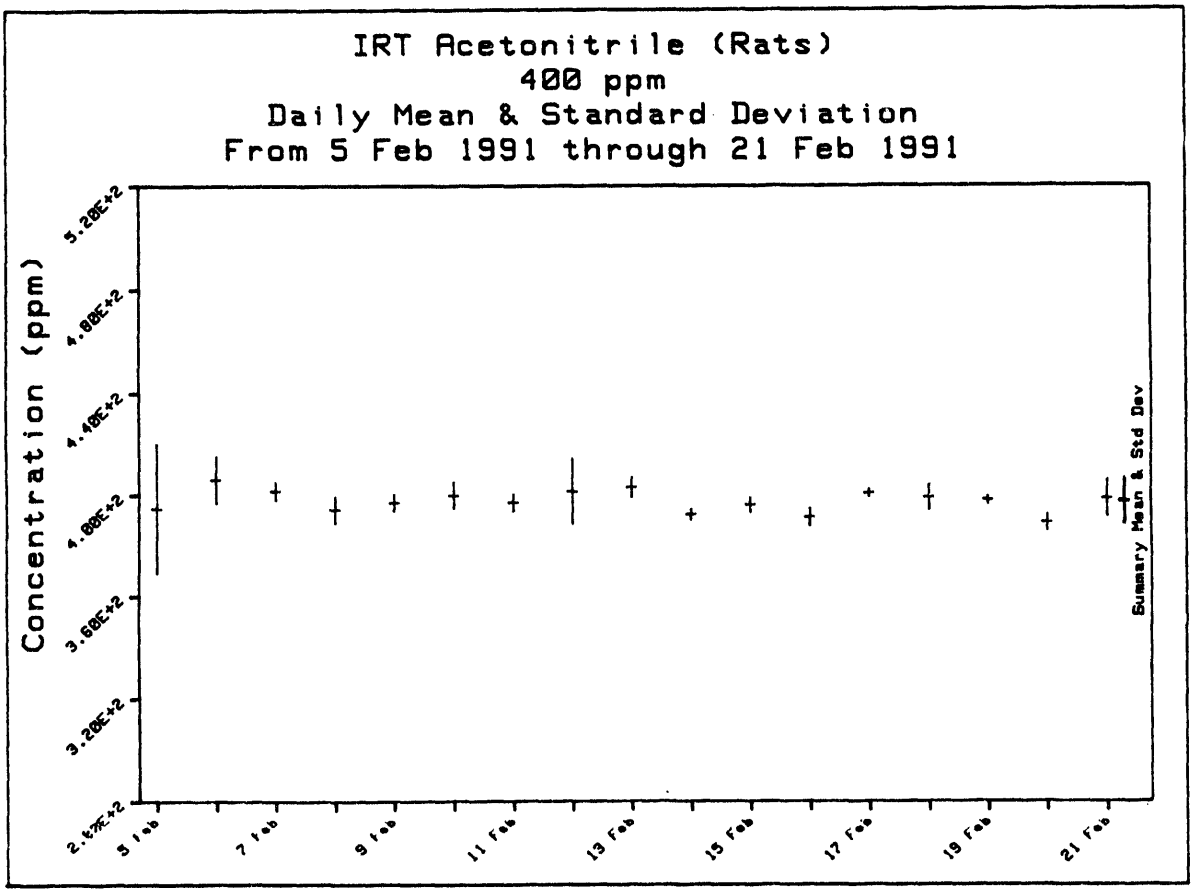


Daily Summation for IRT Acetonitrile (Rats) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 400 ppm/Concentration Range=3.60E+2 to 4.40E+2

Minimum Detectable Limit: 1.100E+00 Quantifiable Limit: 8.300E+00

Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	3.95E+02	98.6%	2.550E+01	6.5%	4.37E+02	3.41E+02	12	11	91.7%
6 Feb 1991	4.06E+02	101.5%	9.277E+00	2.3%	4.30E+02	3.95E+02	12	12	100.0%
7 Feb 1991	4.01E+02	100.3%	3.578E+00	.9%	4.08E+02	3.97E+02	12	12	100.0%
8 Feb 1991	3.94E+02	98.5%	5.236E+00	1.3%	4.00E+02	3.81E+02	11	11	100.0%
9 Feb 1991	3.97E+02	99.2%	3.264E+00	.8%	4.02E+02	3.89E+02	11	11	100.0%
10 Feb 1991	4.00E+02	99.9%	5.192E+00	1.3%	4.06E+02	3.94E+02	10	10	100.0%
11 Feb 1991	3.97E+02	99.2%	3.377E+00	.9%	4.00E+02	3.90E+02	10	10	100.0%
12 Feb 1991	4.01E+02	100.3%	1.280E+01	3.2%	4.22E+02	3.88E+02	8	8	100.0%
13 Feb 1991	4.03E+02	100.7%	3.905E+00	1.0%	4.08E+02	3.97E+02	9	9	100.0%
14 Feb 1991	3.92E+02	98.0%	2.090E+00	.5%	3.95E+02	3.88E+02	12	12	100.0%
15 Feb 1991	3.96E+02	99.0%	2.837E+00	.7%	4.01E+02	3.90E+02	12	12	100.0%
16 Feb 1991	3.91E+02	97.8%	3.480E+00	.9%	3.98E+02	3.84E+02	12	12	100.0%
17 Feb 1991	4.01E+02	100.2%	1.455E+00	.4%	4.03E+02	3.98E+02	12	12	100.0%
18 Feb 1991	3.99E+02	99.7%	5.012E+00	1.3%	4.04E+02	3.88E+02	12	12	100.0%
19 Feb 1991	3.98E+02	99.5%	1.547E+00	.4%	4.00E+02	3.95E+02	12	12	100.0%
20 Feb 1991	3.89E+02	97.2%	3.211E+00	.8%	3.94E+02	3.84E+02	11	11	100.0%
21 Feb 1991	3.98E+02	99.6%	7.248E+00	1.8%	4.09E+02	3.89E+02	12	12	100.0%
Summary	3.97E+02	99.3%	8.933E+00	2.2%	4.37E+02	3.41E+02	190	189	99.5%

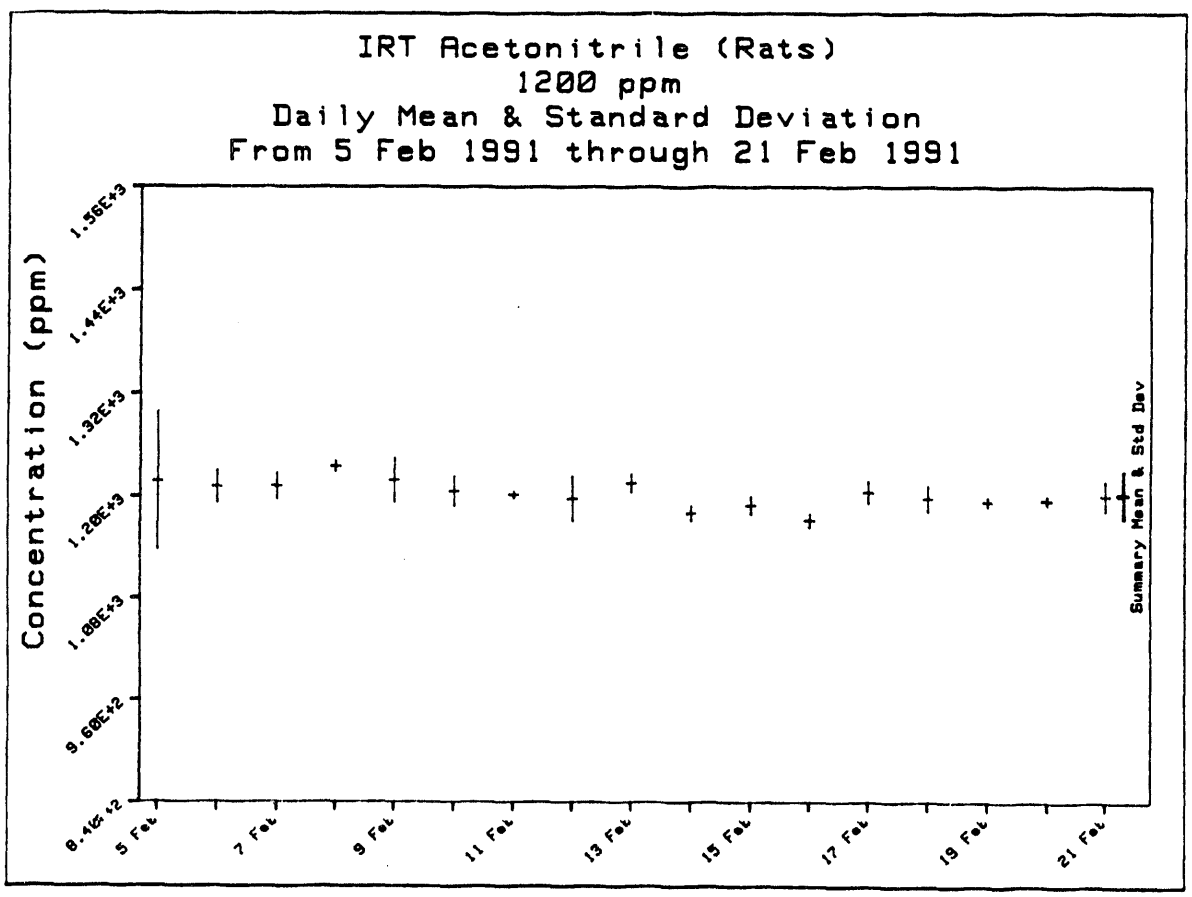


Daily Summation for IRT Acetonitrile (Rats) — From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 1200 ppm/Concentration — Range=1.08E+3 to 1.32E+3

Minimum Detectable Limit: 1.100E+00 — Quantifiable Limit: 8.300E+00

Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	1.22E+03	101.5%	8.051E+01	6.6%	1.42E+03	1.13E+03	12	10	83.3%
6 Feb 1991	1.21E+03	101.0%	1.898E+01	1.6%	1.26E+03	1.19E+03	12	12	100.0%
7 Feb 1991	1.21E+03	101.0%	1.504E+01	1.2%	1.24E+03	1.19E+03	12	12	100.0%
8 Feb 1991	1.24E+03	103.0%	5.801E+00	.5%	1.25E+03	1.23E+03	11	11	100.0%
9 Feb 1991	1.22E+03	101.6%	2.572E+01	2.1%	1.25E+03	1.18E+03	11	11	100.0%
10 Feb 1991	1.21E+03	100.5%	1.747E+01	1.4%	1.24E+03	1.18E+03	10	10	100.0%
11 Feb 1991	1.20E+03	100.2%	3.644E+00	.3%	1.21E+03	1.20E+03	9	9	100.0%
12 Feb 1991	1.20E+03	99.8%	2.609E+01	2.2%	1.23E+03	1.17E+03	8	8	100.0%
13 Feb 1991	1.22E+03	101.4%	1.019E+01	.8%	1.24E+03	1.21E+03	9	9	100.0%
14 Feb 1991	1.18E+03	98.4%	8.730E+00	.7%	1.19E+03	1.16E+03	12	12	100.0%
15 Feb 1991	1.19E+03	99.2%	1.017E+01	.9%	1.20E+03	1.17E+03	12	12	100.0%
16 Feb 1991	1.17E+03	97.7%	8.001E+00	.7%	1.19E+03	1.16E+03	12	12	100.0%
17 Feb 1991	1.21E+03	100.5%	1.307E+01	1.1%	1.24E+03	1.19E+03	12	12	100.0%
18 Feb 1991	1.20E+03	99.8%	1.521E+01	1.3%	1.22E+03	1.17E+03	12	12	100.0%
19 Feb 1991	1.19E+03	99.5%	5.237E+00	.4%	1.20E+03	1.18E+03	12	12	100.0%
20 Feb 1991	1.20E+03	99.7%	5.202E+00	.4%	1.21E+03	1.19E+03	12	12	100.0%
21 Feb 1991	1.20E+03	100.1%	1.726E+01	1.4%	1.23E+03	1.18E+03	12	12	100.0%
Summary	1.20E+03	100.3%	2.783E+01	2.3%	1.42E+03	1.13E+03	190	188	98.9%

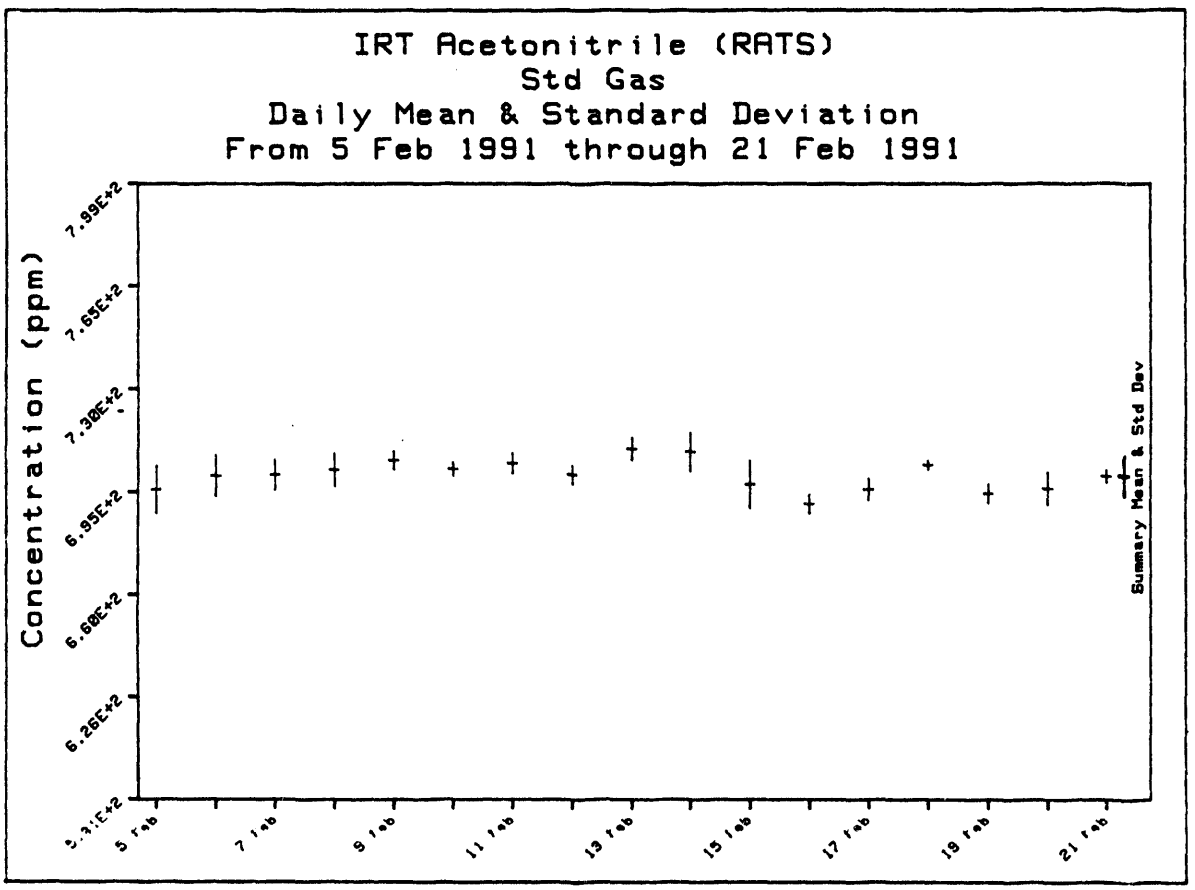


Daily Summation for IRT Acetonitrile (RATS) — From 5 Feb 1991 through 21 Feb 1991

Summary Data for: Std Gas/Concentration — Range=6.60E+2 to 7.30E+2

Minimum Detectable Limit: 1.100E+00 — Quantifiable Limit: 8.300E+00

Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	6.96E+02	100.1%	7.969E+00	1.1%	7.08E+02	6.84E+02	13	13	100.0%
6 Feb 1991	7.01E+02	100.8%	6.879E+00	1.0%	7.15E+02	6.93E+02	12	12	100.0%
7 Feb 1991	7.01E+02	100.8%	5.062E+00	.7%	7.12E+02	6.95E+02	12	12	100.0%
8 Feb 1991	7.03E+02	101.1%	5.465E+00	.8%	7.14E+02	6.95E+02	12	12	100.0%
9 Feb 1991	7.06E+02	101.5%	3.107E+00	.4%	7.10E+02	7.02E+02	10	10	100.0%
10 Feb 1991	7.03E+02	101.1%	2.212E+00	.3%	7.07E+02	7.01E+02	11	11	100.0%
11 Feb 1991	7.05E+02	101.4%	3.342E+00	.5%	7.12E+02	7.01E+02	9	9	100.0%
12 Feb 1991	7.01E+02	100.9%	3.155E+00	.5%	7.04E+02	6.95E+02	10	10	100.0%
13 Feb 1991	7.10E+02	102.1%	3.799E+00	.5%	7.15E+02	7.05E+02	10	10	100.0%
14 Feb 1991	7.09E+02	102.0%	6.388E+00	.9%	7.21E+02	7.02E+02	12	12	100.0%
15 Feb 1991	6.98E+02	100.4%	8.007E+00	1.1%	7.15E+02	6.90E+02	12	12	100.0%
16 Feb 1991	6.91E+02	99.4%	3.157E+00	.5%	6.96E+02	6.87E+02	12	12	100.0%
17 Feb 1991	6.96E+02	100.2%	3.588E+00	.5%	7.02E+02	6.92E+02	12	12	100.0%
18 Feb 1991	7.04E+02	101.3%	1.500E+00	.2%	7.08E+02	7.02E+02	13	13	100.0%
19 Feb 1991	6.95E+02	99.9%	3.160E+00	.5%	7.02E+02	6.91E+02	12	12	100.0%
20 Feb 1991	6.96E+02	100.2%	5.415E+00	.8%	7.08E+02	6.91E+02	12	12	100.0%
21 Feb 1991	7.00E+02	100.8%	2.075E+00	.3%	7.05E+02	6.97E+02	12	12	100.0%
Summary	7.01E+02	100.8%	6.780E+00	1.0%	7.21E+02	6.84E+02	196	196	100.0%

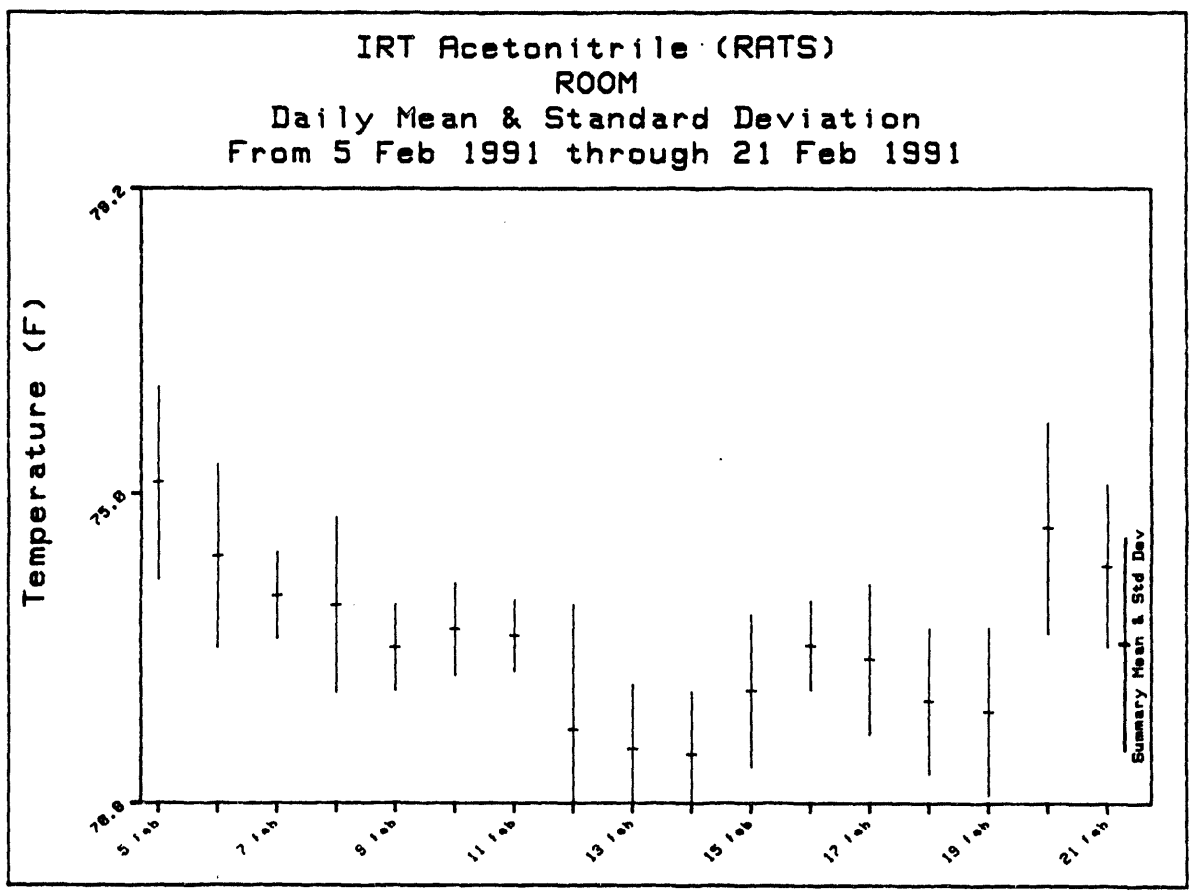


Temperature Data

Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: ROOM/Temperature Range varied during study

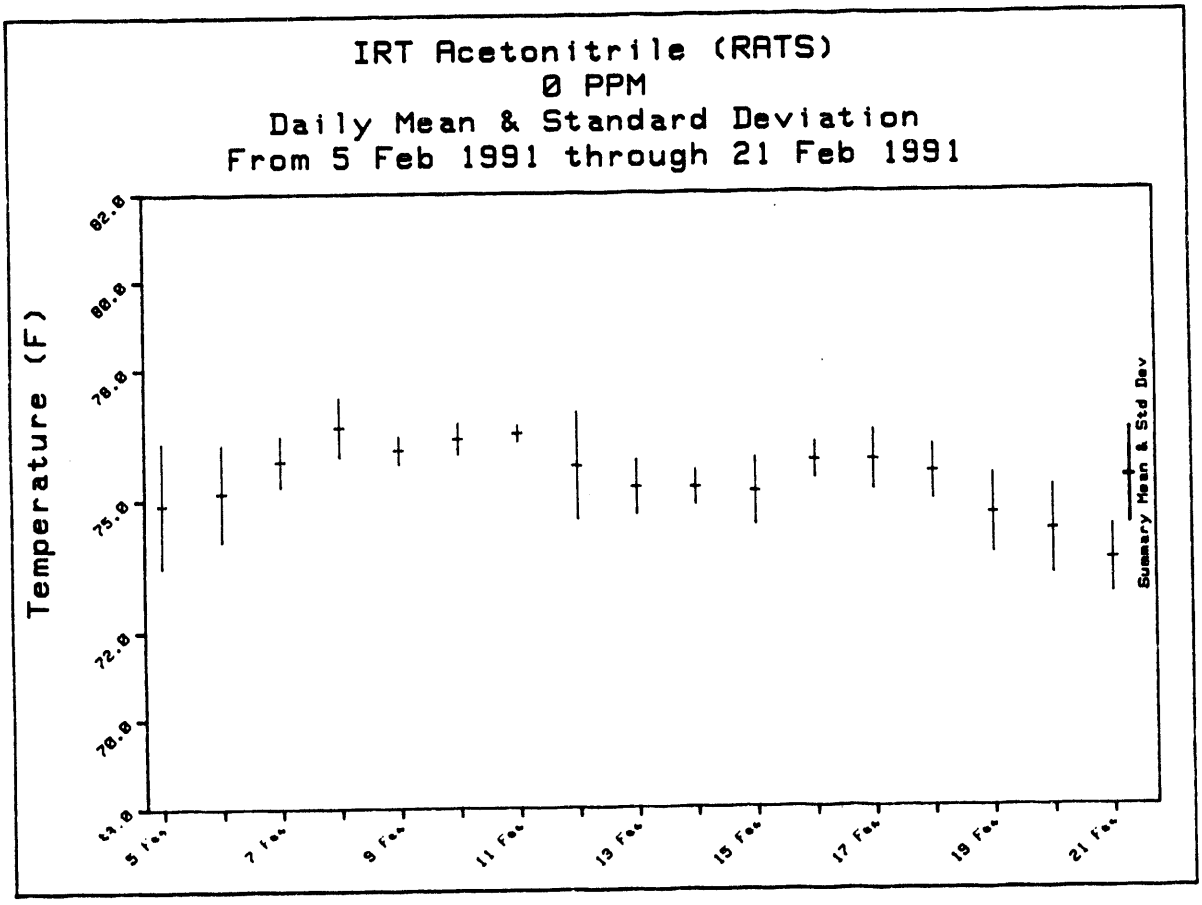
Date	Mean	Std Dev	% RSD	Maximum	Minimum	N
5 Feb 1991	75.2	1.31	1.7%	77.8	73.9	25
6 Feb 1991	74.2	1.24	1.7%	76.7	72.9	35
7 Feb 1991	73.6	.58	.8%	75.4	73.1	26
8 Feb 1991	73.5	1.17	1.6%	77.1	71.6	33
9 Feb 1991	72.9	.58	.8%	74.2	72.3	30
10 Feb 1991	73.2	.62	.8%	74.0	72.5	36
11 Feb 1991	73.1	.48	.7%	74.7	72.3	30
12 Feb 1991	71.8	1.68	2.3%	75.0	70.3	30
13 Feb 1991	71.6	.85	1.2%	73.4	70.7	34
14 Feb 1991	71.5	.83	1.2%	72.9	70.5	34
15 Feb 1991	72.3	1.02	1.4%	73.8	69.7	33
16 Feb 1991	73.0	.60	.8%	73.9	71.5	30
17 Feb 1991	72.8	1.01	1.4%	74.0	70.8	30
18 Feb 1991	72.2	.97	1.3%	73.7	70.8	33
19 Feb 1991	72.1	1.12	1.6%	74.0	70.8	32
20 Feb 1991	74.5	1.43	1.9%	77.2	72.6	27
21 Feb 1991	74.0	1.09	1.5%	75.8	72.2	34
Summary	73.0	1.42	2.0%	77.8	69.7	532



Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 0 PPM/Temperature Range= 72.0 to 78.0

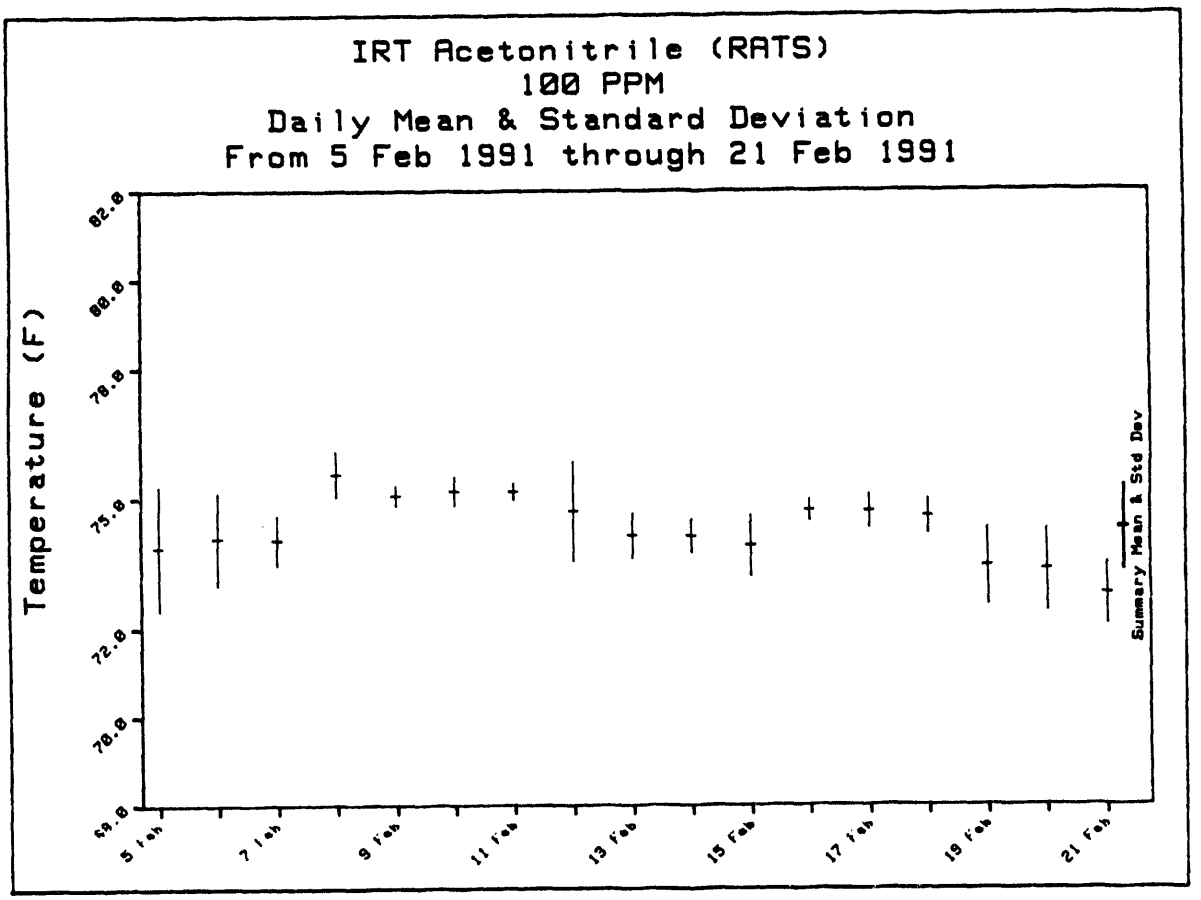
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	74.9	99.8%	1.42	1.9%	77.2	73.5	6	6	100.0%
6 Feb 1991	75.2	100.2%	1.11	1.5%	77.1	74.0	8	8	100.0%
7 Feb 1991	75.9	101.2%	.57	.8%	76.7	75.4	6	6	100.0%
8 Feb 1991	76.7	102.2%	.67	.9%	77.4	75.7	7	7	100.0%
9 Feb 1991	76.1	101.5%	.32	.4%	76.7	75.7	7	7	100.0%
10 Feb 1991	76.4	101.8%	.35	.5%	76.7	75.8	8	8	100.0%
11 Feb 1991	76.5	102.0%	.18	.2%	76.7	76.3	6	6	100.0%
12 Feb 1991	75.8	101.0%	1.22	1.6%	77.6	74.6	7	7	100.0%
13 Feb 1991	75.3	100.3%	.62	.8%	76.2	74.7	7	7	100.0%
14 Feb 1991	75.2	100.3%	.39	.5%	76.0	74.9	7	7	100.0%
15 Feb 1991	75.2	100.2%	.77	1.0%	76.1	74.3	7	7	100.0%
16 Feb 1991	75.9	101.2%	.40	.5%	76.2	75.0	8	8	100.0%
17 Feb 1991	75.9	101.1%	.67	.9%	76.5	74.6	8	8	100.0%
18 Feb 1991	75.6	100.8%	.62	.8%	76.3	74.7	8	8	100.0%
19 Feb 1991	74.6	99.5%	.91	1.2%	75.8	73.4	7	7	100.0%
20 Feb 1991	74.2	99.0%	1.01	1.4%	75.8	73.0	7	7	100.0%
21 Feb 1991	73.6	98.1%	.76	1.0%	74.5	72.8	8	8	100.0%
Summary	75.4	100.6%	1.08	1.4%	77.6	72.8	122	122	100.0%



Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 100 PPM/Temperature Range= 72.0 to 78.0

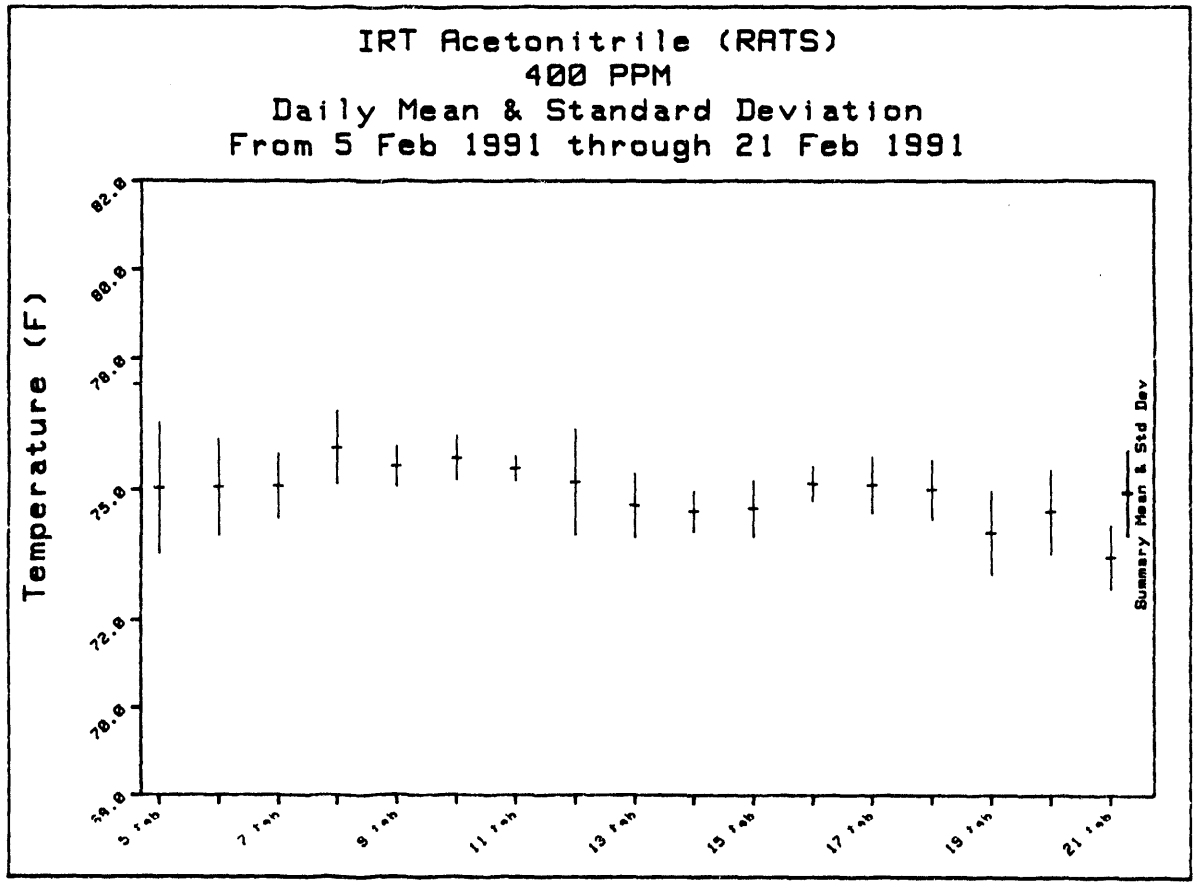
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	73.8	98.5%	1.43	1.9%	76.2	72.4	6	6	100.0%
6 Feb 1991	74.1	98.7%	1.07	1.4%	75.9	72.9	8	8	100.0%
7 Feb 1991	74.0	98.7%	.57	.8%	75.0	73.5	6	6	100.0%
8 Feb 1991	75.6	100.7%	.52	.7%	76.2	74.9	7	7	100.0%
9 Feb 1991	75.1	100.1%	.23	.3%	75.4	74.8	7	7	100.0%
10 Feb 1991	75.2	100.2%	.34	.4%	75.4	74.5	8	8	100.0%
11 Feb 1991	75.2	100.2%	.19	.2%	75.3	74.9	6	6	100.0%
12 Feb 1991	74.7	99.6%	1.16	1.6%	76.5	73.4	7	7	100.0%
13 Feb 1991	74.1	98.8%	.52	.7%	74.8	73.5	7	7	100.0%
14 Feb 1991	74.1	98.8%	.39	.5%	74.8	73.6	7	7	100.0%
15 Feb 1991	73.9	98.5%	.70	1.0%	74.8	72.7	7	7	100.0%
16 Feb 1991	74.7	99.6%	.25	.3%	75.0	74.2	8	8	100.0%
17 Feb 1991	74.7	99.6%	.39	.5%	75.1	74.1	8	8	100.0%
18 Feb 1991	74.6	99.4%	.40	.5%	75.1	74.1	8	8	100.0%
19 Feb 1991	73.4	97.9%	.88	1.2%	74.5	72.3	7	7	100.0%
20 Feb 1991	73.3	97.8%	.93	1.3%	74.8	72.3	7	7	100.0%
21 Feb 1991	72.8	97.0%	.70	1.0%	73.7	72.0	8	8	100.0%
Summary	74.3	99.1%	.98	1.3%	76.5	72.0	122	122	100.0%



Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 400 PPM/Temperature Range= 72.0 to 78.0

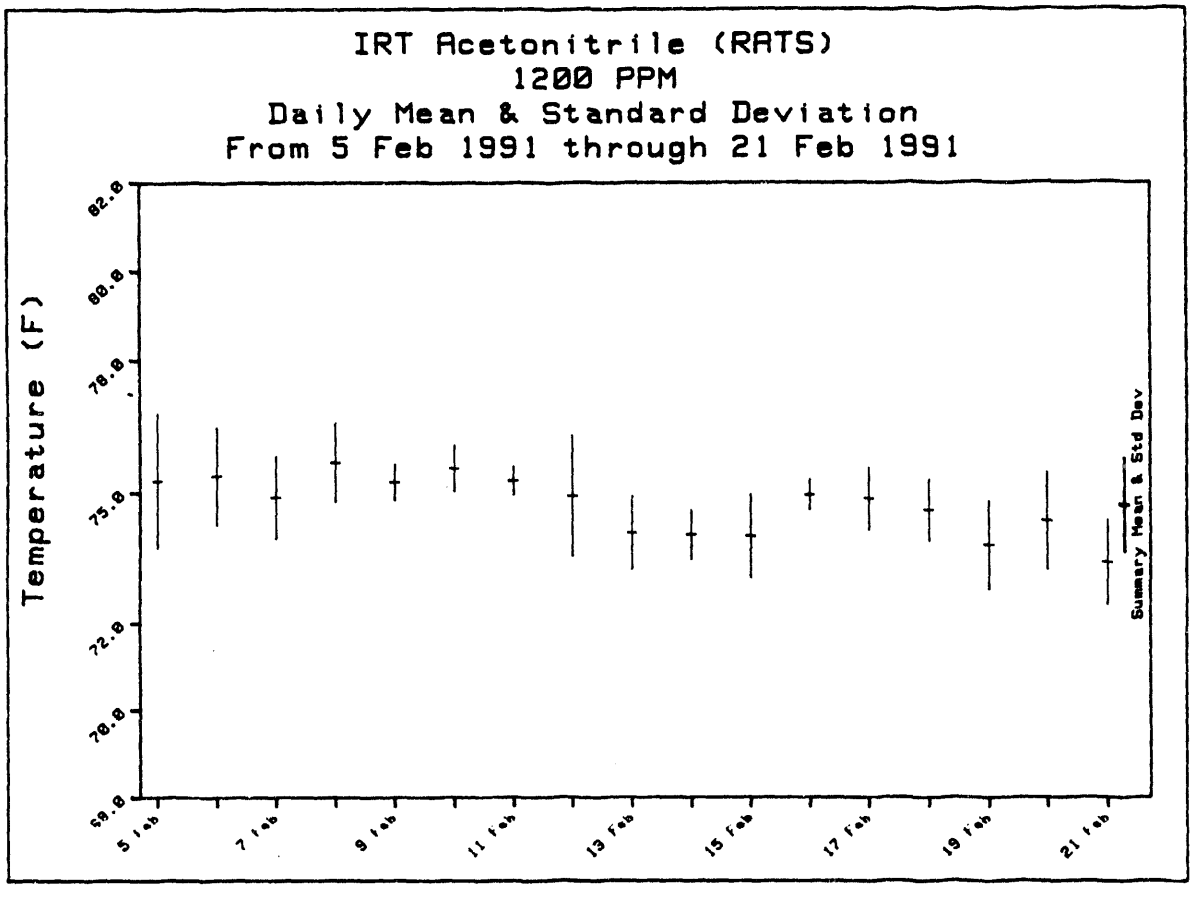
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	75.0	100.0%	1.50	2.0%	77.5	73.7	6	6	100.0%
6 Feb 1991	75.1	100.1%	1.11	1.5%	76.9	74.0	8	8	100.0%
7 Feb 1991	75.1	100.1%	.74	1.0%	76.5	74.6	6	6	100.0%
8 Feb 1991	76.0	101.3%	.83	1.1%	77.0	74.9	7	7	100.0%
9 Feb 1991	75.5	100.7%	.46	.6%	76.4	75.1	7	7	100.0%
10 Feb 1991	75.7	101.0%	.51	.7%	76.3	74.9	8	8	100.0%
11 Feb 1991	75.5	100.6%	.28	.4%	75.8	75.1	6	6	100.0%
12 Feb 1991	75.2	100.2%	1.21	1.6%	76.9	74.0	7	7	100.0%
13 Feb 1991	74.6	99.5%	.72	1.0%	75.6	73.9	7	7	100.0%
14 Feb 1991	74.5	99.3%	.46	.6%	75.4	74.2	7	7	100.0%
15 Feb 1991	74.6	99.4%	.64	.9%	75.4	74.0	6	6	100.0%
16 Feb 1991	75.1	100.2%	.40	.5%	75.6	74.3	8	8	100.0%
17 Feb 1991	75.1	100.1%	.65	.9%	75.9	73.9	8	8	100.0%
18 Feb 1991	75.0	100.0%	.68	.9%	75.9	74.2	8	8	100.0%
19 Feb 1991	74.0	98.7%	.94	1.3%	75.3	72.9	7	7	100.0%
20 Feb 1991	74.5	99.3%	.96	1.3%	75.9	73.3	7	7	100.0%
21 Feb 1991	73.4	97.9%	.71	1.0%	74.5	72.7	8	8	100.0%
Summary	74.9	99.9%	.97	1.3%	77.5	72.7	121	121	100.0%



Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 1200 PPM/Temperature Range= 72.0 to 78.0

Date	Mean	% Target	St: Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	75.3	100.4%	1.53	2.0%	77.8	74.0	6	6	100.0%
6 Feb 1991	75.4	100.5%	1.11	1.5%	77.3	74.2	8	8	100.0%
7 Feb 1991	74.9	99.9%	.94	1.3%	76.7	74.2	6	6	100.0%
8 Feb 1991	75.7	100.9%	.89	1.2%	76.9	74.7	7	7	100.0%
9 Feb 1991	75.2	100.3%	.41	.5%	76.0	74.9	7	7	100.0%
10 Feb 1991	75.6	100.7%	.52	.7%	76.3	74.8	8	8	100.0%
11 Feb 1991	75.3	100.4%	.32	.4%	75.6	74.8	6	6	100.0%
12 Feb 1991	74.9	99.9%	1.38	1.8%	76.8	73.4	8	8	100.0%
13 Feb 1991	74.1	98.8%	.84	1.1%	75.2	73.2	7	7	100.0%
14 Feb 1991	74.0	98.7%	.56	.8%	75.2	73.6	7	7	100.0%
15 Feb 1991	74.0	98.7%	.95	1.3%	74.9	72.4	7	7	100.0%
16 Feb 1991	75.0	99.9%	.34	.5%	75.4	74.2	8	8	100.0%
17 Feb 1991	74.9	99.8%	.71	.9%	75.6	73.7	8	8	100.0%
18 Feb 1991	74.6	99.5%	.70	.9%	75.6	73.9	8	8	100.0%
19 Feb 1991	73.8	98.4%	1.01	1.4%	75.3	72.5	7	7	100.0%
20 Feb 1991	74.3	99.1%	1.11	1.5%	76.0	73.0	7	7	100.0%
21 Feb 1991	73.4	97.9%	.97	1.3%	74.6	72.4	8	8	100.0%
Summary	74.7	99.6%	1.07	1.4%	77.8	72.4	123	123	100.0%

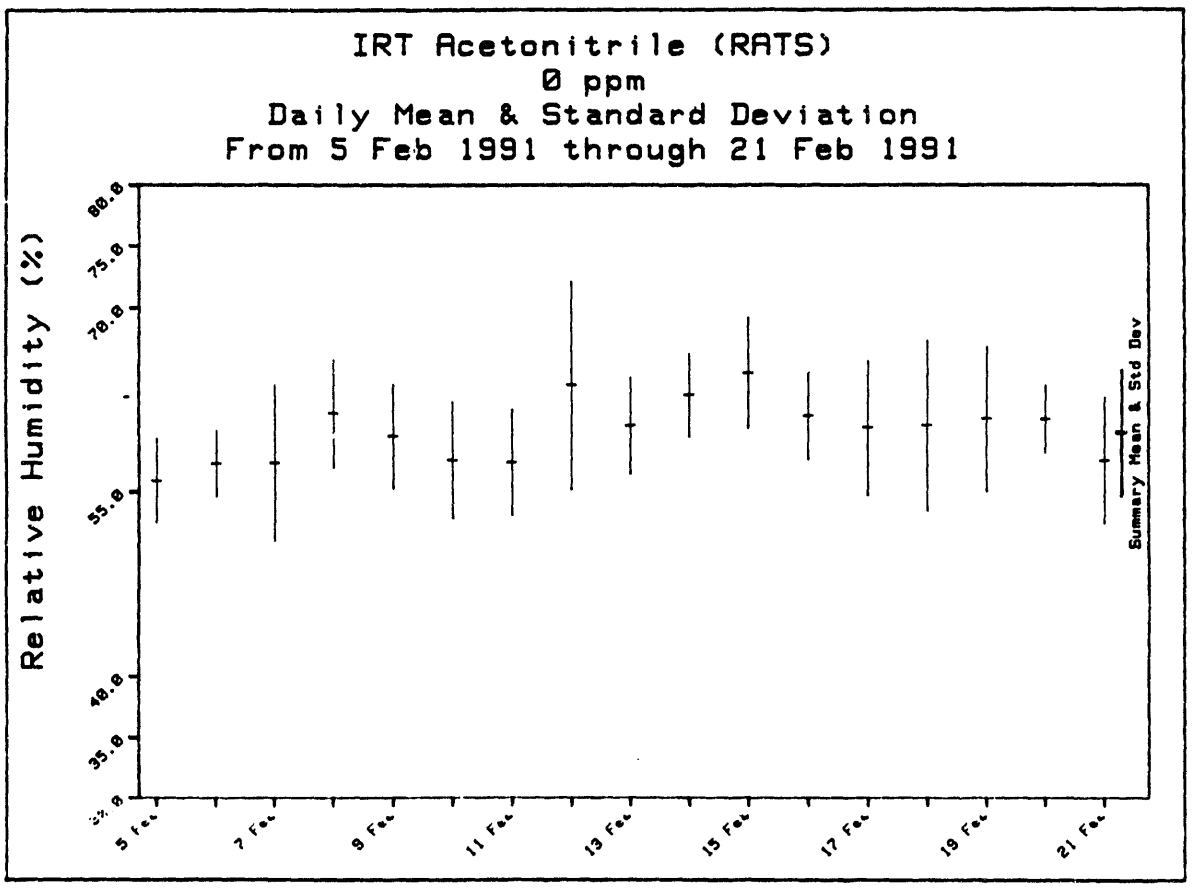


Relative Humidity Data

Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 0 ppm/Relative Humidity Range= 40.0 to 70.0

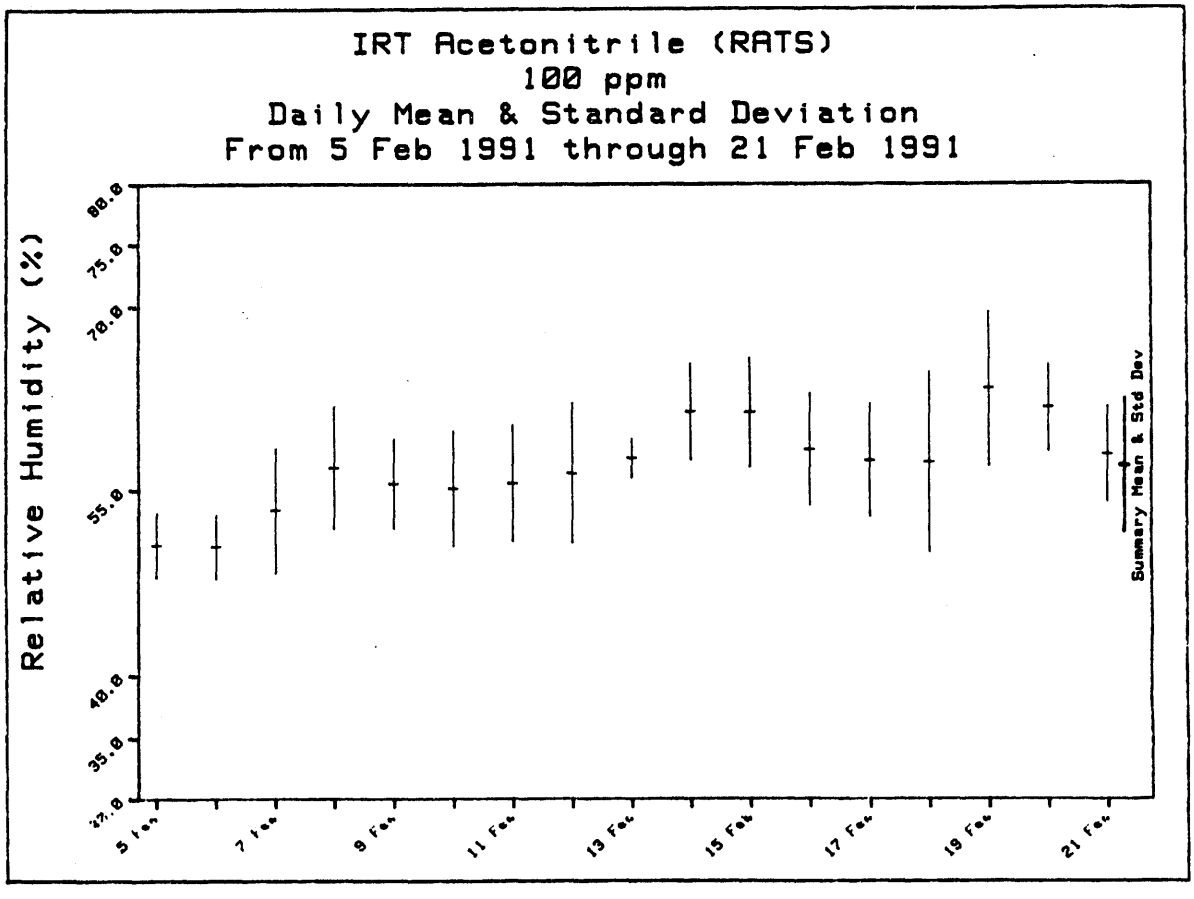
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	55.9	101.6%	3.38	6.0%	60.6	51.0	7	7	100.0%
6 Feb 1991	57.2	104.1%	2.65	4.6%	62.1	53.8	8	8	100.0%
7 Feb 1991	57.3	104.2%	6.29	11.0%	64.3	48.7	6	6	100.0%
8 Feb 1991	61.3	111.5%	4.37	7.1%	66.8	54.6	7	7	100.0%
9 Feb 1991	59.5	108.1%	4.20	7.1%	65.2	54.8	7	7	100.0%
10 Feb 1991	57.5	104.6%	4.69	8.2%	62.6	50.3	8	8	100.0%
11 Feb 1991	57.4	104.3%	4.24	7.4%	62.5	51.3	7	7	100.0%
12 Feb 1991	63.6	115.7%	8.45	13.3%	72.3	54.1	6	4	66.7%
13 Feb 1991	60.4	109.7%	3.90	6.5%	65.5	55.9	6	6	100.0%
14 Feb 1991	62.8	114.2%	3.41	5.4%	66.3	58.7	7	7	100.0%
15 Feb 1991	64.6	117.5%	4.51	7.0%	68.1	57.6	6	6	100.0%
16 Feb 1991	61.1	111.2%	3.52	5.8%	65.4	56.5	7	7	100.0%
17 Feb 1991	60.2	109.5%	5.46	9.1%	64.8	52.1	8	8	100.0%
18 Feb 1991	60.4	109.8%	6.91	11.4%	66.5	50.7	6	6	100.0%
19 Feb 1991	60.9	110.8%	5.87	9.6%	66.7	51.9	6	6	100.0%
20 Feb 1991	60.9	110.6%	2.70	4.4%	64.6	56.7	7	7	100.0%
21 Feb 1991	57.5	104.6%	5.07	8.8%	64.9	51.7	8	8	100.0%
Summary	59.8	108.7%	5.07	8.5%	72.3	48.7	117	115	98.3%



Daily Summation for IRT Acetonitrile (RATS) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 100 ppm/Relative Humidity Range= 40.0 to 70.0

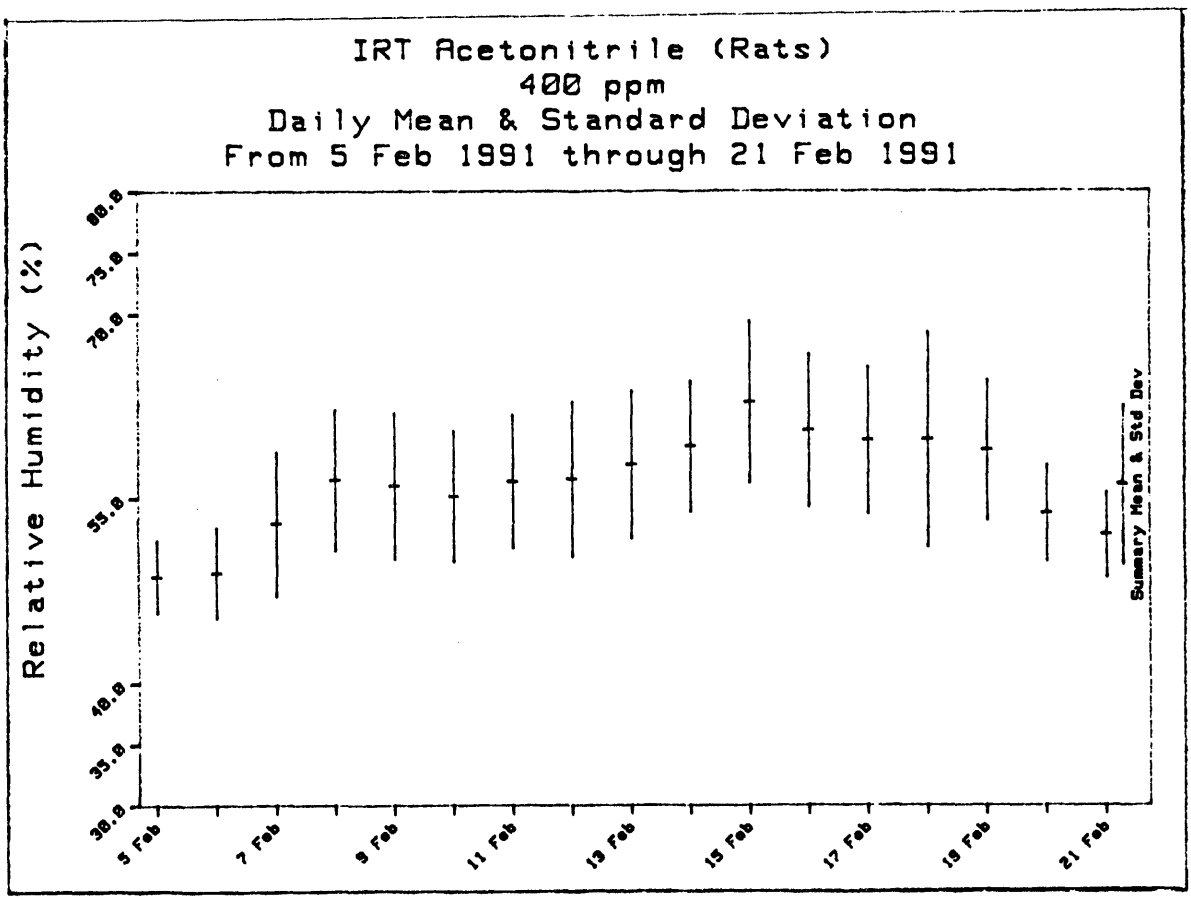
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	50.5	91.8%	2.59	5.1%	53.9	47.3	7	7	100.0%
6 Feb 1991	50.4	91.6%	2.54	5.0%	53.7	47.4	8	8	100.0%
7 Feb 1991	53.3	97.0%	5.05	9.5%	59.3	46.2	5	5	100.0%
8 Feb 1991	56.8	103.3%	4.96	8.7%	61.3	48.6	7	7	100.0%
9 Feb 1991	55.5	100.9%	3.63	6.5%	61.1	50.4	8	8	100.0%
10 Feb 1991	55.1	100.2%	4.68	8.5%	62.2	48.6	8	8	100.0%
11 Feb 1991	55.6	101.0%	4.71	8.5%	60.1	48.8	7	7	100.0%
12 Feb 1991	56.4	102.5%	5.68	10.1%	63.7	49.6	6	6	100.0%
13 Feb 1991	57.6	104.8%	1.56	2.7%	59.7	55.6	7	7	100.0%
14 Feb 1991	61.4	111.6%	3.93	6.4%	65.4	56.2	7	7	100.0%
15 Feb 1991	61.3	111.5%	4.43	7.2%	65.0	54.1	7	7	100.0%
16 Feb 1991	58.3	106.0%	4.54	7.8%	64.0	52.8	7	7	100.0%
17 Feb 1991	57.4	104.4%	4.58	8.0%	61.8	51.3	8	8	100.0%
18 Feb 1991	57.3	104.2%	7.31	12.8%	64.7	48.3	6	6	100.0%
19 Feb 1991	63.3	115.0%	6.29	9.9%	69.4	55.0	6	6	100.0%
20 Feb 1991	61.7	112.2%	3.52	5.7%	66.1	57.5	7	7	100.0%
21 Feb 1991	57.9	105.2%	3.86	6.7%	63.3	52.4	8	8	100.0%
Summary	57.0	103.6%	5.42	9.5%	69.4	46.2	119	119	100.0%



Daily Summation for IRT Acetonitrile (Rats) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 400 ppm/Relative Humidity Range= 40.0 to 70.0

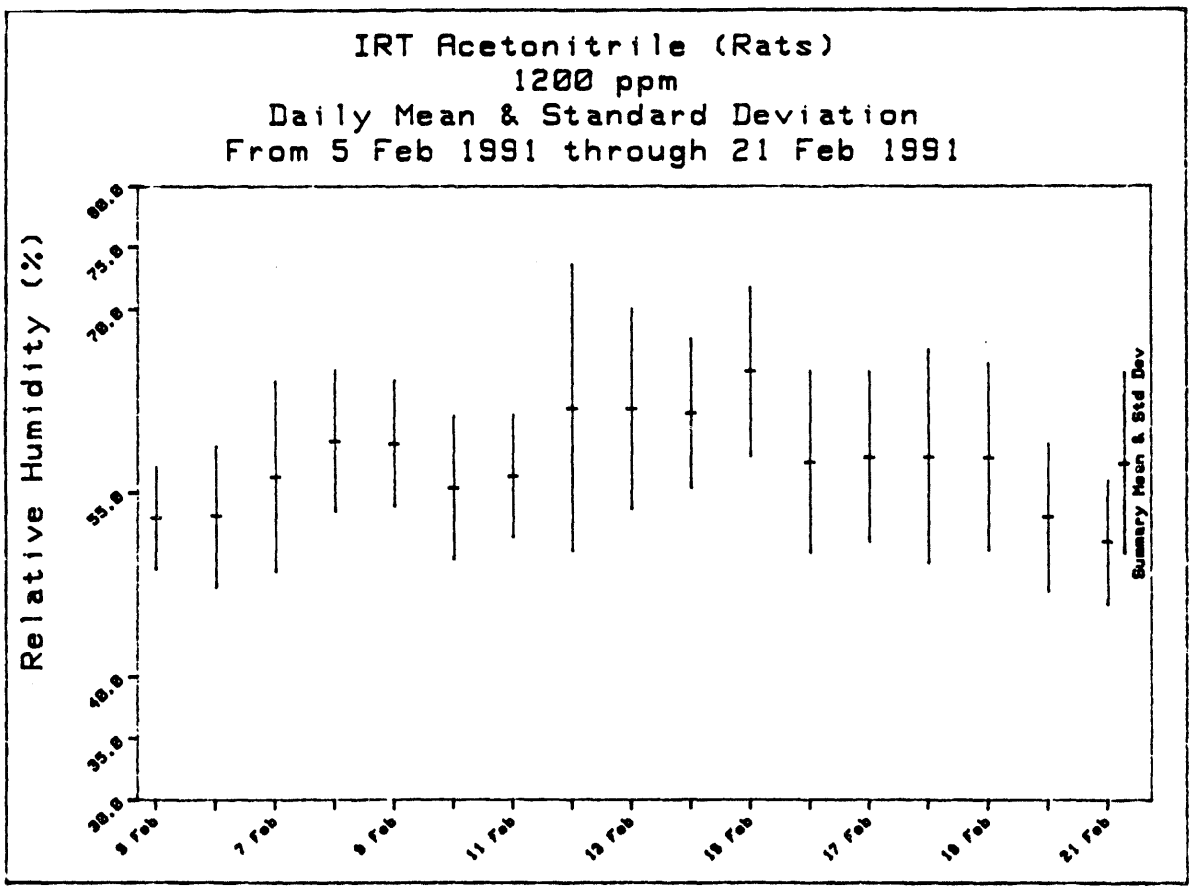
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	48.6	88.4%	2.90	6.0%	51.9	45.0	7	7	100.0%
6 Feb 1991	49.0	89.0%	3.62	7.4%	53.5	44.3	8	8	100.0%
7 Feb 1991	52.9	96.2%	5.84	11.0%	58.8	44.6	6	6	100.0%
8 Feb 1991	56.5	102.7%	5.72	10.1%	61.7	47.6	7	7	100.0%
9 Feb 1991	56.0	101.8%	5.93	10.6%	61.9	48.2	8	8	100.0%
10 Feb 1991	55.1	100.2%	5.29	9.6%	62.7	48.7	8	8	100.0%
11 Feb 1991	56.4	102.5%	5.40	9.6%	61.4	48.0	7	7	100.0%
12 Feb 1991	56.6	102.8%	6.30	11.1%	63.0	48.8	6	6	100.0%
13 Feb 1991	57.8	105.0%	6.02	10.4%	65.0	51.0	7	7	100.0%
14 Feb 1991	59.2	107.7%	5.33	9.0%	64.5	52.4	7	7	100.0%
15 Feb 1991	62.8	114.2%	6.59	10.5%	69.3	53.0	7	7	100.0%
16 Feb 1991	60.5	110.1%	6.24	10.3%	69.7	50.9	7	7	100.0%
17 Feb 1991	59.7	108.6%	5.99	10.0%	65.0	51.1	8	8	100.0%
18 Feb 1991	59.8	108.7%	8.74	14.6%	67.5	49.1	6	6	100.0%
19 Feb 1991	58.9	107.1%	5.74	9.8%	64.4	51.2	6	6	100.0%
20 Feb 1991	53.7	97.7%	3.90	7.3%	59.6	47.8	7	7	100.0%
21 Feb 1991	52.0	94.5%	3.43	6.6%	57.1	46.6	8	8	100.0%
Summary	56.1	102.0%	6.48	11.5%	69.7	44.3	120	120	100.0%



Daily Summation for IRT Acetonitrile (Rats) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 1200 ppm/Relative Humidity Range= 40.0 to 70.0

Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	53.0	96.3%	4.20	7.9%	58.8	48.7	7	7	100.0%
6 Feb 1991	53.1	96.5%	5.79	10.9%	60.7	43.2	8	8	100.0%
7 Feb 1991	56.3	102.4%	7.77	13.8%	65.1	45.4	6	6	100.0%
8 Feb 1991	59.3	107.8%	5.74	9.7%	65.7	50.6	7	7	100.0%
9 Feb 1991	59.1	107.4%	5.11	8.6%	64.3	51.9	8	8	100.0%
10 Feb 1991	55.4	100.8%	5.87	10.6%	65.4	47.4	8	8	100.0%
11 Feb 1991	56.4	102.5%	5.01	8.9%	61.7	49.5	7	7	100.0%
12 Feb 1991	61.9	112.6%	11.66	18.8%	74.9	47.3	7	4	57.1%
13 Feb 1991	61.9	112.6%	8.15	13.2%	71.3	49.9	7	5	71.4%
14 Feb 1991	61.5	111.9%	6.05	9.8%	72.3	54.5	7	6	85.7%
15 Feb 1991	64.9	118.0%	6.85	10.5%	70.7	54.8	7	4	57.1%
16 Feb 1991	57.5	104.6%	7.41	12.9%	69.0	46.8	7	7	100.0%
17 Feb 1991	57.9	105.3%	6.93	12.0%	64.3	47.1	8	8	100.0%
18 Feb 1991	58.0	105.4%	8.72	15.0%	65.7	46.8	6	6	100.0%
19 Feb 1991	57.9	105.3%	7.63	13.2%	64.4	47.3	6	6	100.0%
20 Feb 1991	53.1	96.5%	6.05	11.4%	59.6	43.1	7	7	100.0%
21 Feb 1991	51.0	92.7%	5.06	9.9%	57.0	41.7	8	8	100.0%
Summary	57.5	104.5%	7.37	12.8%	74.9	41.7	121	112	92.6%

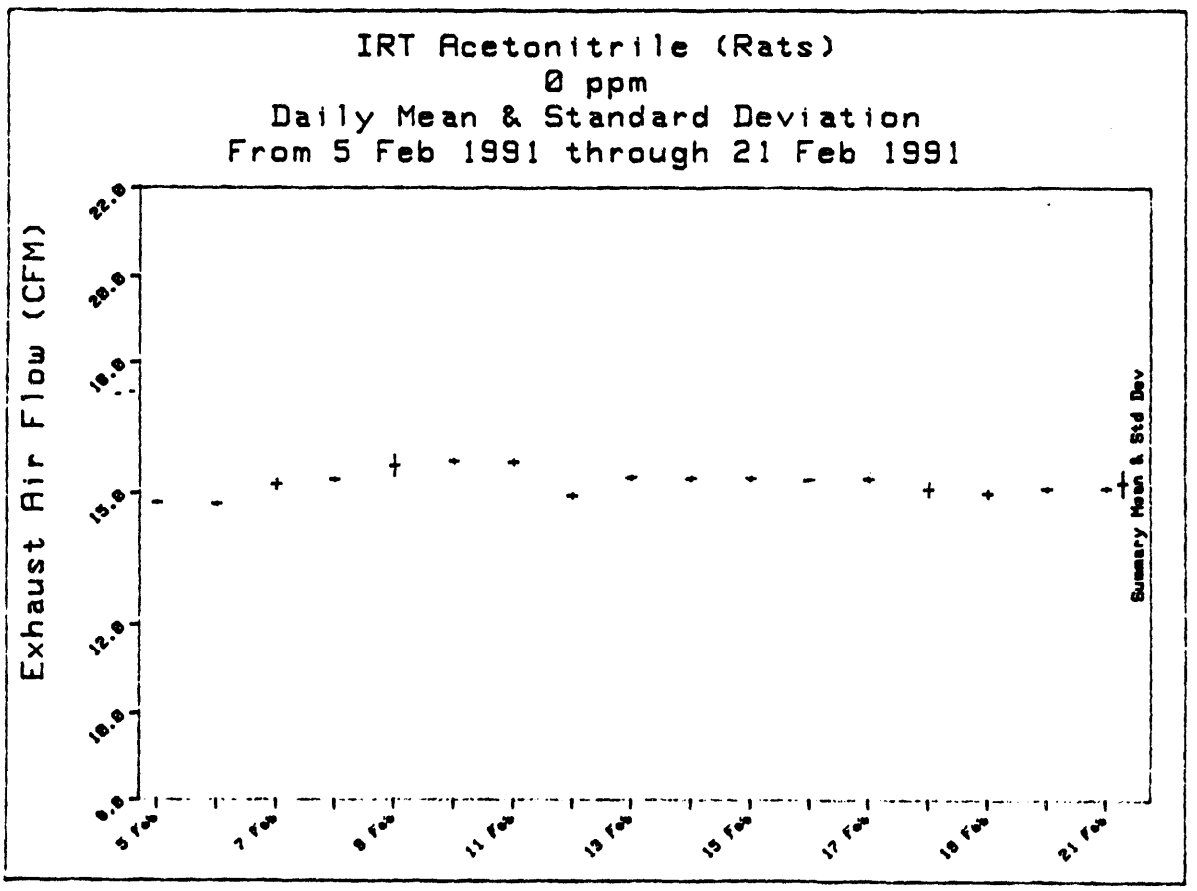


Exhaust Airflow Data

Daily Summation for IRT Acetonitrile (Rats) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 0 ppm/Exhaust Air Flow Range= 12.0 to 18.0

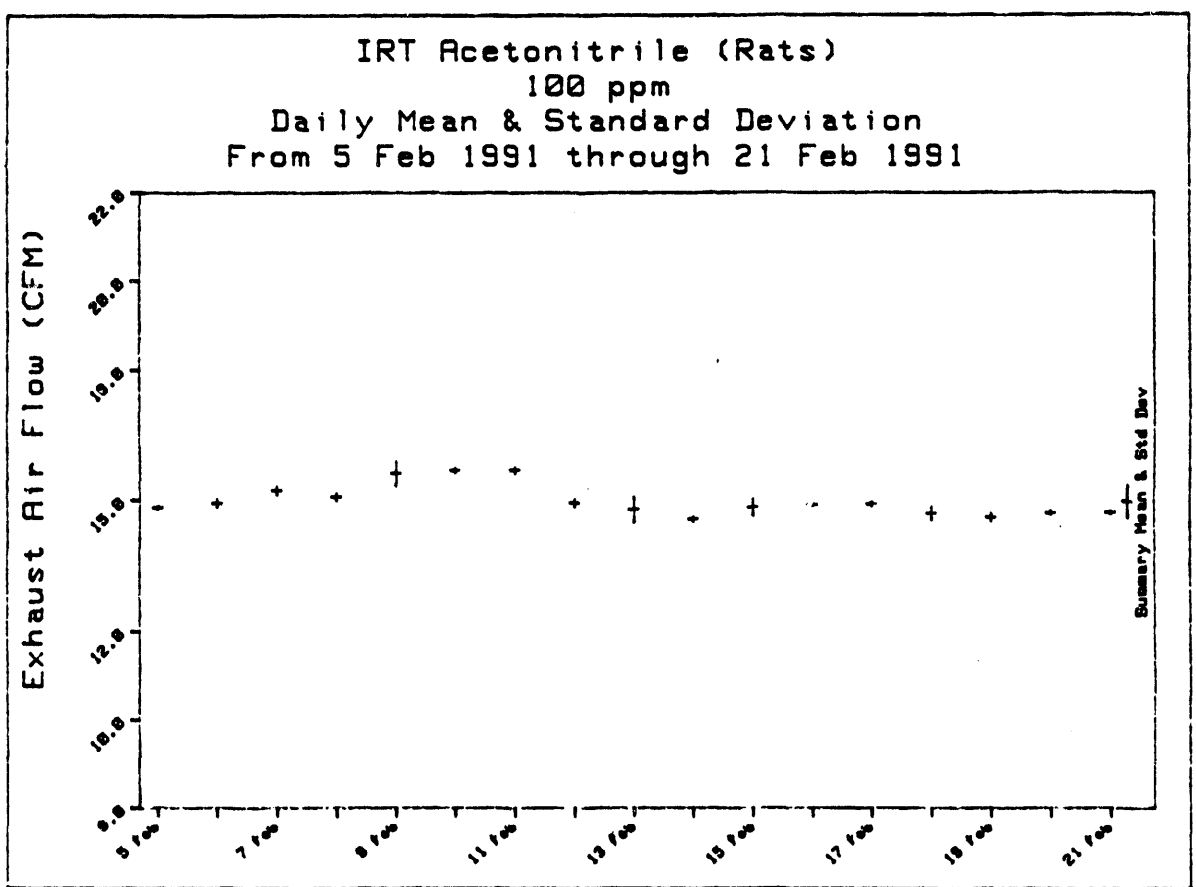
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	14.8	98.5%	.02	.1%	14.8	14.7	6	6	100.0%
6 Feb 1991	14.7	98.3%	.03	.2%	14.8	14.7	8	8	100.0%
7 Feb 1991	15.2	101.3%	.11	.7%	15.3	15.0	7	7	100.0%
8 Feb 1991	15.3	102.0%	.03	.2%	15.3	15.3	8	8	100.0%
9 Feb 1991	15.6	104.2%	.23	1.5%	15.8	15.3	7	7	100.0%
10 Feb 1991	15.7	104.9%	.04	.3%	15.8	15.7	8	8	100.0%
11 Feb 1991	15.7	104.8%	.06	.4%	15.8	15.6	7	7	100.0%
12 Feb 1991	14.9	99.6%	.05	.3%	15.0	14.8	6	6	100.0%
13 Feb 1991	15.4	102.4%	.03	.2%	15.4	15.3	7	7	100.0%
14 Feb 1991	15.3	102.2%	.04	.3%	15.4	15.3	8	8	100.0%
15 Feb 1991	15.3	102.3%	.04	.3%	15.4	15.3	8	8	100.0%
16 Feb 1991	15.3	102.0%	.01	.1%	15.3	15.3	6	6	100.0%
17 Feb 1991	15.3	102.2%	.05	.3%	15.4	15.2	8	8	100.0%
18 Feb 1991	15.1	100.6%	.15	1.0%	15.3	15.0	8	8	100.0%
19 Feb 1991	15.0	100.0%	.09	.6%	15.1	14.9	8	8	100.0%
20 Feb 1991	15.1	100.7%	.04	.2%	15.2	15.1	7	7	100.0%
21 Feb 1991	15.1	100.8%	.03	.2%	15.2	15.1	8	8	100.0%
Summary	15.2	101.6%	.29	1.9%	15.8	14.7	125	125	100.0%



Daily Summation for IRT Acetonitrile (Rats) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 100 ppm/Exhaust Air Flow Range= 12.0 to 18.0

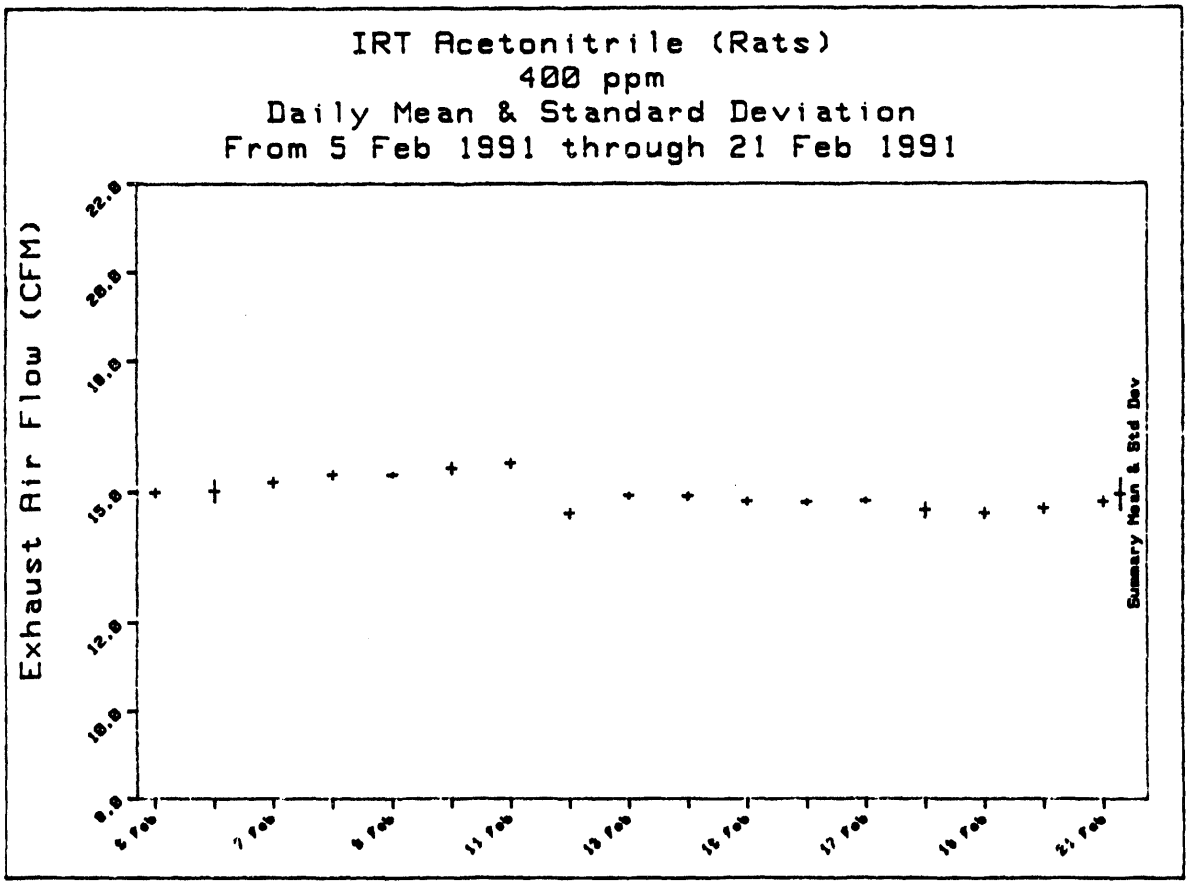
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	14.8	98.8%	.03	.2%	14.9	14.8	6	6	100.0%
6 Feb 1991	14.9	99.5%	.08	.6%	15.1	14.8	8	8	100.0%
7 Feb 1991	15.2	101.4%	.09	.6%	15.3	15.0	7	7	100.0%
8 Feb 1991	15.1	100.4%	.08	.6%	15.2	15.0	8	8	100.0%
9 Feb 1991	15.6	104.0%	.27	1.8%	15.9	15.0	7	7	100.0%
10 Feb 1991	15.7	104.5%	.04	.3%	15.8	15.6	8	8	100.0%
11 Feb 1991	15.7	104.5%	.06	.4%	15.8	15.6	7	7	100.0%
12 Feb 1991	14.9	99.5%	.08	.5%	15.0	14.8	6	6	100.0%
13 Feb 1991	14.8	98.5%	.28	1.9%	15.3	14.6	7	7	100.0%
14 Feb 1991	14.6	97.1%	.05	.3%	14.7	14.5	8	8	100.0%
15 Feb 1991	14.8	98.9%	.19	1.3%	15.0	14.4	8	8	100.0%
16 Feb 1991	14.9	99.2%	.02	.2%	14.9	14.9	6	6	100.0%
17 Feb 1991	14.9	99.4%	.05	.3%	15.0	14.8	8	8	100.0%
18 Feb 1991	14.7	97.9%	.15	1.0%	15.0	14.6	8	8	100.0%
19 Feb 1991	14.6	97.4%	.09	.6%	14.7	14.5	8	8	100.0%
20 Feb 1991	14.7	98.0%	.04	.3%	14.8	14.7	7	7	100.0%
21 Feb 1991	14.7	98.1%	.03	.2%	14.8	14.7	8	8	100.0%
Summary	15.0	99.8%	.37	2.5%	15.9	14.4	125	125	100.0%



Daily Summation for IRT Acetonitrile (Rats) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 400 ppm/Exhaust Air Flow Range= 12.0 to 18.0

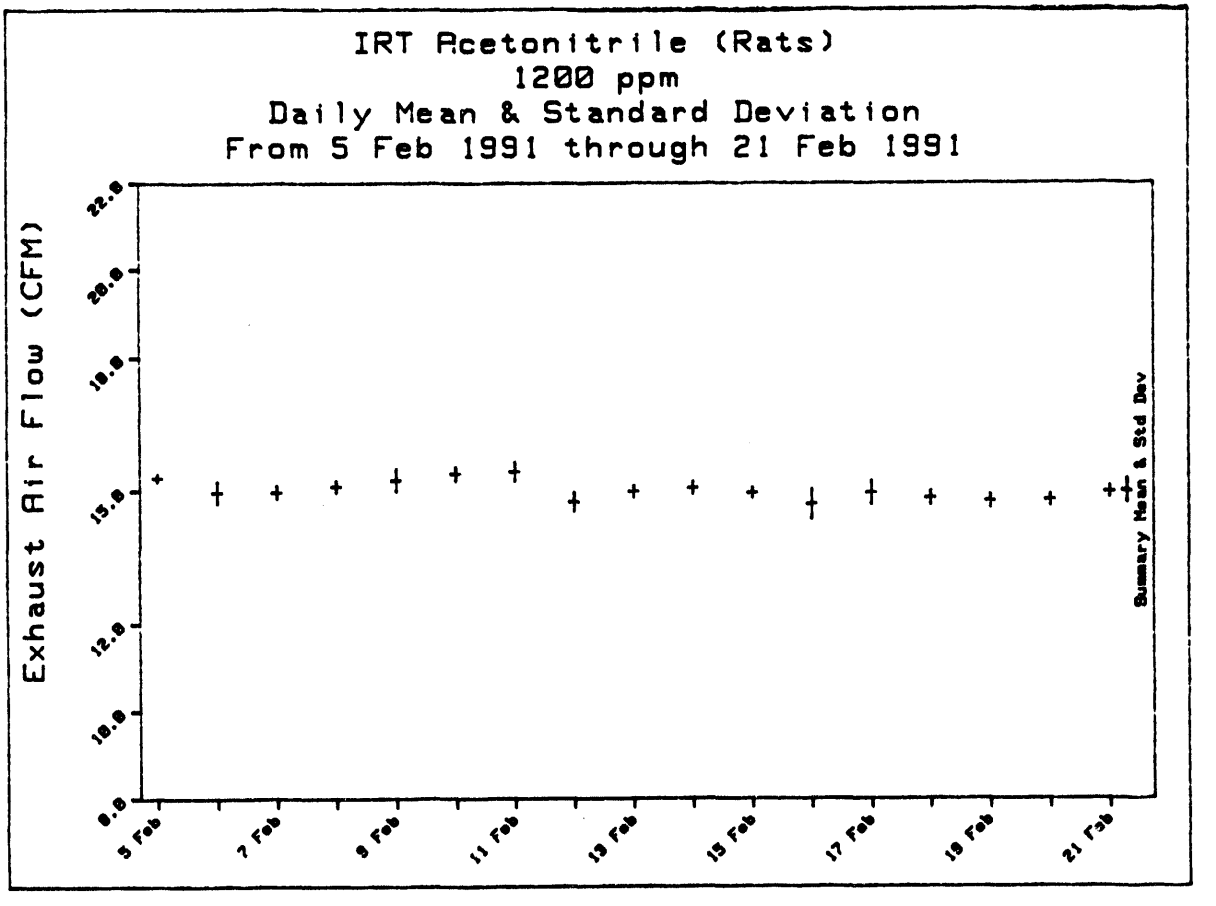
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	15.0	99.9%	.08	.6%	15.1	14.9	6	6	100.0%
6 Feb 1991	15.0	100.1%	.24	1.6%	15.6	14.9	8	8	100.0%
7 Feb 1991	15.2	101.5%	.10	.6%	15.4	15.1	7	7	100.0%
8 Feb 1991	15.4	102.7%	.08	.5%	15.5	15.3	8	8	100.0%
9 Feb 1991	15.4	102.6%	.04	.3%	15.5	15.4	7	7	100.0%
10 Feb 1991	15.5	103.7%	.12	.8%	15.7	15.4	8	8	100.0%
11 Feb 1991	15.7	104.4%	.09	.6%	15.8	15.5	7	7	100.0%
12 Feb 1991	14.5	96.6%	.10	.7%	14.6	14.4	5	5	100.0%
13 Feb 1991	14.9	99.4%	.06	.4%	15.0	14.8	7	7	100.0%
14 Feb 1991	14.9	99.3%	.08	.5%	15.0	14.8	8	8	100.0%
15 Feb 1991	14.8	98.5%	.07	.5%	14.9	14.7	8	8	100.0%
16 Feb 1991	14.8	98.4%	.05	.4%	14.8	14.7	6	6	100.0%
17 Feb 1991	14.8	98.6%	.05	.3%	14.9	14.7	8	8	100.0%
18 Feb 1991	14.6	97.1%	.17	1.1%	14.9	14.5	8	8	100.0%
19 Feb 1991	14.5	96.7%	.11	.8%	14.7	14.3	8	8	100.0%
20 Feb 1991	14.6	97.5%	.10	.7%	14.8	14.5	8	8	100.0%
21 Feb 1991	14.8	98.5%	.11	.7%	14.9	14.6	8	8	100.0%
Summary	15.0	99.8%	.37	2.5%	15.8	14.3	125	125	100.0%



Daily Summation for IRT Acetonitrile (Rats) From 5 Feb 1991 through 21 Feb 1991

Summary Data for: 1200 ppm/Exhaust Air Flow Range= 12.0 to 18.0

Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
5 Feb 1991	15.3	101.9%	.09	.6%	15.5	15.2	6	6	100.0%
6 Feb 1991	14.9	99.6%	.25	1.7%	15.4	14.8	8	8	100.0%
7 Feb 1991	14.9	99.7%	.15	1.0%	15.2	14.7	7	7	100.0%
8 Feb 1991	15.1	100.6%	.13	.9%	15.3	15.0	8	8	100.0%
9 Feb 1991	15.2	101.5%	.25	1.7%	15.8	15.0	7	7	100.0%
10 Feb 1991	15.4	102.5%	.16	1.0%	15.7	15.1	8	8	100.0%
11 Feb 1991	15.4	102.8%	.22	1.4%	15.8	15.3	7	7	100.0%
12 Feb 1991	14.7	98.2%	.21	1.5%	15.0	14.5	6	6	100.0%
13 Feb 1991	15.0	99.8%	.13	.9%	15.2	14.9	7	7	100.0%
14 Feb 1991	15.1	100.3%	.14	.9%	15.3	14.9	8	8	100.0%
15 Feb 1991	14.9	99.6%	.12	.8%	15.2	14.8	8	8	100.0%
16 Feb 1991	14.7	97.9%	.34	2.3%	15.1	14.5	6	6	100.0%
17 Feb 1991	14.9	99.6%	.26	1.8%	15.3	14.5	8	8	100.0%
18 Feb 1991	14.8	98.8%	.16	1.0%	15.1	14.7	8	8	100.0%
19 Feb 1991	14.7	98.3%	.15	1.0%	15.0	14.6	8	8	100.0%
20 Feb 1991	14.8	98.5%	.13	.9%	15.0	14.7	8	8	100.0%
21 Feb 1991	15.0	99.7%	.13	.9%	15.2	14.7	8	8	100.0%
Summary	15.0	100.0%	.27	1.8%	15.8	14.5	126	126	100.0%



Exposure Operation Discussion Sheets

EXPOSURE OPERATION DISCUSSION SHEET

INCLUDES DISCUSSIONS AND/OR EXPLANATIONS OF PROBLEMS AFFECTING ANIMAL ENVIRONMENT AND EXPOSURES. EXPLANATIONS ARE INCLUDED FOR DATA IN WHICH THERE WERE EXCURSIONS OF DAILY MEAN OR STANDARD DEVIATION BEYOND ALLOWABLE OPERATING LIMITS OR EXCURSIONS OF INDIVIDUAL DATUM BEYOND CRITICAL LIMITS.

STUDY: Acetonitrile Inhalation Reproductive Toxicity Study

REPORTING PERIOD: February 4 - February 21, 1991
NOTE: 24 Hour Collection Period extends from ~5:00 a.m. to ~5:00 a.m.

COMPILED BY: R.J. Weigel **DATE:** 3/4/91

CHAMBER CONCENTRATION

DATE	DISCUSSION OR EXPLANATION
	No problems or excursions to report this month.

TEMPERATURE & RELATIVE HUMIDITY

DATE	DISCUSSION OR EXPLANATION
	No problems or excursions to report this month.

CHAMBER FLOW & VACUUM

DATE	DISCUSSION OR EXPLANATION
	No problems or excursions to report this month.

Chamber Uniformity Data

CHAMBER UNIFORMITY DATA SHEET

COMPOUND: Acetonitrile IRT

EXPOSURE ROOM NUMBER: 332

TPV MEASUREMENTS

CHAMBER:	1200 ppm R		400 ppm R		100 ppm R					
DATE:	10/30/90		10/30/90		10/29/90					
SAMPLE PORT	MONITOR READING	% of Mean	MONITOR READING	% of Mean	MONITOR READING	% of Mean	MONITOR READING	% of Mean	MONITOR READING	% of Mean
BACK: 1B	462800	98.4%	157100	99.2%	43230	99.5%				
2B	464200	98.7%	156500	98.9%	43390	99.9%				
3B	467300	99.4%	156800	99.0%	43610	100.4%				
4B	469600	99.9%	157400	99.4%	43470	100.1%				
5B	469000	99.7%	158300	100.0%	43390	99.9%				
6B	470100	100.0%	158300	100.0%	43310	99.7%				
FRONT: 1F	470100	100.0%	159000	100.4%	43460	100.1%				
2F	470900	100.1%	158400	100.1%	43500	100.2%				
3F	473400	100.7%	159600	100.8%	43430	100.0%				
4F	473400	100.7%	159000	100.4%	43360	99.8%				
5F	474200	100.8%	159700	100.9%	43510	100.2%				
6F	477600	101.6%	159700	100.9%	43550	100.3%				
MEAN:	470216.7	100.0%	158316.7	100.0%	43434.2	100.0%				
TPV:	4185.65	0.9%	1141.64	0.7%	105.70	0.2%				
BPV:	////////////	*	////////////	*	////////////	*	////////////		////////////	

* *Indistinguishable from WPV*

WPV MEASUREMENTS

IN-LINE 1st	479700	102.0%	160400	101.2%	43730	100.7%		
2nd	471800	100.3%	158600	100.0%	43310	99.7%		
3rd	459400	97.7%	156600	98.8%	43270	99.6%		
MEAN:	470300.0	100.0%	158533.3	100.0%	43436.7	100.0%		
WPV:	10232.79	2.2%	1900.88	1.2%	254.82	0.6%		

INLET AND EXHAUST MEASUREMENTS

Inlet	464900		155200		44000			
Exhaust	457600		155700		43040			
% Difference:	1.6%		-0.3%		2.2%			

[Data located in appropriate daily data file]

MONITOR TYPE: S840A **BNW #:** N809568

COMMENTS: Prestart measurements without animals in chambers.

ENTERED BY: ML Fallon **DATE:** 10/31/90 **REVIEWED BY:** RJ Weigel **DATE:** 11/16/90

CHAMBER UNIFORMITY DATA SHEET

COMPOUND: Acetonitrile IRT **EXPOSURE ROOM NUMBER:** 332

TPV MEASUREMENTS

CHAMBER: / DATE: / SAMPLE PORT	1200 ppm R 02/11/91 MONITOR READING	% of Mean	400 ppm R 02/12/91 MONITOR READING	% of Mean	100 ppm R 02/13/91 MONITOR READING	% of Mean	MONITOR READING	% of Mean	MONITOR READING	% of Mean
BACK: 1B										
2B										
3B	481900	99.9%	164600	99.6%	41340	99.6%				
4B	479700	99.4%	165500	100.2%	41420	99.7%				
5B	484400	100.4%	165100	99.9%	41550	100.1%				
6B										
FRONT: 1F										
2F	487100	100.9%	165300	100.1%	41620	100.2%				
3F	479200	99.3%	165000	99.9%	41560	100.1%				
4F	481600	99.8%	165400	100.1%	41570	100.1%				
5F	484400	100.4%	165500	100.2%	41610	100.2%				
6F										
MEAN:	482614.3	100.0%	165200.0	100.0%	41524.3	100.0%				
TPV:	2833.98	0.6%	326.60	0.2%	104.38	0.3%				
BPV:	//////////	0.3%	//////////	*	//////////	0.2%	//////////		//////////	

* *Indistinguishable from WPV*

WPV MEASUREMENTS

IN-LINE 1st	482600	99.4%	165700	100.4%	41740	100.1%		
2nd	487100	100.3%	165300	100.1%	41620	99.8%		
3rd	486700	100.3%	164200	99.5%	41760	100.1%		
MEAN:	485466.7	100.0%	165066.7	100.0%	41706.7	100.0%		
WPV:	2490.65	0.5%	776.75	0.5%	75.72	0.2%		

INLET AND EXHAUST MEASUREMENTS

Inlet	505900	167200	45180		
Exhaust	479100	163000	41260		
% Difference:	5.3%	2.5%	8.7%		

[Data located in appropriate daily data file]

MONITOR TYPE: HP 5840 A GC BNW #: N809568

COMMENTS: Measurements done with animals in chambers.

ENTERED BY: ML Fallon DATE: 02/19/91 REVIEWED BY: RJ Weigel DATE: 2/19/91

APPENDIX C

ANIMAL DATA

Animal Health Screen Reports

Animal Health Screen Reports

ARS RODENT HEALTH SCREEN REPORT

Investigator: Mast
Study: Acetonitrile IRT
Building: LSL II
Room: 429
Date initiated: 1/28/91

Lab no: U-12
Animal/Shipment no: 910006
Date rc'd: 1/8/91
Source: CR R10
Species/Strain: Rat/CD
Sex: M/F Age: BD 11/15/90

Status: Ten rats (5M.5F) for pre-exposure health screen to include gross necropsy, nasopharyngeal wash for culture, ectoparasite exam for pinworm, serology and histopathology.

Gross Necropsy
No significant lesions

Endoparasite/Ectoparasite exam
0/10 * Anal tape exam for pinworm ova

*Number positive/number examined

Nasopharyngeal culture

0/10 *	Beta hemolytic streptococci
0/10	*Bordetella bronchiseptica*
0/10	*Citrobacter freundii*
0/10	Coagulase positive staphylococci
0/10	*Klebsiella oxytoca*
0/10	*Klebsiella pneumoniae*
0/10	*Pasturella* sp.
0/10	*Pseudomonas aeruginosa*
0/10	*Streptococcus pneumoniae*
0/10	*Corynebacterium kutscheri*

*Number of positive cultures/number cultured

Serology: Rat

0/10 *	*Mycoplasma pulmonis*
0/10	Sendai virus
0/10	Pneumonia virus of mice (Testing done at M.A.)
0/10	RCV/SDAV (Testing done at M.A.)
0/10	KRV/H1

*Number of positive tests/number tested

Histopathology

1/10 *	Hard.gl.	Occasional tiny focus of chronic inflammation not typical of SDA virus infection (#2)
1/10	Trachea	Focal inflammation in lamina propria (#4)
1/10	Liver	Rare tiny focus of inflammation in hepatic parenchyma
1/10	Hard.gl.	Rare tiny focus of inflammation, not typical of SDA virus infection
1/10	Liver	Occasional tiny to small focus of inflammation in hepatic parenchyma

Correlation/Summary

None of the histopathologic findings are considered significant. There are no indications of significant infection or other disease.

Released for Study on 2/4/91.

Released from Quarantine on 2/14/91.

Irene E. Jarrell 2/14/91
Technologist

2/15/91
Veterinarian

Mast
Evanoff
Gorham

MICROBIOLOGICAL ASSOCIATES, INC.

Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Fax (301) 738-1036

K.J

Our Code NTP 1613
Page 1

TO: Dr. Steve Rowe
Battelle Northwest Laboratory
P.O. Box 999
LSL-II
Richland, WA 99352

FROM: Robert L. Peters, Ph.D. rlp
DATE: February 14, 1991
SPECIMEN: 10 rat sera
RECEIVED: February 12, 1991

Acetonitrile IRT
Pre-exposure
Health screen

RESULTS: None of the tests were positive.

Sample ID	--- ELISA --- PVM	RCV/SDA
U12 - 1	0.00	0.02
U12 - 2	0.16	0.02
U12 - 3	0.01	0.02
U12 - 4	0.00	0.02
U12 - 5	0.00	0.02
U12 - 6	0.00	0.02
U12 - 7	0.00	0.01
U12 - 8	0.00	0.01
U12 - 9	0.01	0.00
U12 - 10	0.02	0.03

ELISA: positive value is >= 0.17 OD units

Recd 2/25/91
SEA

Orig: Prog. Office
cc: Mast
Evanoff
Gorham

ARS RODENT HEALTH SCREEN REPORT

Investigator: Mast
Study: Acetonitrile-IRT
Building: LSL2
Room: 332
Date initiated: 2/14/91

Lab no: U-23
Animal/Shipment no: 910006
Date rc'd: 1/8/91
Source: Charles River
Species/Strain: Rat/CD
Sex: F Age: BD 11/15/90

Status: One rat, #231-400PPM, was found dead on study and submitted for necropsy.

Gross Necropsy

Focus of acute hemorrhage in cerebrum. Hemorrhage seems to be confined largely to the ventricles. No other gross abmormalities.

Correlation/Summary

There were no indications of trauma other than the hemorrhage in the brain. Therefore, the hemorrhage may have occurred spontaneously.

3/22/91

Veterinarian

Mast
Evanoff
Boyd
Gorham

ARS RODENT HEALTH SCREEN REPORT

Investigator: Mast
Study: Acetonitrile-IRT
Building: LSL2
Room: 332
Date initiated: 2/15/91

Lab no: U-24
Animal/Shipment no: 910006
Date rc'd: 1/8/91
Source: Charles River
Species/Strain: Rat/CD
Sex: F Age: BD 11/15/90

Status: Two female high-dose rats, #84 and #88, were submitted moribund for necropsy.

Gross Necropsy

2/2*	Thin, rough hair coat
2/2	Only small to moderate quantities of ingesta in gastrointestinal tract. No gross evidence of pregnancy; reproductive tract normal.

*Number affected/number examined

Histopathology
None

Correlation/Summary
Food and water consumption were apparently poor. The cause was not determined. There were no indications of infectious disease.

Veterinarian 3/22/91

Mast
Evanoff
Boyd
Gorham

ARS RODENT HEALTH SCREEN REPORT

Investigator: Mast
Study: Acetonitrile-IRT
Building: LSL2
Room: 332
Date initiated: 2/19/91

Lab no: U-32
Animal/Shipment no: 910006
Date rc'd: 1/8/91
Source: Charles River
Species/Strain: Rat/CD
Sex: F Age:BD 11/15/90

Status: Ten terminal sacrifice rat sera (5 controls, 5-1200PPM) were submitted for viral antibody testing.

Serology (BNW): Rat

0/10 *	Mycoplasma pulmonis
0/10	Sendai virus
0/10	Pneumonia virus of mice
0/10	RCV/SDAV
0/10	KRV/H1

*Number of positive tests/number tested

Correlation/Summary

Tests for antibodies were negative indicating the rats had not been exposed to any of the pathogens for which they were tested.

K.G. Pauly
Technologist

Veterinarian 3/21/91

(Mast)
Evanoff
Boyd
Gorham

APPENDIX D

DEVELOPMENTAL TOXICITY DATA

Calendar of Events
Maternal Body and Organ Weights
Nonpregnant Female Body and Organ Weights
Reproductive Measures and Fetal Data

Calendar of Events

Inhalation Developmental Toxicity Study of Acetonitrile in Rats:
CALENDAR OF EVENTS

Exposure levels; treatments 1-4	0, 100, 400, 1200 ppm
Ordered rats	11/21/90
Received rats (ARS#II-91-02-910006)	1/8/91
Health screen	1/28/91
Tail tattoo females	1/22/91
Released from quarantine	2/14/91
Weighed female rats	1/23/91
0dg (# positive) weighed, individually caged	(A) 1/30/91 (72) (B) 1/31/91 (80) (C) 2/1/91 (50) (D) 2/2/91 (34)
randomized all groups	2/2/91
Moved to exposure room (6dg)	(A) 2/5/91 (B) 2/6/91 (C) 2/7/91 (D) 2/8/91 (non-pregnant group) 2/8/91
Exposure 6 hours/day; 7 days/week; 4-17dg	
Weighed (6dg) started exposure	(A-D) 2/5/91 to 2/8/91
Weighed (10dg)	(A-D) 2/9/91 to 2/12/91
Weighed (14dg)	(A-D) 2/13/91 to 2/16/91
Weighed (17dg)	(A-D) 2/16/91 to 2/19/91
Weighed (20dg)	(A-D) 2/19/91 to 2/22/91
Teratology sacrifice (20dg)	(A) 2/19/91 (B) 2/20/91 (C) 2/21/91 (D) 2/22/91
Blood distribution sacrifice (8dg)	2/7/91
Blood distribution sacrifice (18dg)	2/19/91
Non-pregnant group	
Selected, randomized, and individually caged	2/2/91
Weighed (exposure day 1) started exposure	2/8/91
Weighed (exposure day 5)	2/12/91
Weighed (exposure day 10)	2/17/91
Weighed and sacrificed	2/22/91
Fetal specimen exams completed	4/11/91

Maternal Body and Organ Weights

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Body and Organ Weights (g) for Sperm-positive Females

TMT=0 ppm Acetonitrile

MATNO	Pre-study Wt (g)	0 dg Wt (g)	6 dg Wt (g)	10 dg Wt (g)	14 dg Wt (g)	17 dg Wt (g)	20 dg Wt (g)	Uter Wt (g)	Liver Wt (g)	Kidney Wt (g)	Adrenal Wt	Pregnant
3	240.0	265.3	308.1	327.0	339.1	366.3	412.5	75.09	16.4378	2.0385	0.0706	P
11	276.4	271.7	320.7	328.8	342.4	367.0	395.0	59.05	14.4457	2.1007	0.0617	P
16	260.3	284.0	315.8	333.5	353.1	369.8	409.1	71.32	14.7444	2.1243	0.0778	P
32	238.2	259.1	292.8	302.4	324.8	345.4	384.8	92.80	14.1353	2.0197	0.0783	P
38	231.0	260.0	291.5	306.7	333.5	365.1	411.9	80.10	18.3357	1.9727	0.0608	P
48	252.3	270.1	302.2	316.6	337.3	369.6	417.4	90.28	16.0187	1.8592	0.0836	P
66	242.9	258.4	301.3	316.3	338.6	362.5	400.3	68.06	15.7575	1.8687	0.0753	P
89	262.3	274.6	323.9	341.0	357.6	383.9	439.5	87.04	18.5454	2.2675	0.0810	P
95	267.3	297.1	343.6	356.3	377.9	410.4	453.7	76.62	17.6701	2.2346	0.0868	P
99	250.5	273.1	316.7	325.7	331.8	315.6	316.0	1.06	11.5296	2.5092	0.0493	NP
108	251.2	262.6	296.9	312.3	336.6	358.6	401.7	80.67	15.6574	1.8132	0.0717	P
113	252.3	266.4	301.4	316.0	330.2	372.4	412.2	93.89	14.8302	1.8971	0.0762	P
115	262.7	291.3	321.4	330.5	319.9	318.5	327.4	1.13	11.3920	2.1401	0.0771	NP
125	241.8	259.2	298.2	307.8	301.2	308.3	311.5	0.81	11.8234	2.0362	0.0637	NP
139	244.0	267.1	314.7	328.9	355.0	383.3	440.5	101.36	17.0680	2.1396	0.0815	P
158	246.7	273.6	303.4	309.2	337.9	369.7	420.3	93.59	15.4595	2.0809	0.0653	P
181	254.4	275.0	309.4	309.0	304.8	302.7	300.0	0.76	11.9054	2.2927	0.0628	NP
184	275.6	287.1	322.9	325.8	351.8	376.5	401.9	45.71	16.2368	2.4036	0.0813	P
185	237.4	241.2	282.9	299.6	327.4	352.9	409.2	96.09	16.5710	1.8713	0.0796	P
203	240.3	250.3	283.7	296.7	326.3	361.3	406.9	87.53	15.8465	2.0938	0.0710	P
219	266.1	279.6	309.4	315.9	338.9	372.2	420.7	86.75	15.3107	1.8043	0.0692	P
236	260.5	282.2	313.0	317.0	334.3	361.1	414.7	76.89	17.1357	2.1122	0.0778	P
243	254.9	298.8	340.9	355.4	381.3	424.0	465.8	89.20	19.0529	2.0011	0.0677	P
278	246.3	262.2	311.2	314.9	338.8	359.9	383.3	62.60	14.7356	2.1726	0.0743	P
289	245.0	254.8	285.5	283.6	275.6	258.9	245.5	0.62	18.5156	1.7749	0.0738	NP
292	231.8	255.5	294.9	309.5	340.4	374.0	421.1	92.43	15.9628	2.1593	0.0836	P
293	227.1	243.9	279.2	292.9	314.0	341.4	381.0	69.02	14.3782	2.0168	0.0626	P
312	250.7	271.4	301.7	312.9	341.7	379.4	433.5	84.75	19.3793	2.1170	0.0681	P
314	224.1	235.2	267.1	281.2	301.1	333.4	381.6	90.60	15.5357	1.8208	0.0767	P
319	240.3	261.2	302.3	312.9	311.0	301.2	302.5	0.81	11.9121	2.3348	0.0658	NP
323	241.0	258.9	298.9	306.0	327.9	344.9	372.9	24.21	16.5566	2.2078	0.0759	P
336	276.9	295.5	325.7	331.4	360.5	395.3	447.1	103.51	16.0662	2.2035	0.0793	P
373	233.5	249.8	280.7	283.9	277.1	274.8	279.2	1.00	10.1032	1.8986	0.0722	NP

Code for Pregnant Column: P=Pregnant NP=Non-Pregnant

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Body and Organ Weights (g) for Sperm-positive Females

TMT=100 ppm Acetonitrile

MATNO	Pre-study Wt (g)	0 dg Wt (g)	6 dg Wt (g)	10 dg Wt (g)	14 dg Wt (g)	17 dg Wt (g)	20 dg Wt (g)	Uter Wt (g)	Liver Wt (g)	Kidney Wt (g)	Adrenal Wt	Pregnant
1	222.8	249.8	286.5	305.5	326.5	350.5	370.3	32.01	15.6794	2.0349	0.0625	P
42	263.1	296.4	338.3	350.9	379.1	421.3	481.7	98.29	20.5389	2.3031	0.0845	P
85	243.4	253.9	287.1	296.1	313.0	339.8	381.6	69.30	14.4130	1.6776	0.0420	P
86	254.4	284.1	331.9	337.6	321.7	311.8	312.0	1.04	12.8985	2.3518	0.0581	NP
92	250.2	277.8	310.5	326.9	349.8	382.9	437.8	89.30	20.7097	2.3688	0.0813	P
122	244.0	263.5	296.0	297.3	323.2	344.2	387.5	75.71	15.3015	2.0022	0.0628	P
126	229.7	241.3	274.5	284.3	311.2	339.2	379.9	72.31	13.9242	1.8178	0.0566	P
162	252.5	267.7	318.7	323.4	351.9	379.5	420.3	84.89	17.8962	2.0353	0.0747	P
171	252.7	276.7	319.4	333.8	341.0	331.3	327.7	0.76	12.5738	2.1120	0.0838	NP
176	276.2	289.2	292.5	300.4	330.0	353.6	397.5	54.41	19.9102	2.5174	0.0698	P
190	261.3	273.1	313.3	333.5	355.0	384.9	422.4	69.22	18.1620	2.3971	0.0865	P
208	245.6	267.4	300.9	319.2	351.2	380.3	427.8	83.52	16.6906	1.9230	0.0550	P
221	246.9	271.0	298.7	314.8	338.1	363.4	409.5	86.89	15.0951	1.8873	0.0858	P
224	245.7	257.6	285.1	299.3	332.4	364.6	411.8	76.16	17.6544	2.2498	0.0626	P
239	243.7	265.7	299.9	314.8	321.4	350.4	368.9	40.77	16.3314	2.2924	0.0831	P
258	253.4	275.0	311.4	324.5	353.8	377.8	431.8	82.30	17.7302	2.0789	0.0642	P
267	260.0	277.4	313.3	333.6	352.7	375.2	422.0	85.78	15.3910	1.9120	0.0727	P
271	249.1	272.8	304.8	320.4	335.1	370.6	421.9	83.94	17.3997	1.9138	0.0709	P
273	231.5	236.9	283.9	300.5	319.7	353.1	388.8	79.85	14.7330	1.9418	0.0716	P
274	248.8	262.5	310.0	324.7	346.0	371.9	420.0	92.31	15.6407	2.0503	0.0725	P
275	241.1	260.3	279.6	294.3	309.2	331.3	369.1	56.34	15.6282	2.1433	0.0710	P
276	243.6	264.1	301.3	330.0	359.0	388.0	422.7	97.42	15.7932	2.2471	0.0878	P
281	264.7	285.6	311.4	336.9	360.9	350.8	411.3	78.84	16.2243	2.1256	0.0733	P
288	264.0	290.2	336.9	338.3	358.2	377.5	419.5	45.08	18.9769	2.4135	0.1069	P
290	237.9	258.5	288.5	303.8	320.1	356.0	397.1	84.71	16.7347	2.1541	0.0951	P
291	235.8	260.6	287.9	305.9	324.1	350.4	385.9	74.40	15.5630	1.9454	0.0821	P
296	226.7	235.5	273.4	291.0	307.3	326.1	356.6	48.53	15.1758	1.9039	0.0755	P
321	226.0	241.5	272.1	279.9	304.0	334.0	382.1	78.32	14.6787	1.8846	0.0570	P
326	260.0	285.6	327.1	344.0	375.0	406.2	461.2	98.74	17.2066	2.2462	0.0896	P
343	235.7	254.2	296.8	304.9	337.0	365.8	417.6	93.16	15.8628	1.9407	0.0561	P
348	256.8	269.1	300.6	311.1	332.4	365.8	419.0	95.33	14.7700	2.0242	0.0636	P
353	252.9	276.2	307.9	321.5	350.6	384.1	442.0	101.73	16.1699	1.9731	0.0727	P
368	236.5	261.3	314.5	340.6	333.6	331.3	341.3	0.71	14.5088	2.2811	0.0662	NP

Code for Pregnant Column: P=Pregnant NP=Non-Pregnant

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Body and Organ Weights (g) for Sperm-positive Females

TMT=400 ppm Acetonitrile

MATNO	Pre-study Wt (g)	0 dg Wt (g)	6 dg Wt (g)	10 dg Wt (g)	14 dg Wt (g)	17 dg Wt (g)	20 dg Wt (g)	Uter Wt (g)	Liver Wt (g)	Kidney Wt (g)	Adrenal Wt	Pregnant
17	241.1	268.5	319.2	335.4	365.4	392.6	420.5	47.01	18.1738	2.3582	0.0751	P
28	258.1	273.0	299.9	307.7	300.1	303.8	310.6	0.73	11.9634	1.9625	0.0634	NP
30	264.4	287.9	322.9	344.4	371.2	406.9	460.3	92.41	19.1444	2.2123	0.1043	P
46	217.2	235.4	261.1	265.4	284.7	302.1	324.1	36.52	13.4965	1.9604	0.0644	P
47	253.0	265.1	302.1	308.0	331.7	356.7	401.6	70.41	18.6044	2.2138	0.1001	P
52	240.7	267.1	305.5	312.2	337.4	366.0	410.8	67.58	17.7583	1.9943	0.0717	P
73	246.3	246.8	288.2	304.3	327.7	352.5	388.6	66.14	15.9805	1.9543	0.0943	P
93	250.0	265.8	313.9	322.0	343.5	377.3	422.4	86.13	16.7963	2.0497	0.0835	P
123	235.4	251.9	280.1	292.4	320.3	344.0	385.8	66.75	15.4491	2.1843	0.0668	P
133	244.9	274.0	316.6	317.9	326.1	329.0	315.9	0.86	13.2141	2.3838	0.0706	NP
135	253.1	278.8	330.6	346.4	372.2	398.8	450.3	91.21	18.2592	2.2722	0.0804	P
137	257.3	273.0	303.4	311.3	333.9	364.8	402.9	88.23	14.3974	0.9943	0.0856	P
140	264.6	292.0	344.8	342.9	362.9	369.8	372.4	0.88	16.9917	2.9984	0.0868	NP
156	228.7	238.8	274.9	282.3	305.8	331.7	376.4	72.98	15.1821	1.9569	0.0791	P
165	229.5	257.0	289.1	305.1	327.4	355.1	408.1	87.01	16.3570	1.9788	0.0715	P
174	246.1	265.4	290.8	312.2	332.1	355.8	393.3	81.44	15.2898	2.0399	0.0734	P
188	249.8	262.7	293.5	305.9	334.9	368.3	430.6	96.37	17.1070	2.0818	0.0912	P
198	238.1	243.0	271.8	283.6	303.6	330.6	377.5	78.15	14.0007	1.9450	0.0601	P
205	242.7	255.0	285.4	312.3	332.0	365.5	416.9	80.01	18.4802	2.2222	0.0661	P
210	243.5	255.5	297.2	287.9	288.6	288.1	295.1	0.69	12.5078	2.2321	0.0602	NP
223	219.6	224.4	262.9	269.2	289.7	308.0	346.3	60.31	14.3050	1.8722	0.0617	P
235	251.8	284.0	327.5	325.3	317.4	321.5	320.5	0.66	13.0487	2.0926	0.0608	NP
270	266.0	295.8	351.9	372.9	399.4	431.6	476.5	77.29	21.0031	2.3399	0.0984	P
286	260.5	267.8	263.1	301.3	324.1	360.3	392.7	52.47	17.1173	2.0204	0.0761	P
315	250.5	276.4	321.5	334.8	362.6	392.2	446.9	80.41	18.7537	1.9371	0.0625	P
320	249.4	277.0	318.6	329.2	357.8	394.6	423.2	98.53	15.0852	1.8727	0.0970	P
342	240.5	270.4	319.3	337.7	374.4	409.6	473.9	48.08	20.3278	2.2926	0.1054	P
349	238.5	263.7	299.8	308.7	324.2	307.7	313.2	0.69	13.3444	2.0512	0.0570	NP
356	253.1	261.6	300.0	312.4	338.2	366.6	404.2	61.75	16.0404	2.0801	0.0651	P

Code for Pregnant Column: P=Pregnant NP=Non-Pregnant

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Body and Organ Weights (g) for Sperm-positive Females

TMT=1200 ppm Acetonitrile

MATNO	Pre-study Wt (g)	0 dg Wt (g)	6 dg Wt (g)	10 dg Wt (g)	14 dg Wt (g)	17 dg Wt (g)	20 dg Wt (g)	Uter Wt (g)	Liver Wt (g)	Kidney Wt (g)	Adrenal Wt	Pregnant
14	257.2	286.6	312.7	325.5	343.1	377.9	441.5	96.28	18.7359	2.0798	0.0883	P
21	249.8	271.2	305.4	317.5	338.1	373.3	352.2	83.06	12.2329	2.3296	0.1029	P
23	243.1	271.1	302.0	295.6	315.8	349.7	392.8	77.83	15.1846	2.1701	0.0697	P
25	268.2	273.7	319.9	334.9	347.3	379.5	428.7	78.76	19.4970	2.1230	0.0728	P
31	223.9	251.5	284.1	292.5	303.3	294.9	284.0	0.79	11.5328	1.9924	0.0863	NP
34	246.8	267.4	303.1	306.7	327.8	349.1	392.5	54.13	17.7091	2.2543	0.0658	P
60	266.4	297.5	330.0	332.0	358.6	380.7	407.5	40.89	17.9237	2.2866	0.0737	P
64	242.6	267.8	318.4	334.1	351.6	381.8	430.0	85.51	18.0051	2.0588	0.0733	P
65	253.3	267.8	307.9	328.9	352.7	381.4	414.3	80.21	16.1283	2.0561	0.0943	P
87	236.5	256.0	303.4	314.2	341.3	369.4	428.8	93.11	18.7140	2.0661	0.0615	P
106	276.9	291.9	326.2	320.4	342.4	380.3	424.7	78.06	19.4136	2.0879	0.0613	P
109	236.8	264.1	306.4	311.4	284.4	245.9	213.6	0.43	10.6127	1.9928	0.1151	NP
110	239.5	262.6	293.3	295.0	284.1	245.1	228.9	0.98	12.1970	2.1838	0.1120	NP
151	230.9	246.5	283.6	285.9	311.8	343.2	381.8	77.11	14.7724	1.7718	0.0753	P
157	255.5	270.1	310.7	309.5	312.5	277.7	277.4	7.33	14.6889	2.5048	0.0941	P
169	258.4	279.0	315.8	336.4	368.6	405.9	457.7	94.37	18.7851	2.1463	0.0870	P
170	260.7	283.7	334.7	333.3	373.4	401.6	462.4	103.66	19.7023	2.6097	0.0702	P
218	277.7	294.3	334.1	343.6	366.3	392.4	434.6	77.74	17.2370	1.9829	0.0807	P
247	249.1	257.7	287.8	299.0	324.5	357.1	405.8	3.89	16.3491	1.9079	0.0627	P
263	236.3	243.3	275.4	288.5	305.7	334.4	372.5	78.29	13.5705	1.8577	0.0681	P
282	267.9	283.4	327.8	340.6	365.9	394.4	448.7	93.62	17.8795	2.1503	0.0681	P
311	234.4	245.5	278.6	294.4	318.0	343.3	386.4	85.08	14.6425	1.8186	0.0788	P
339	240.0	265.9	305.2	315.8	298.6	273.6	240.7	0.49	11.7154	2.0686	0.0964	NP
340	259.0	279.6	333.2	348.1	378.2	406.3	461.4	97.46	18.8514	2.2747	0.0710	P
344	243.9	268.1	315.0	325.4	320.8	322.9	314.2	0.64	12.6254	2.0427	0.0867	NP
350	249.2	261.7	302.5	313.3	345.1	375.0	415.2	87.96	16.4325	2.0856	0.0722	P
355	223.5	246.3	263.7	272.9	296.9	322.2	363.0	73.83	13.9108	1.8454	0.0650	P
358	238.9	263.9	295.6	313.3	341.5	368.4	419.3	77.52	16.9004	2.0934	0.0789	P
361	220.2	227.5	247.5	251.9	271.7	297.6	338.8	80.47	12.8425	1.6739	0.0736	P
362	267.9	300.7	349.1	354.5	378.8	408.5	456.0	96.58	17.9807	2.1849	0.0763	P
363	239.2	261.3	299.7	303.3	325.6	347.7	386.9	72.78	14.8882	1.8693	0.0716	P

Code for Pregnant Column: P=Pregnant NP=Non-Pregnant

Nonpregnant Female Body and Organ Weights

Inhalation Developmental Toxicity Study in Rodents: Body and Organ Weights(g) for Nonpregnant Rats

TMT=0 ppm Acetonitrile

MATNO	Pre-study Wt (g)	Exposure Day 1 Wt (g)	Exposure Day 5 Wt (g)	Exposure Day 10 Wt (g)	Sacrifice Wt (g)	Liver Wt (g)	Kidney Wt (g)	Adrenal Wt (g)
40	252.7	262.4	275.6	281.6	280.5	11.8629	2.2059	0.0735
79	232.5	249.9	270.6	275.8	280.8	9.8434	1.9555	0.0556
96	249.4	285.0	280.5	286.7	288.2	10.8610	2.2084	0.0777
127	229.3	256.3	268.4	269.3	283.4	10.3713	1.8781	0.0541
128	262.6	299.0	315.0	313.6	320.6	12.6562	2.5277	0.0956
175	240.3	272.4	286.9	281.8	288.5	11.1536	2.0651	0.0702
212	235.1	267.6	304.1	303.2	296.7	11.0456	2.1028	0.0635
369	246.3	293.2	339.6	336.3	326.7	13.7628	2.5804	0.0874

Inhalation Developmental Toxicity Study in Rodents: Body and Organ Weights(g) for Nonpregnant Rats

TMT=100 ppm Acetonitrile

MATNO	Pre-study Wt (g)	Exposure Day 1 Wt (g)	Exposure Day 5 Wt (g)	Exposure Day 10 Wt (g)	Sacrifice Wt (g)	Liver Wt (g)	Kidney Wt (g)	Adrenal Wt (g)
13	247.9	270.6	302.2	293.8	300.3	10.2010	2.0013	0.0626
98	250.4	267.4	289.9	296.4	293.9	12.2979	2.2562	0.0605
105	247.0	275.0	302.8	296.4	302.0	12.2178	2.0700	0.0559
138	259.8	289.6	326.7	333.3	335.4	13.2024	2.5007	0.0693
149	238.2	259.7	271.4	278.1	281.4	10.4132	1.8865	0.0873
200	243.3	255.9	290.9	301.0	306.5	11.3218	2.2278	0.0717
250	240.9	248.8	255.0	254.1	255.4	9.2846	1.8622	0.0694
324	258.7	281.3	287.5	291.7	302.3	10.8748	2.2732	0.0682
334	284.1	309.6	355.9	367.7	362.1	15.7153	2.5785	0.0807
352	245.7	278.2	330.0	352.1	335.0	12.3276	2.6497	0.0721

Inhalation Developmental Toxicity Study in Rodents: Body and Organ Weights(g) for Nonpregnant Rats

TMT=400 ppm Acetonitrile

MATNO	Pre-study Wt (g)	Exposure Day 1 Wt (g)	Exposure Day 5 Wt (g)	Exposure Day 10 Wt (g)	Sacrifice Wt (g)	Liver Wt (g)	Kidney Wt (g)	Adrenal Wt (g)
12	251.3	265.8	290.4	289.0	297.5	11.1850	2.2257	0.0860
27	226.3	255.6	301.8	319.7	312.3	10.4480	1.9497	0.0634
102	285.5	313.9	353.0	352.0	371.5	14.6108	2.4060	0.0914
112	249.0	276.6	287.5	297.5	320.0	12.8943	2.1097	0.0777
146	226.0	248.7	286.1	295.7	286.2	10.6676	1.8706	0.0733
153	260.4	262.7	283.4	286.8	297.7	12.6961	2.2026	0.0618
161	262.9	288.9	294.5	302.2	314.6	14.5048	2.1727	0.0364
168	243.4	276.1	321.7	329.9	327.5	11.0710	2.1607	0.1015
199	264.9	286.3	290.7	299.2	317.6	13.7562	2.2185	0.0829
249	237.0	269.8	312.7	324.5	320.8	11.6423	2.0519	0.0622

Inhalation Developmental Toxicity Study in Rodents: Body and Organ Weights(g) for Nonpregnant Rats

TMT=1200 ppm Acetonitrile

MATNO	Pre-study Wt (g)	Exposure Day 1 Wt (g)	Exposure Day 5 Wt (g)	Exposure Day 10 Wt (g)	Sacrifice Wt (g)	Liver Wt (g)	Kidney Wt (g)	Adrenal Wt (g)
5	248.3	274.4	291.9	306.3	317.4	12.9284	2.2525	0.0623
39	263.5	287.8	294.9	296.6	299.8	11.5658	2.1744	0.0713
67	229.9	254.2	295.8	279.9	301.3	13.9674	2.0285	0.0836
69	260.7	286.1	339.5	361.8	346.4	12.3682	1.9491	0.0762
145	245.5	268.6	274.1	277.2	285.5	13.6313	1.9756	0.0731
242	268.5	294.8	339.5	348.8	341.5	15.0893	2.5166	0.0762
347	243.1	277.4	295.8	311.5	329.2	14.7687	2.1655	0.0714
364	239.3	265.0	287.4	286.8	291.2	13.3939	1.8991	0.0693

Reproductive Measures and Fetal Data

Fetal Malformation and Variation Code

ROSK	Reduced Ossification - Skull
DIUR	Dilated Urethra
ROST	Reduced Ossification - Sternebrae
ROVE	Reduced Ossification - Vertebrae
SURB	Supernumerary Rib
RPCA	Renal Pelvic Cavitation
ROPB	Reduced Ossification - Pelvis
ROPH	Reduced Ossification - Phalanges
MAST	Misaligned Sternebrae
ANUR	Anury
ECOV	Ectopic Ovary
MIRB	Missing Rib
MIVE	Missing Vertebrae
FUAD	Fused Adrenals
FUKD	Fused Kidneys
RURB	Rudimentary Rib
EDMA	Edema
MOPT	Microphthalamia
HEAD	Hemorrhagic Adrenals
MAVM	Major Vessel Malformation
FUVA	Fused Vertebrae
ANOR	Anomalous Rib

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=0 ppm Acetonitrile

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
3	1	1	3.285	2	H										
3	2	1	3.484	2	V										
3	3	1	3.488	1	H										
3	4	1	3.310	2	V										
3	5	1	3.489	2	H										
3	6	1	3.419	2	V	ROSK									
3	7	1	3.555	2	H										
3	8	1	3.219	2	V	ROSK									
3	9	1	3.469	1	H										
3	10	2	.	.											
3	11	1	3.577	1	V	DIUR	ROVE								
3	12	1	3.606	2	H										
3	13	1	3.764	2	V										
3	14	1	3.474	2	H	ROST									
3	15	1	3.475	1	V	ROSK									
11	1	1	3.617	1	V	ROSK									
11	2	1	3.520	2	H										
11	3	2	.	.											
11	4	2	.	.											
11	5	1	3.692	1	V										
11	6	1	3.691	2	H										
11	7	1	3.531	2	V	ROSK									
11	8	1	3.901	1	H										
11	9	1	3.777	1	V	ROVE									
11	10	1	3.860	1	H										
11	11	1	3.611	1	V										
11	12	1	3.401	2	H										
11	13	1	3.472	2	V										
11	14	1	3.728	1	H										
16	1	2	.	.											
16	2	2	.	.											
16	3	1	3.223	2	V	ROSK									
16	4	1	3.423	2	H										
16	5	1	3.210	2	V	ROVE									
16	6	1	3.322	1	H										
16	7	1	3.545	2	V										
16	8	1	2.994	2	H										
16	9	1	3.266	1	V										
16	10	1	3.265	2	H										
16	11	1	3.418	1	V										
16	12	1	3.232	1	H										
16	13	1	3.180	2	V										
16	14	2	.	.											
16	15	1	3.137	1	H										
16	16	1	3.520	1	V										
16	17	1	3.475	2	H										
32	1	1	3.292	1	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=0 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
32	2	1	3.416	2	H	ROST									
32	3	1	3.445	1	V										
32	4	1	3.047	2	H										
32	5	1	3.277	2	V										
32	6	1	3.501	2	H										
32	7	1	3.391	2	V										
32	8	1	3.613	1	H										
32	9	1	3.354	2	V										
32	10	1	3.113	1	H										
32	11	1	2.623	2	V	ROST									
32	12	1	3.067	2	H										
32	13	1	3.284	2	V										
32	14	1	3.196	2	H										
32	15	1	3.460	1	V										
32	16	1	3.254	1	H										
32	17	1	3.098	2	V										
32	18	1	3.291	1	H										
32	19	1	3.624	1	V										
38	1	1	2.771	1	V	ROSK	ROVE								
38	2	1	3.166	2	H										
38	3	1	3.359	1	V	ROSK									
38	4	1	3.305	2	H										
38	5	1	3.245	1	V	ROSK									
38	6	1	3.173	1	H										
38	7	1	3.335	1	V										
38	8	1	3.251	2	H										
38	9	1	2.957	2	V										
38	10	1	3.014	2	H	ROST									
38	11	1	3.180	1	V										
38	12	1	3.147	1	H										
38	13	1	3.228	2	V										
38	14	1	3.319	1	H	ROVE									
38	15	1	3.189	1	V	ROSK									
38	16	1	3.239	1	H	SURB									
48	1	1	3.781	1	H										
48	2	1	3.751	1	V										
48	3	1	3.592	2	H										
48	4	1	3.802	2	V	SURB									
48	5	1	3.553	2	H										
48	6	1	3.462	2	V	ROST									
48	7	1	3.494	1	H										
48	8	1	3.710	1	V										
48	9	1	3.365	2	H										
48	10	1	3.794	1	V										
48	11	1	3.667	1	H										
48	12	1	3.647	1	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=0 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
48	13	1	3.963	1	H										
48	14	1	3.341	1	V										
48	15	1	3.508	2	H										
48	16	1	3.680	1	V										
66	1	1	2.849	2	V	DIUR	RPCA								
66	2	1	2.989	2	H										
66	3	2	.	.											
66	4	1	3.068	2	V										
66	5	1	2.677	2	H										
66	6	1	3.105	2	V										
66	7	1	3.404	1	H										
66	8	1	3.219	1	V										
66	9	1	3.026	1	H										
66	10	1	3.448	1	V	ROST									
66	11	1	3.580	1	H										
66	12	1	3.284	2	V										
66	13	4	.	.											
66	14	1	3.729	1	H										
66	15	1	3.811	1	V	ROST									
89	1	1	3.711	2	H										
89	2	1	3.689	2	V										
89	3	1	4.078	1	H										
89	4	2	.	.											
89	5	1	3.569	2	V										
89	6	1	3.837	1	H	SURB									
89	7	1	3.673	2	V	ROVE									
89	8	1	3.500	1	H										
89	9	1	3.563	2	V										
89	10	1	3.630	2	H										
89	11	1	4.149	1	V	SURB									
89	12	1	4.104	1	H	SURB									
89	13	1	3.831	1	V										
89	14	1	3.871	2	H										
89	15	1	3.656	2	V										
89	16	1	3.782	2	H	ROVE									
95	1	1	2.935	1	V	DIUR									
95	2	1	3.174	2	H										
95	3	1	3.003	2	V										
95	4	1	3.290	1	H										
95	5	1	3.259	1	V	DIUR									
95	6	1	3.546	1	H										
95	7	1	3.292	1	V										
95	8	1	3.639	1	H										
95	9	1	3.014	1	V										
95	10	1	3.473	1	H										
95	11	1	3.164	1	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=0 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
95	12	1	3.554	2	H										
95	13	1	2.911	2	V										
95	14	4	.	.											
95	15	1	3.603	1	H										
95	16	1	3.340	1	V										
108	1	1	3.666	2	H										
108	2	1	3.827	1	V										
108	3	1	3.912	2	H										
108	4	1	3.537	2	V										
108	5	1	4.014	1	H										
108	6	1	3.947	1	V										
108	7	1	3.758	2	H										
108	8	1	3.746	1	V										
108	9	1	3.907	2	H										
108	10	1	3.593	2	V										
108	11	1	4.076	1	H										
108	12	1	4.013	2	V										
108	13	1	4.072	1	H										
108	14	1	4.197	1	V	DIUR									
113	1	1	3.116	2	V	ROST	ROPB								
113	2	1	3.151	1	H	ROPB	ROPH								
113	3	1	3.645	1	V	ROPB									
113	4	1	3.826	1	H	ROPB	ROPH	SURB							
113	5	1	3.434	2	V	DIUR									
113	6	1	3.681	1	H	ROPB	ROPH								
113	7	1	3.547	1	V	ROSK	ROPB								
113	8	1	3.402	2	H	ROVE	ROPH								
113	9	1	3.891	1	V	ROSK									
113	10	1	3.321	2	H	ROPB									
113	11	1	2.920	2	V	ROSK	ROST								
113	12	1	3.363	2	H										
113	13	1	2.575	2	V										
113	14	1	3.473	2	H	ROST	ROPB								
113	15	1	3.201	2	V	ROSK									
113	16	1	3.453	2	H	ROPB	ROPH								
113	17	1	3.402	2	V	ROST									
113	18	1	3.839	1	H										
139	1	1	3.700	1	H										
139	2	1	3.824	2	V	ROSK									
139	3	1	3.792	2	H										
139	4	1	3.707	1	V	ROST									
139	5	1	3.870	1	H										
139	6	1	3.739	1	V										
139	7	1	3.750	1	H										
139	8	1	3.750	1	V	ROSK	ROST								
139	9	1	3.915	1	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=0 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
139	10	1	3.705	1	V										
139	11	1	3.769	1	H										
139	12	1	3.778	1	V	ROSK									
139	13	1	3.845	1	H										
139	14	1	3.932	1	V	ROSK									
139	15	1	3.197	2	H	ROVE									
139	16	1	3.925	2	V										
139	17	1	3.874	1	H										
139	18	1	3.825	1	V										
158	1	1	3.640	2	H										
158	2	1	3.471	2	V										
158	3	1	3.671	1	H										
158	4	1	3.270	2	V										
158	5	1	3.734	1	H										
158	6	1	3.324	2	V										
158	7	1	3.810	1	H										
158	8	1	3.790	1	V										
158	9	1	3.693	2	H										
158	10	1	3.541	1	V										
158	11	1	3.284	2	H										
158	12	1	3.780	2	V	DIUR									
158	13	1	3.086	2	H	ROST									
158	14	1	3.918	1	V										
158	15	1	2.821	2	H	ROST									
158	16	1	3.397	2	V										
158	17	1	4.076	1	H										
184	1	1	3.511	2	V	ROSK									
184	2	1	3.594	1	H										
184	3	1	3.665	2	V										
184	4	2	.	.											
184	5	1	3.369	2	H										
184	6	1	3.753	1	V	ROSK									
184	7	1	3.635	1	H										
184	8	1	3.688	1	V										
184	9	1	3.426	2	H										
185	1	1	2.770	2	H	ROPB	ROPH								
185	2	1	3.259	2	V	ROSK									
185	3	1	3.406	1	H										
185	4	1	3.445	1	V	ROSK									
185	5	1	3.605	1	H										
185	6	1	3.655	1	V	ROST									
185	7	1	2.806	1	H	ROST	ROPB								
185	8	1	3.311	1	V										
185	9	1	3.306	1	H										
185	10	1	3.292	1	V	ROST									
185	11	1	3.214	1	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=0 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
185	12	1	2.926	2	V	ROST									
185	13	1	2.842	2	H	ROST	ROPB								
185	14	1	3.385	1	V										
185	15	1	3.014	2	H										
185	16	1	3.399	2	V	ROSK									
185	17	1	3.370	2	H										
185	18	2	.	.											
185	19	1	3.681	1	V										
185	20	1	3.476	2	H										
203	1	1	3.167	1	H	ROST									
203	2	1	3.406	2	V										
203	3	1	3.737	1	H										
203	4	1	3.752	1	V	DIUR	ROST								
203	5	1	3.507	2	H										
203	6	1	3.633	1	V	DIUR									
203	7	1	3.593	2	H										
203	8	2	.	.											
203	9	1	3.564	2	V										
203	10	1	3.783	1	H										
203	11	1	3.741	2	V										
203	12	1	3.503	2	H										
203	13	2	.	.											
203	14	1	3.800	1	V	DIUR									
203	15	1	4.093	1	H										
203	16	1	3.992	1	V	DIUR									
203	17	1	3.643	1	V	DIUR									
203	18	1	3.987	1	H										
219	1	1	3.640	2	V										
219	2	1	3.564	2	H										
219	3	1	3.602	2	V	ROSK									
219	4	1	3.694	1	H										
219	5	1	3.644	2	V	ROSK	ROVE								
219	6	1	3.689	1	H										
219	7	1	3.862	1	V	ROVE									
219	8	1	3.854	1	H										
219	9	1	3.401	2	V										
219	10	1	3.359	1	H										
219	11	1	3.558	2	V	ROSK									
219	12	1	3.363	2	H	ROVE									
219	13	1	3.816	1	V										
219	14	1	3.971	1	H										
219	15	1	3.714	1	V	ROVE									
219	16	1	3.630	1	H										
236	1	1	3.284	1	H										
236	2	1	3.371	1	V	ROPB									
236	3	1	3.506	2	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=0 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
236	4	1	3.514	2	V										
236	5	1	3.461	1	H										
236	6	4	.	.											
236	7	1	2.776	1	V	ROST	ROPB								
236	8	1	3.582	1	H										
236	9	1	3.345	2	V										
236	10	1	3.454	2	H										
236	11	2	.	.											
236	12	1	3.261	2	V										
236	13	1	3.567	1	H										
236	14	1	3.956	1	V										
236	15	1	3.706	2	H										
236	16	1	3.062	1	V										
243	1	1	2.898	2	H	ROST									
243	2	1	3.174	2	V										
243	3	1	3.717	1	H										
243	4	1	3.502	1	V										
243	5	2	.	.											
243	6	1	3.846	1	H	SURB									
243	7	2	.	.											
243	8	1	3.605	2	V										
243	9	1	3.041	1	H										
243	10	1	3.607	2	V	SURB									
243	11	1	3.265	2	H										
243	12	1	3.272	2	V										
243	13	1	3.377	2	H										
243	14	1	3.453	1	V										
243	15	1	3.355	2	H										
243	16	1	3.347	2	V										
243	17	1	3.304	2	H										
243	18	1	3.637	1	V										
243	19	1	3.545	2	H										
278	1	1	3.729	1	V										
278	2	1	3.620	2	H	SURB									
278	3	1	3.877	1	V										
278	4	1	3.661	2	H										
278	5	2	.	.											
278	6	1	3.333	2	V										
278	7	1	3.207	2	H	ROVE									
278	8	1	3.563	1	V										
278	9	1	3.659	2	H	SURB									
278	10	1	3.548	2	V										
278	11	1	3.301	1	H										
278	12	1	3.613	2	V										
292	1	1	2.581	1	H	ROPB									
292	2	1	3.228	2	V	ROST	ROVE								

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=0 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
292	3	1	3.413	1	H										
292	4	1	3.366	1	V										
292	5	1	3.374	1	H										
292	6	1	3.346	2	V										
292	7	1	3.319	2	H										
292	8	1	3.190	2	V										
292	9	2	.	.											
292	10	2	.	.											
292	11	1	3.518	1	H	ROVE									
292	12	1	3.375	1	V										
292	13	1	3.302	2	H										
292	14	1	3.239	2	V										
292	15	1	3.357	2	H										
292	16	1	3.354	2	V										
292	17	1	3.225	2	H										
292	18	1	3.344	1	V										
292	19	1	3.252	2	H										
292	20	1	3.474	2	V	ROVE									
293	1	1	3.228	2	H										
293	2	1	3.707	1	V										
293	3	2	.	.											
293	4	1	3.317	2	H										
293	5	1	3.822	1	V	ROVE	ROST	MAST							
293	6	1	3.361	1	H										
293	7	2	.	.											
293	8	2	.	.											
293	9	1	3.478	2	V										
293	10	1	3.494	2	H										
293	11	1	3.388	2	V										
293	12	1	3.528	1	H	ROVE									
293	13	1	3.302	2	V	ROSK									
293	14	1	3.458	2	H										
293	15	1	3.292	2	V										
293	16	1	4.108	1	H										
312	1	1	2.702	1	V	ROSK									
312	2	1	2.706	2	H	ROST									
312	3	1	3.384	2	V										
312	4	1	3.312	2	H										
312	5	1	3.377	1	V										
312	6	1	3.606	1	H										
312	7	1	3.496	1	V										
312	8	1	3.366	1	H										
312	9	1	3.319	1	V										
312	10	1	3.143	1	H										
312	11	1	3.307	1	V										
312	12	1	2.928	2	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=0 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
312	13	1	3.055	2	V										
312	14	1	2.946	2	H										
312	15	1	3.049	2	V	ROST									
312	16	1	3.285	2	H										
314	1	1	3.061	1	H										
314	2	1	3.335	2	V										
314	3	1	3.280	1	H										
314	4	1	3.351	1	V										
314	5	1	3.594	1	H										
314	6	1	3.770	1	V										
314	7	1	3.754	1	H										
314	8	1	3.602	1	V	ROVE									
314	9	1	3.571	2	H										
314	10	1	3.552	2	V										
314	11	1	3.739	1	H										
314	12	1	3.472	2	V										
314	13	1	3.832	1	H										
314	14	1	3.489	1	V										
314	15	1	3.443	2	H										
314	16	1	3.480	2	V										
314	17	1	3.429	2	H										
314	18	2	.	.											
323	1	1	3.795	1	V										
323	2	1	3.826	1	H										
323	3	2	.	.											
323	4	1	3.884	1	V	ROSK									
323	5	1	3.381	2	H										
336	1	1	3.785	2	H										
336	2	1	3.995	1	V										
336	3	1	3.823	2	H										
336	4	1	3.550	2	V										
336	5	1	3.766	2	H										
336	6	1	4.411	1	V	ROST									
336	7	1	4.084	1	H										
336	8	1	3.939	2	V	ROSK									
336	9	1	4.147	2	H										
336	10	1	4.108	1	V	ROST									
336	11	1	4.150	1	H										
336	12	1	4.031	1	V										
336	13	1	4.010	2	H										
336	14	1	3.842	1	V										
336	15	1	4.402	1	H										
336	16	1	3.838	2	V										
336	17	1	4.273	1	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=100 ppm Acetonitrile

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
1	1	1	3.756	1	H										
1	2	1	4.077	1	V	SURB									
1	3	1	3.661	2	H										
1	4	2	.	.											
1	5	1	3.681	2	V										
1	6	1	3.805	1	H	SURB									
42	1	1	3.755	2	V										
42	2	1	3.575	1	H	ROVE									
42	3	1	3.727	1	V	ROVE	ROPB								
42	4	1	3.895	1	H	ROVE									
42	5	1	3.780	1	V										
42	6	1	3.844	2	H										
42	7	1	3.852	2	V	ROVE									
42	8	1	4.100	1	H	ROPB									
42	9	1	3.559	2	V										
42	10	1	3.546	1	H	ROVE	ROPB	ROPH							
42	11	1	3.582	2	V										
42	12	1	3.507	2	H										
42	13	1	3.999	1	V										
42	14	1	3.802	2	H										
42	15	1	3.464	2	V										
42	16	1	3.520	2	H	ROVE									
42	17	1	3.389	2	V	ROVE									
85	1	1	3.060	1	H										
85	2	1	3.286	2	V										
85	3	1	3.540	1	H										
85	4	1	3.273	2	V										
85	5	2	.	.											
85	6	1	3.592	1	H										
85	7	2	.	.											
85	8	1	3.691	1	V										
85	9	1	3.771	1	H										
85	10	1	3.556	2	V										
85	11	1	3.527	2	H										
85	12	1	3.448	1	V	DIUR	RPCA								
85	13	1	2.984	2	H										
85	14	1	3.460	1	V										
85	15	1	3.236	2	H										
92	1	1	3.224	1	H	ROST									
92	2	1	3.399	2	V	DIUR	ROST	ROVE							
92	3	1	3.588	1	H										
92	4	1	3.640	1	V										
92	5	1	3.723	1	H										
92	6	1	3.374	1	V										
92	7	1	3.109	2	H										
92	8	1	2.461	2	V	ROST									
92	9	1	3.171	2	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=100 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
92	10	2	.	.											
92	11	1	3.265	2	V										
92	12	1	3.612	1	H										
92	13	1	3.389	1	V										
92	14	1	3.243	2	H	ROVE									
92	15	1	3.608	1	V	ROST									
92	16	1	3.399	2	H	ROST									
92	17	1	3.318	1	V	ROST									
92	18	1	3.374	1	H										
122	1	1	3.156	2	V										
122	2	1	3.592	2	H										
122	3	1	3.588	2	V										
122	4	1	3.773	1	H										
122	5	1	3.673	2	V	SURB									
122	6	2	.	.											
122	7	1	3.345	2	H	ROVE									
122	8	1	3.514	2	V	DIUR									
122	9	1	3.757	2	H										
122	10	1	3.327	2	V	SURB									
122	11	1	3.589	2	H										
122	12	1	3.551	2	V										
122	13	1	3.905	1	H										
122	14	1	3.736	2	V	SURB									
122	15	1	3.814	1	H										
126	1	1	2.904	2	V										
126	2	1	3.229	2	H										
126	3	1	3.340	1	V	ROSK	ROST								
126	4	1	3.334	2	H										
126	5	1	3.337	2	V										
126	6	1	3.473	1	H										
126	7	1	3.422	2	V										
126	8	1	3.314	2	H										
126	9	1	3.297	2	V										
126	10	1	3.494	1	H										
126	11	1	3.169	2	V	ROST	MAST								
126	12	1	3.353	2	H										
126	13	1	3.355	1	V										
126	14	1	3.402	1	H										
162	1	2	.	.											
162	2	1	3.532	1	H										
162	3	1	3.544	1	V										
162	4	1	3.199	2	H	SURB									
162	5	1	3.384	2	V										
162	6	1	3.657	1	H	SURB									
162	7	1	3.455	2	V										
162	8	1	3.446	1	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=100 ppm Acetonitrile (continued)

no	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
162	9	1	3.650	1	V	SURB									
162	10	1	3.537	1	H	ROST	SURB								
162	11	1	3.544	1	V										
162	12	1	3.632	1	H										
162	13	1	3.205	2	V										
162	14	1	3.849	1	H	ROVE									
162	15	1	3.338	2	V	ROVE									
162	16	1	3.623	1	H										
162	17	1	3.038	2	V	ROST									
176	1	1	2.863	1	H	ROPB									
176	2	1	3.013	1	V	ROPB									
176	3	1	3.227	2	H	ROST									
176	4	2	.	.											
176	5	1	3.098	2	V										
176	6	1	3.082	1	H										
176	7	1	2.860	1	V										
176	8	1	2.519	2	H	ROST	ROPB								
176	9	1	2.911	2	V										
176	10	2	.	.											
176	11	1	2.931	2	H	ROST	SURB								
176	12	1	2.414	1	V	ROST	ROPB	ROPH							
176	13	4	.	.											
176	14	2	.	.											
176	15	1	3.372	1	H										
176	16	2	.	.											
190	1	4	.	.											
190	2	1	3.377	2	H										
190	3	1	3.908	2	V	ROVE									
190	4	1	4.065	1	H										
190	5	1	3.357	2	V										
190	6	1	4.041	1	H										
190	7	1	3.824	2	V										
190	8	1	3.202	2	H										
190	9	1	3.503	2	V										
190	10	2	.	.											
190	11	1	3.477	2	H										
190	12	1	3.931	2	V										
190	13	1	3.725	1	H										
190	14	1	3.638	2	V										
208	1	1	3.481	1	H										
208	2	1	3.436	2	V	ROSK									
208	3	1	3.600	2	H										
208	4	1	3.573	1	V	ROSK									
208	5	2	.	.											
208	6	1	3.567	2	H										
208	7	1	3.432	2	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=100 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
208	8	1	3.457	1	H										
208	9	1	3.646	1	V										
208	10	1	3.293	1	H										
208	11	1	3.697	1	V	ROSK									
208	12	1	3.357	2	H										
208	13	1	3.264	2	V										
208	14	1	3.308	2	H										
208	15	1	3.533	1	V										
208	16	1	3.517	2	H										
208	17	1	3.486	2	V										
221	1	1	3.523	1	H										
221	2	1	3.579	2	V										
221	3	1	3.548	1	H										
221	4	1	3.457	2	V										
221	5	1	3.807	1	H										
221	6	1	3.599	1	V	ROSK									
221	7	1	3.728	1	H										
221	8	1	3.690	1	V										
221	9	2	.	.											
221	10	1	3.490	1	H										
221	11	1	3.618	1	V	ROSK									
221	12	1	2.797	2	H	ROST	SURB								
221	13	1	3.688	2	V	ROSK									
221	14	1	3.552	2	H										
221	15	1	3.690	1	V	ROSK									
221	16	1	3.725	2	H										
221	17	1	3.537	2	V	ROSK									
224	1	1	2.952	2	V	ROST									
224	2	1	3.453	2	H										
224	3	1	3.321	1	V	ROST									
224	4	2	.	.											
224	5	1	2.685	2	H	ROST									
224	6	1	2.976	1	V										
224	7	1	3.404	2	H	ROVE									
224	8	1	3.589	1	V										
224	9	4	.	.											
224	10	1	3.497	2	H										
224	11	1	3.335	2	V										
224	12	1	2.898	2	H										
224	13	1	3.567	1	V										
224	14	1	3.327	2	H										
224	15	1	3.599	1	V										
224	16	1	3.556	1	H										
239	1	1	1.453	2	V	ROSK	ROST	SURB	ROPB	ROVE					
239	2	1	3.295	2	H	SURB									
239	3	1	3.501	1	V	ROSK									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=100 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
239	4	1	3.641	2	H										
239	5	1	3.243	2	V	ROVE									
239	6	1	3.339	2	H										
239	7	1	3.731	1	V	ROSK									
258	1	1	3.600	1	V										
258	2	1	3.332	2	H										
258	3	1	3.537	2	V										
258	4	1	3.561	2	H										
258	5	1	3.443	2	V	ROST	ROVE	SURB							
258	6	1	3.959	1	H										
258	7	1	2.997	2	V	SURB									
258	8	1	4.008	1	H										
258	9	1	3.676	1	V										
258	10	1	3.696	1	H										
258	11	1	3.433	2	V	DIUR	ROSK								
258	12	1	3.923	1	H										
258	13	1	3.592	1	V	ROST	SURB								
258	14	1	3.730	1	H	ROST									
258	15	1	3.663	1	V										
267	1	1	3.722	2	H	SURB	ROPH								
267	2	1	3.857	2	V	DIUR									
267	3	1	3.799	2	H										
267	4	1	3.620	2	V	DIUR									
267	5	1	3.981	2	H										
267	6	1	3.854	2	V										
267	7	1	3.479	2	H										
267	8	1	3.143	2	V	ROVE									
267	9	1	4.110	1	H										
267	10	1	3.788	2	V										
267	11	1	3.701	2	H										
267	12	1	3.668	2	V										
267	13	1	3.589	2	H										
267	14	1	3.993	2	V	DIUR	ROVE								
267	15	1	3.947	1	H										
271	1	4	.	.											
271	2	1	3.743	1	H										
271	3	1	4.183	1	V										
271	4	1	4.115	1	H										
271	5	1	3.469	2	V										
271	6	1	3.675	2	H										
271	7	1	4.011	1	V										
271	8	1	3.796	2	H										
271	9	1	3.923	1	V	SURB									
271	10	1	4.039	1	H										
271	11	1	4.042	1	V										
271	12	1	4.059	1	H	SURB									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=100 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
271	13	1	3.916	1	V										
271	14	1	3.912	2	H										
271	15	1	4.052	1	V										
273	1	1	4.060	1	H	SURB									
273	2	1	3.257	1	V	ROVE	FURB								
273	3	1	3.937	1	H										
273	4	1	3.213	2	V	ROVE									
273	5	1	3.558	1	H										
273	6	1	3.828	2	V										
273	7	1	3.684	2	H										
273	8	1	4.119	1	V	ROSK									
273	9	1	3.597	1	H										
273	10	1	3.815	2	V										
273	11	2	.	.											
273	12	1	3.821	2	H										
273	13	1	3.719	1	V										
273	14	1	3.601	2	H										
273	15	1	3.329	2	V	ROSK									
274	1	2	.	.											
274	2	2	.	.											
274	3	2	.	.											
274	4	1	3.482	1	V	ROSK	ROPB								
274	5	1	3.586	2	H										
274	6	1	3.728	1	V										
274	7	1	3.487	2	H										
274	8	1	3.556	1	V	ROST									
274	9	1	2.974	1	H	ROST									
274	10	1	3.709	1	V	ROST	ROSK								
274	11	1	3.443	2	H										
274	12	1	3.629	1	V	ROPB									
274	13	1	2.298	2	H	ROPB	ROST	SURB	ROPH						
274	14	1	3.045	1	V	ROSK									
274	15	1	3.073	2	H										
274	16	1	3.066	2	V	SURB									
274	17	1	3.601	1	H	SURB									
274	18	1	3.638	2	V										
274	19	1	3.436	2	H										
274	20	1	3.303	1	V										
275	1	1	2.757	2	H	ROST									
275	2	1	3.296	2	V										
275	3	1	3.245	1	H										
275	4	1	2.809	2	V										
275	5	1	3.062	1	H										
275	6	1	3.177	1	V	ROST									
275	7	1	3.265	1	H										
275	8	1	3.121	2	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=100 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
275	9	1	3.632	1	H										
275	10	1	3.125	2	V										
275	11	1	3.034	1	H										
276	1	1	3.666	2	V										
276	2	1	3.584	2	H										
276	3	1	3.782	2	V	ROVE									
276	4	1	3.795	1	H										
276	5	1	3.916	1	V										
276	6	1	3.671	2	H										
276	7	1	3.949	2	V										
276	8	1	4.151	1	H										
276	9	1	3.959	2	V	ROSK									
276	10	1	3.909	1	H										
276	11	1	4.043	2	V										
276	12	1	3.950	1	H										
276	13	1	3.915	2	V										
276	14	1	3.639	2	H										
276	15	1	4.179	1	V										
276	16	2	.	.											
276	17	1	3.679	2	H										
276	18	1	3.515	2	V										
281	1	1	3.513	1	V	DIUR	RPCA								
281	2	2	.	.											
281	3	1	2.911	1	H										
281	4	1	1.969	2	V	ROSK	ROST	ROVE	ROPB	SURB	ROPH				
281	5	1	3.763	1	H										
281	6	1	3.631	1	V	DIUR									
281	7	1	3.611	2	H										
281	8	2	.	.											
281	9	2	.	.											
281	10	1	3.611	1	V										
281	11	1	3.398	2	H										
281	12	1	3.117	1	V										
281	13	1	3.552	2	H										
281	14	1	3.434	2	V										
281	15	1	3.068	2	H										
281	16	1	2.709	2	V	ANUR	ECOV	MIRB	MIVE	FUAD	FUKD				
281	17	1	3.954	1	H										
281	18	1	3.718	1	V										
288	1	1	3.448	1	H	SURB	ROPB								
288	2	1	3.158	2	V	SURB									
288	3	1	3.361	1	H	SURB									
288	4	2	.	.											
288	5	1	2.885	2	V										
288	6	1	3.319	1	H										
288	7	1	3.323	2	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=100 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
288	8	1	2.892	1	H	ROST	ROPB	SURB							
288	9	1	3.179	2	V	SURB									
290	1	1	2.690	2	V	ROVE									
290	2	1	3.335	1	H										
290	3	1	3.495	2	V										
290	4	1	3.688	2	H										
290	5	1	3.323	2	V										
290	6	1	3.528	2	H										
290	7	1	3.727	1	V										
290	8	1	3.583	1	H										
290	9	1	3.533	1	V										
290	10	1	3.220	2	H	RURB									
290	11	1	3.310	2	V										
290	12	1	3.442	1	H										
290	13	1	3.496	2	V										
290	14	1	3.636	2	H										
290	15	1	3.707	1	V										
290	16	1	3.433	2	H	SURB									
291	1	4	.	.											
291	2	1	3.333	1	H										
291	3	1	3.857	1	V										
291	4	1	3.658	1	H										
291	5	1	3.418	2	V										
291	6	1	3.803	1	H										
291	7	4	.	.											
291	8	1	3.549	2	V										
291	9	1	3.053	2	H										
291	10	1	3.931	1	V										
291	11	1	3.889	1	H	ROST									
291	12	1	3.429	2	V										
291	13	1	3.296	2	H	ROST									
291	14	1	3.543	2	V	ROVE									
291	15	1	3.782	1	H										
291	16	1	3.614	2	V										
296	1	1	4.023	1	H										
296	2	1	3.927	2	V										
296	3	1	4.217	1	H										
296	4	1	4.007	2	V										
296	5	1	3.940	2	H										
296	6	1	4.145	1	V										
296	7	1	3.840	2	H										
296	8	1	4.089	1	V										
321	1	1	3.446	1	H										
321	2	1	3.881	1	V	DIUR									
321	3	1	3.669	1	H										
321	4	1	3.766	1	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=100 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
321	5	1	3.382	1	H										
321	6	1	3.760	1	V										
321	7	1	3.826	1	H										
321	8	1	3.347	1	V										
321	9	1	3.289	2	H	ROPB									
321	10	1	3.640	1	V										
321	11	1	3.488	2	H										
321	12	1	3.529	2	V										
321	13	1	3.777	1	H										
321	14	1	3.486	1	V										
326	1	1	3.371	2	V										
326	2	1	3.800	1	H	ROVE									
326	3	1	2.786	1	V										
326	4	1	3.810	2	H	ROVE									
326	5	1	3.909	1	V										
326	6	1	3.538	2	H										
326	7	1	3.525	1	V										
326	8	1	3.710	2	H										
326	9	1	3.761	2	V	ROVE									
326	10	1	3.673	1	H										
326	11	1	3.645	2	V	ROSK									
326	12	1	3.754	1	H	ROST									
326	13	1	3.812	1	V	ROVE									
326	14	1	3.493	2	H										
326	15	1	3.502	1	V	DIUR									
326	16	1	3.673	1	H										
326	17	1	3.890	2	V										
326	18	1	4.124	1	H										
343	1	1	3.540	2	V	SURB									
343	2	1	3.867	1	H	SURB									
343	3	1	3.933	1	V										
343	4	1	3.492	2	H	SURB	ROVE								
343	5	1	3.652	2	V										
343	6	1	3.626	1	H										
343	7	1	3.691	1	V	ROVE									
343	8	1	3.699	2	H										
343	9	1	3.749	1	V	ROVE	SURB	ROST							
343	10	1	3.590	1	H	SURB									
343	11	1	3.651	1	V	SURB									
343	12	1	3.417	2	H										
343	13	1	3.459	2	V										
343	14	1	3.694	1	H										
343	15	1	3.597	1	V										
343	16	1	3.629	2	H	ROVE									
343	17	1	3.686	1	V	SURB									
348	1	1	3.794	1	H	ROST									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=100 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
348	2	1	3.142	1	V	ROVE	SURB								
348	3	1	3.696	1	H										
348	4	1	3.404	2	V										
348	5	1	3.693	1	H										
348	6	1	3.340	2	V										
348	7	1	3.984	1	H										
348	8	1	3.907	1	V	ROVE									
348	9	1	3.777	1	H										
348	10	1	3.596	2	V										
348	11	1	3.901	1	H	ROPH									
348	12	1	3.149	2	V										
348	13	1	3.668	1	H										
348	14	1	3.658	1	V										
348	15	1	3.548	2	H										
348	16	1	3.823	1	V	DIUR									
348	17	1	3.489	2	H										
348	18	1	3.525	2	V										
353	1	1	3.601	1	H	ROVE	ROPB	ROPH							
353	2	1	3.722	2	V	ROVE									
353	3	1	3.942	1	H	ROVE									
353	4	1	3.995	1	V	ROSK	ROPH								
353	5	1	3.974	1	H										
353	6	1	3.969	2	V										
353	7	1	4.011	1	H										
353	8	1	4.031	1	V	ROVE									
353	9	1	3.038	2	H	ROST	ROPH	ROPB	ROVE						
353	10	1	3.642	2	V	ROVE									
353	11	1	3.934	1	H										
353	12	1	3.546	2	V	ROSK	ROVE								
353	13	1	3.347	2	H										
353	14	1	3.499	2	V										
353	15	1	3.463	2	H	ROST	ROVE								
353	16	1	3.755	1	V										
353	17	1	3.962	2	H										
353	18	1	3.244	2	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=400 ppm Acetonitrile

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
17	1	1	3.724	2	V										
17	2	1	3.411	2	H										
17	3	1	3.783	2	V										
17	4	1	3.747	2	H										
17	5	1	4.130	2	V										
17	6	1	3.605	2	H	SURB									
17	7	1	3.823	1	V	ROVE									
17	8	1	3.374	2	H										
30	1	1	3.534	1	V	ROSK									
30	2	1	3.822	2	H										
30	3	1	3.336	2	V										
30	4	1	3.514	1	H										
30	5	1	3.806	2	V										
30	6	1	3.928	1	H										
30	7	1	3.455	1	V										
30	8	1	3.605	2	H										
30	9	1	3.292	2	V	ROVE									
30	10	1	3.190	2	H										
30	11	1	3.739	1	V										
30	12	1	3.461	1	H										
30	13	2	.	.											
30	14	1	3.390	2	V										
30	15	1	3.379	2	H										
30	16	1	3.371	2	V										
30	17	1	3.180	2	H										
30	18	1	3.323	2	V										
46	1	1	3.743	2	H										
46	2	1	3.738	2	V										
46	3	1	3.727	2	H										
46	4	1	3.842	1	V										
46	5	1	4.258	1	H										
46	6	1	3.994	2	V										
47	1	1	3.468	1	V										
47	2	1	3.701	2	H										
47	3	1	3.443	2	V										
47	4	1	3.426	2	H										
47	5	1	3.600	2	V	SURB									
47	6	1	3.086	1	H										
47	7	1	3.390	1	V										
47	8	1	3.440	1	H										
47	9	2	.	.											
47	10	1	3.677	2	V										
47	11	1	3.546	2	H										
47	12	1	3.827	2	V										
47	13	1	3.506	1	H										
47	14	1	3.595	2	V										
52	1	1	2.845	2	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=400 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
52	2	1	3.441	1	H										
52	3	1	3.556	1	V										
52	4	1	3.633	1	H										
52	5	1	3.408	1	V	ROST									
52	6	1	3.680	1	H										
52	7	2	.	.											
52	8	1	2.944	2	V										
52	9	1	2.924	2	H	ROST									
52	10	1	3.177	2	V	ROST									
52	11	1	3.213	2	H	ROST									
52	12	1	3.212	1	V	ROST									
52	13	1	3.237	2	H	ROST									
52	14	1	3.581	1	V										
73	1	1	3.082	1	V										
73	2	2	.	.											
73	3	1	3.187	1	H										
73	4	1	3.145	1	V										
73	5	1	2.684	2	H										
73	6	1	3.178	1	V										
73	7	1	3.003	2	H										
73	8	1	3.088	2	V										
73	9	1	3.395	2	H										
73	10	1	3.432	2	V										
73	11	2	.	.											
73	12	1	3.377	2	H										
73	13	1	3.667	1	V										
73	14	1	3.309	1	H										
73	15	1	3.103	1	V	ROSK									
93	1	1	2.971	2	V										
93	2	1	3.376	2	H										
93	3	1	3.615	2	V										
93	4	1	3.837	1	H										
93	5	1	3.618	2	V										
93	6	1	3.721	1	H										
93	7	1	3.518	2	V										
93	8	1	3.998	1	H										
93	9	1	3.583	2	V										
93	10	1	2.728	2	H	ROST									
93	11	1	3.013	2	V										
93	12	1	3.596	2	H										
93	13	1	3.697	2	V										
93	14	1	3.687	2	H										
93	15	1	3.394	2	V										
93	16	1	3.811	2	H										
123	1	1	3.279	2	V	SURB									
123	2	1	3.342	2	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=400 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
123	3	1	2.881	2	V										
123	4	1	3.183	1	H										
123	5	1	3.198	1	V										
123	6	2	.	.											
123	7	1	3.382	1	H										
123	8	1	3.233	1	V										
123	9	1	3.111	1	H										
123	10	1	3.042	1	V										
123	11	1	3.438	1	H										
123	12	1	3.416	1	V										
123	13	1	3.433	1	H										
123	14	1	2.807	2	V										
135	1	1	3.766	1	H										
135	2	1	3.223	2	V										
135	3	2	.	.											
135	4	1	3.877	1	H	ROST									
135	5	1	3.409	2	V										
135	6	1	3.379	2	H										
135	7	1	3.258	2	V										
135	8	1	3.260	2	H										
135	9	1	3.471	2	V										
135	10	1	3.143	2	H	ROST									
135	11	1	3.462	1	V	ROST									
135	12	1	3.584	1	H	ROST									
135	13	1	3.643	2	V										
135	14	1	3.401	2	H										
135	15	1	3.453	2	V										
135	16	1	3.521	2	H										
135	17	1	3.192	2	V										
135	18	1	3.163	2	H										
137	1	1	3.266	2	V										
137	2	1	3.281	1	H	ROST									
137	3	1	3.196	1	V	SURB									
137	4	1	3.216	1	H										
137	5	1	3.606	1	V										
137	6	1	3.434	1	H										
137	7	1	3.232	2	V	SURB									
137	8	1	3.348	1	H	SURB									
137	9	1	3.359	2	V										
137	10	1	3.332	1	H										
137	11	1	3.520	1	V	SURB									
137	12	1	3.252	1	H										
137	13	1	3.262	2	V										
137	14	1	3.333	1	H										
137	15	1	3.386	1	V										
137	16	1	3.026	2	H	SURB									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=400 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
137	17	1	3.145	2	V										
156	1	1	3.621	2	H	ROST									
156	2	1	3.738	1	V	ROSK									
156	3	1	3.639	2	H										
156	4	1	3.695	1	V	ROSK									
156	5	1	3.627	2	H										
156	6	1	3.440	2	V										
156	7	1	3.835	1	H										
156	8	1	3.860	1	V										
156	9	1	3.721	1	H										
156	10	1	3.499	1	V	ROSK	ROVE								
156	11	1	3.409	2	H										
156	12	1	3.722	1	V										
156	13	1	3.627	1	H										
165	1	1	3.092	2	V	ROSK									
165	2	1	3.435	1	H										
165	3	1	3.603	1	V										
165	4	1	2.697	1	H	ROST									
165	5	1	3.159	2	V										
165	6	1	3.540	1	H										
165	7	1	3.334	2	V										
165	8	1	3.462	2	H										
165	9	1	3.567	2	V	ROSK									
165	10	1	3.324	2	H										
165	11	1	3.296	2	V										
165	12	1	3.430	2	H										
165	13	1	3.419	2	V										
165	14	1	3.632	1	H										
165	15	1	3.651	2	V										
165	16	1	3.619	2	H										
174	1	1	3.492	2	V	ROSK									
174	2	1	3.512	1	H										
174	3	1	3.311	2	V	EDMA	MOPT	ECOV	HEAD	MAVM	ROST	ROSK	ROVE	ROPB	FUVA
174	4	1	3.761	1	H										
174	5	1	3.903	1	V										
174	6	1	2.745	1	H	ROST									
174	7	1	3.756	1	V	ROSK									
174	8	1	3.647	1	H										
174	9	1	3.509	2	V										
174	10	1	3.428	2	H										
174	11	1	3.301	2	V										
174	12	1	3.608	1	H										
174	13	1	3.587	2	V	ROSK									
174	14	1	3.480	2	H										
174	15	1	3.871	1	V										
188	1	1	3.325	1	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=400 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
188	2	1	3.516	1	H										
188	3	1	3.493	1	V										
188	4	1	3.395	1	H										
188	5	1	3.318	2	V										
188	6	1	3.073	1	H										
188	7	1	3.531	1	V										
188	8	1	3.277	1	H										
188	9	1	3.126	2	V										
188	10	1	3.068	1	H										
188	11	1	3.291	2	V										
188	12	1	3.055	2	H										
188	13	1	3.587	1	V										
188	14	1	3.154	2	H										
188	15	1	3.136	1	V										
188	16	1	3.405	2	H										
188	17	1	3.331	2	V										
188	18	1	2.967	1	H										
188	19	1	3.265	2	V										
198	1	1	3.360	2	H										
198	2	1	3.421	1	V	ROSK									
198	3	1	3.642	1	H										
198	4	1	3.444	2	V	ROVE	SURB								
198	5	1	3.258	2	H										
198	6	1	3.235	1	V	ROST	SURB								
198	7	1	3.658	1	H	SURB									
198	8	1	3.665	2	V										
198	9	1	3.384	2	H										
198	10	1	3.610	2	V										
198	11	1	3.503	2	H										
198	12	1	3.566	2	V	SURB									
198	13	1	3.075	2	H	SURB									
198	14	1	3.519	1	V	SURB									
198	15	1	3.143	2	H	SURB									
205	1	1	3.566	1	H										
205	2	1	3.334	1	V										
205	3	1	3.234	2	H										
205	4	1	3.602	1	V										
205	5	1	3.420	1	H	ROST									
205	6	1	3.352	1	V	ROVE									
205	7	1	3.297	2	H	ROST									
205	8	1	3.484	2	V										
205	9	1	2.901	2	H	ROST									
205	10	1	3.180	2	V										
205	11	1	3.385	2	H	MAST									
205	12	2	.	.											
205	13	1	3.492	2	V	ROVE									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=400 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
205	14	1	3.402	2	H										
205	15	1	3.558	2	V	MAST									
205	16	1	3.747	2	H										
223	1	1	3.805	1	V										
223	2	1	3.708	2	H										
223	3	1	4.086	2	V										
223	4	2	.	.											
223	5	1	3.634	2	H										
223	6	1	3.825	2	V	ROST									
223	7	1	3.777	2	H										
223	8	2	.	.											
223	9	1	3.899	1	V										
223	10	1	3.562	2	H	SURB									
223	11	1	3.920	1	V										
223	12	1	3.642	2	H										
270	1	1	3.162	2	H										
270	2	1	3.579	2	V										
270	3	1	3.710	1	H										
270	4	2	.	.											
270	5	2	.	.											
270	6	1	3.608	1	V										
270	7	1	3.590	1	H										
270	8	1	3.273	2	V										
270	9	1	3.307	2	H	ROVE									
270	10	1	3.439	2	V										
270	11	1	3.305	2	H										
270	12	1	3.697	1	V										
270	13	1	3.266	2	H										
270	14	1	3.349	1	V										
270	15	1	3.302	2	H										
270	16	1	3.370	2	V										
286	1	1	3.482	2	V	MAST	ROST								
286	2	1	3.415	2	H										
286	3	1	3.806	2	V	ROVE									
286	4	1	3.476	1	H										
286	5	4	.	.											
286	6	1	3.838	1	V	SURB									
286	7	1	3.404	1	H										
286	8	1	3.108	2	V										
286	9	1	3.789	2	H										
286	10	1	3.435	2	V										
315	1	1	3.134	2	H	ROST									
315	2	1	3.176	2	V	ROST									
315	3	1	3.292	2	H										
315	4	1	3.281	2	V										
315	5	1	3.354	2	H	ROST									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=400 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
315	6	1	3.338	1	V										
315	7	1	3.504	2	H										
315	8	1	3.147	2	V										
315	9	1	3.601	1	H										
315	10	1	3.318	2	V										
315	11	1	3.276	2	H										
315	12	1	3.526	2	V										
315	13	1	3.290	1	H										
315	14	1	3.485	1	V										
315	15	1	3.229	2	H										
320	1	1	3.375	2	V										
320	2	1	3.482	1	H	SURB									
320	3	1	3.606	2	V										
320	4	1	3.600	1	H										
320	5	1	3.858	1	V	SURB									
320	6	1	3.593	2	H										
320	7	1	4.095	1	V										
320	8	1	3.920	1	H										
320	9	1	3.989	1	V	ROSK	SURB								
320	10	1	3.605	2	H										
320	11	1	3.754	2	V	ROSK									
320	12	1	3.561	2	H	SURB									
320	13	1	3.966	1	V	ROSK	ROST	ROPB							
320	14	1	3.690	1	H										
320	15	1	3.483	2	V	ROSK									
320	16	1	3.795	1	H										
320	17	1	3.718	2	V										
320	18	1	3.578	2	H										
342	1	1	3.412	2	V										
342	2	1	3.764	1	H										
342	3	1	3.904	1	V										
342	4	1	3.673	2	H										
342	5	1	3.707	2	V										
342	6	1	3.834	1	H										
342	7	1	4.001	1	V										
342	8	1	3.929	1	H	ROST									
342	9	1	3.903	2	V	DIUR									
342	10	1	3.565	2	H										
342	11	1	3.967	1	V	DIUR	RURB								
342	12	1	3.902	1	H										
342	13	1	3.815	2	V										
342	14	1	3.649	2	H										
342	15	1	3.741	2	V	RURB									
342	16	1	3.892	1	H										
342	17	1	3.741	1	V										
356	1	2	.	.											

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=400 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
356	2	1	2.913	1	V	ROVE	ROPB								
356	3	1	3.700	1	H										
356	4	1	3.425	2	V										
356	5	1	2.674	2	H	ROST									
356	6	1	2.704	1	V	ROSK	ROST	ROPB							
356	7	1	3.204	1	H										
356	8	2	.	.											
356	9	1	3.138	2	V										
356	10	2	.	.											
356	11	1	2.714	2	H										
356	12	1	2.871	2	V	ROVE									
356	13	1	3.472	1	H										
356	14	1	2.935	2	V										
356	15	1	3.455	2	H										
356	16	2	.	.											

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=1200 ppm Acetonitrile

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
14	1	1	2.439	1	V	ROSK	ROPB								
14	2	4	.	.											
14	3	1	3.544	1	H										
14	4	1	3.683	1	V										
14	5	1	3.422	1	H										
14	6	2	.	.											
14	7	1	3.705	1	V	DIUR									
14	8	1	3.390	1	H										
14	9	1	3.326	2	V	ROST									
14	10	1	3.541	1	H										
14	11	1	3.818	1	V	MAST	ROST								
14	12	1	2.932	2	H	ROST									
14	13	1	3.264	1	V										
14	14	1	3.326	1	H	ROST									
14	15	1	3.389	1	V	ROST									
14	16	1	3.449	1	H										
14	17	1	3.468	2	V										
14	18	1	3.597	1	H										
14	19	1	3.911	1	V	ROVE									
14	20	1	3.335	1	H										
21	1	1	2.352	1	V	ROSK	ROST	ROPB	ROPH						
21	2	1	2.744	1	H										
21	3	1	2.854	2	V										
21	4	1	3.259	1	H										
21	5	1	3.092	2	V	ROSK									
21	6	1	2.976	2	H										
21	7	1	2.860	2	V										
21	8	1	3.306	1	H	ROPH									
21	9	1	2.950	2	V										
21	10	1	2.949	2	H										
21	11	1	2.930	1	V										
21	12	1	2.778	2	H										
21	13	1	2.959	2	V										
21	14	1	3.146	1	H	MAST									
21	15	1	3.059	1	V	SURB									
21	16	1	2.816	2	H										
21	17	1	3.298	1	V										
21	18	1	2.932	2	H	MAST									
23	1	1	2.934	2	H										
23	2	1	3.117	1	V	ROPB	ROST	ROPB							
23	3	1	3.020	2	H										
23	4	1	3.445	1	V										
23	5	1	3.227	2	H	ROPB	ROST								
23	6	1	3.583	1	V	DIUR									
23	7	1	2.453	1	H										
23	8	1	3.106	1	V										
23	9	1	3.618	1	H	ROPB									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=1200 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
23	10	1	3.332	1	V										
23	11	1	3.197	2	H										
23	12	1	3.301	2	V										
23	13	1	3.122	2	H										
23	14	1	3.492	1	V	SURB									
23	15	1	3.198	1	H	ROST	ROPB								
23	16	1	3.207	2	V										
25	1	1	3.591	2	H										
25	2	1	3.566	1	V										
25	3	1	3.595	2	H	ROVE									
25	4	1	3.683	2	V										
25	5	1	3.899	1	H										
25	6	1	3.651	1	V										
25	7	1	3.717	1	H										
25	8	1	3.427	2	V										
25	9	1	3.513	2	H										
25	10	1	3.764	2	V										
25	11	1	3.601	2	H										
25	12	1	3.641	2	V	DIUR									
25	13	1	3.778	1	H	ROST									
34	1	2	.	.											
34	2	1	3.367	1	H	ROVE									
34	3	1	3.426	1	V										
34	4	1	3.034	1	H										
34	5	2	.	.											
34	6	1	3.345	2	V										
34	7	1	2.865	2	H										
34	8	1	2.019	2	V	ROPB	ROST								
34	9	1	2.942	2	H	ROST									
34	10	2	.	.											
34	11	1	2.824	1	V	ROPB									
34	12	1	2.711	2	H										
34	13	1	2.954	2	V	ROPB									
60	1	1	3.464	2	H										
60	2	1	3.631	1	V										
60	3	1	3.213	2	H										
60	4	1	3.363	2	V										
60	5	1	3.699	1	H										
60	6	1	3.628	1	V										
60	7	1	3.845	1	H										
64	1	1	3.184	1	H										
64	2	1	3.245	2	V										
64	3	1	3.403	1	H	SURB									
64	4	1	2.969	2	V										
64	5	1	3.288	1	H										
64	6	1	3.464	1	V	ROSK									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=1200 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
64	7	1	3.107	2	H										
64	8	1	3.134	1	V										
64	9	1	3.554	1	H										
64	10	1	3.070	2	V										
64	11	1	3.388	1	H										
64	12	1	3.186	2	V										
64	13	1	3.177	2	H										
64	14	1	3.520	1	V	ROSK									
64	15	1	3.177	1	H										
64	16	1	3.084	2	V	ROSK									
64	17	1	3.128	1	H										
65	1	1	3.302	2	H										
65	2	1	3.485	1	V	ROSK									
65	3	1	3.389	2	H										
65	4	1	3.917	1	V	ROSK									
65	5	1	3.137	2	H										
65	6	1	3.526	2	V										
65	7	1	3.500	2	H	ROST									
65	8	1	3.474	2	V	ROSK									
65	9	1	3.699	2	H										
65	10	1	3.898	1	V										
65	11	1	3.846	1	H										
65	12	1	3.581	2	V										
65	13	1	3.568	1	H										
65	14	1	3.080	1	V										
65	15	1	3.365	2	H										
87	1	1	3.463	2	H										
87	2	1	3.386	1	V										
87	3	1	3.327	1	H										
87	4	1	3.364	1	V	SURB									
87	5	1	3.410	1	H										
87	6	1	3.561	2	V										
87	7	1	3.171	2	H										
87	8	1	3.336	2	V										
87	9	1	3.467	1	H	SURB									
87	10	1	3.485	1	V										
87	11	1	3.251	2	H										
87	12	1	3.842	1	V										
87	13	1	3.436	1	H										
87	14	1	3.733	1	V	SURB									
87	15	1	3.495	1	H										
87	16	1	3.536	1	V										
87	17	1	3.649	1	H										
87	18	2	.	.											
106	1	1	3.280	1	V										
106	2	1	3.152	2	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=1200 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
106	3	1	3.173	2	V	ROVE									
106	4	2	.	.											
106	5	1	3.158	1	H	SURB									
106	6	1	3.124	1	V										
106	7	1	3.044	2	H										
106	8	1	2.807	1	V	ROPB	ROST								
106	9	1	3.079	2	H	ROST									
106	10	1	2.885	2	V										
106	11	1	3.341	1	H	ROPB									
106	12	1	3.090	2	V										
106	13	1	2.789	2	H										
106	14	1	3.056	2	V	ROST									
106	15	1	3.000	2	H										
106	16	1	3.238	1	V	DIUR	ROVE								
106	17	1	3.006	1	H	ROST	MAST	ROVE							
151	1	1	3.054	2	H										
151	2	1	3.116	2	V										
151	3	1	3.145	2	H										
151	4	1	3.439	2	V										
151	5	1	3.608	2	H	SURB									
151	6	1	3.506	1	V										
151	7	1	3.469	1	H										
151	8	1	2.773	2	V	ROSK									
151	9	1	3.318	1	H										
151	10	1	3.064	2	V										
151	11	1	3.405	1	H										
151	12	1	3.226	2	V										
151	13	1	3.524	1	H										
151	14	1	2.913	2	V	ROSK									
151	15	1	3.557	1	H										
157	1	4	.	.											
157	2	4	.	.											
157	3	4	.	.											
157	4	4	.	.											
157	5	4	.	.											
157	6	4	.	.											
157	7	4	.	.											
157	8	4	.	.											
157	9	4	.	.											
157	10	4	.	.											
157	11	4	.	.											
157	12	4	.	.											
157	13	4	.	.											
157	14	4	.	.											
169	1	4	.	.											
169	2	1	3.215	2	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=1200 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
169	3	1	3.584	2	H										
169	4	1	3.674	1	V	ROVE									
169	5	1	3.126	1	H	ROST									
169	6	1	3.118	2	V										
169	7	1	3.413	2	H										
169	8	1	3.580	1	V										
169	9	1	2.556	1	H	ROPB	ROST	ROPH							
169	10	1	3.364	1	V										
169	11	1	3.102	1	H	ROST									
169	12	1	3.181	2	V										
169	13	1	3.548	2	H										
169	14	1	3.350	2	V										
169	15	1	3.415	2	H										
169	16	1	3.468	1	V	ROSK									
169	17	1	3.375	1	H										
169	18	1	3.588	2	V	ROSK									
170	1	1	2.797	1	V	DIUR									
170	2	1	3.384	1	H										
170	3	1	3.007	2	V										
170	4	1	3.294	1	H										
170	5	1	3.503	1	V										
170	6	1	3.335	2	H	SURB									
170	7	1	3.331	1	V	DIUR									
170	8	1	3.183	1	H										
170	9	1	3.247	2	V										
170	10	2	.	.											
170	11	1	3.187	2	H										
170	12	1	3.453	1	V										
170	13	1	3.284	2	H										
170	14	1	3.394	2	V	SURB									
170	15	1	3.327	2	H										
170	16	4	.	.											
170	17	1	3.780	1	V										
170	18	1	3.347	2	H										
170	19	1	3.537	1	V										
170	20	1	3.372	2	H										
170	21	1	3.447	1	V	ROVE									
170	22	1	4.032	1	H										
218	1	1	3.591	1	V										
218	2	1	3.681	2	H										
218	3	1	3.805	1	V										
218	4	1	3.620	2	H										
218	5	2	.	.											
218	6	1	3.606	2	V										
218	7	1	3.413	2	H										
218	8	1	3.727	2	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=1200 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
218	9	1	3.613	2	H										
218	10	1	3.647	2	V										
218	11	1	3.911	1	H										
218	12	1	3.544	2	V										
218	13	1	3.600	1	H										
218	14	1	3.381	2	V										
218	15	1	3.723	2	H	ROVE									
247	1	1	3.570	1	H										
247	2	1	3.253	2	V										
247	3	1	3.654	1	H										
247	4	1	2.784	2	V										
247	5	4	.	.											
247	6	1	3.430	2	H										
247	7	1	3.331	1	V										
247	8	1	3.541	2	H										
247	9	1	3.533	2	V										
247	10	1	3.515	2	H										
247	11	1	2.883	1	V										
247	12	1	3.280	2	H										
247	13	1	3.829	2	V										
247	14	1	3.585	1	H	ROVE									
247	15	1	3.562	2	V										
263	1	1	3.793	1	V	DIUR	RURB								
263	2	1	3.431	2	H										
263	3	1	3.602	1	V										
263	4	1	3.527	2	H										
263	5	1	3.522	2	V	ROSK	RURB	ROVE							
263	6	1	3.304	2	H										
263	7	1	3.538	2	V	ROSK									
263	8	1	3.820	1	H										
263	9	1	3.653	2	V										
263	10	1	3.884	1	H										
263	11	1	3.859	1	V										
263	12	1	3.568	2	H										
263	13	1	3.710	1	V										
263	14	1	3.672	2	H										
282	1	1	3.489	1	V										
282	2	1	3.710	1	H										
282	3	1	3.770	2	V	DIUR									
282	4	1	3.736	2	H										
282	5	1	3.653	2	V										
282	6	1	3.821	2	H										
282	7	1	3.652	2	V	DIUR									
282	8	1	3.909	1	H										
282	9	1	3.980	1	V	DIUR									
282	10	1	3.980	1	H										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

TMT=1200 ppm Acetonitrile (continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
282	11	1	3.477	1	V	DIUR									
282	12	1	3.339	2	H										
282	13	1	3.590	2	V										
282	14	1	3.524	2	H										
282	15	1	3.352	2	V										
282	16	1	3.614	2	H										
282	17	1	3.939	1	V	DIUR									
311	1	1	3.352	1	H										
311	2	1	3.231	1	V										
311	3	1	3.121	1	H	ROST									
311	4	1	3.182	1	V	ROST									
311	5	1	3.098	1	H	ROPB									
311	6	1	3.635	1	V	ROSK									
311	7	1	3.044	2	H	ROPH									
311	8	1	3.196	2	V										
311	9	1	2.955	2	H										
311	10	1	3.181	1	V	ROST									
311	11	1	3.106	2	H	ROVE									
311	12	1	3.470	1	V	ROSK									
311	13	2	.	.											
311	14	1	3.349	1	H										
311	15	1	3.201	2	V										
311	16	1	3.160	2	H										
311	17	1	3.554	1	V										
311	18	1	2.944	2	H										
340	1	1	3.316	2	V	ROST									
340	2	1	3.150	2	H	ROST	ROVE								
340	3	1	3.784	1	V	ROVE									
340	4	1	4.060	1	H										
340	5	1	3.598	2	V										
340	6	1	3.006	1	H	ROST	ROPB								
340	7	1	3.664	2	V	ROVE									
340	8	1	3.080	2	H	ROST									
340	9	1	3.439	2	V										
340	10	1	3.773	1	H	ROST									
340	11	1	3.397	1	V	ROPB									
340	12	1	3.395	2	H	ROST									
340	13	1	3.616	1	V	ROVE	ANOR								
340	14	1	3.463	2	H										
340	15	1	3.556	2	V										
340	16	1	3.714	2	H	ROVE									
340	17	1	3.599	2	V	ROVE									
340	18	1	3.770	1	H										
350	1	1	3.926	1	V										
350	2	1	3.761	2	H										
350	3	1	3.459	2	V										

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=1200 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
350	4	1	3.806	1	H										
350	5	1	3.624	2	V										
350	6	1	3.713	2	H										
350	7	1	3.545	2	V										
350	8	1	3.704	2	H										
350	9	1	3.974	1	V										
350	10	1	3.946	2	H										
350	11	1	3.748	2	V										
350	12	1	3.633	2	H										
350	13	1	3.983	1	V										
350	14	1	3.956	1	H										
350	15	1	3.987	1	V										
355	1	1	3.705	1	H										
355	2	1	3.583	2	V	DIUR	SURB								
355	3	1	3.453	2	H										
355	4	1	3.603	1	V	DIUR									
355	5	1	3.700	2	H										
355	6	1	3.766	2	V	DIUR									
355	7	1	3.278	1	H										
355	8	1	3.489	2	V										
355	9	1	3.784	2	H										
355	10	1	3.796	1	V	DIUR	SURB								
355	11	1	3.510	2	H										
355	12	1	3.531	1	V										
355	13	1	3.614	1	H										
355	14	1	3.694	1	V	DIUR									
358	1	1	3.550	1	V										
358	2	1	3.803	1	H										
358	3	2	.	.											
358	4	1	3.700	1	V	ROSK									
358	5	1	3.671	2	H										
358	6	1	3.739	1	V										
358	7	1	3.748	2	H										
358	8	1	3.673	1	V										
358	9	1	3.825	1	H										
358	10	1	3.693	1	V	ROSK									
358	11	1	3.841	1	H										
358	12	1	3.764	1	V	ROSK									
358	13	1	3.522	2	H										
358	14	1	3.444	2	V										
358	15	1	3.693	1	H										
358	16	2	.	.											
361	1	1	3.666	1	H										
361	2	1	3.608	1	V	SURB									
361	3	1	3.630	1	H										
361	4	1	3.402	2	V	ROSK	SURB								

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=1200 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
361	5	1	3.108	2	H										
361	6	1	3.418	1	V										
361	7	1	3.220	2	H	ROST									
361	8	1	3.470	1	V	ROST									
361	9	1	3.054	1	H										
361	10	1	3.655	1	V										
361	11	1	3.013	2	H	ROST									
361	12	1	2.597	2	V	ROSK	ROST								
361	13	1	3.272	2	H										
361	14	1	3.667	1	V										
361	15	1	3.508	2	H										
361	16	1	3.188	2	V										
362	1	1	2.833	2	V	ROSK	ROST	ROPB							
362	2	1	3.533	1	H										
362	3	1	3.071	2	V	ROVE									
362	4	1	3.096	1	H	ROST	SURB								
362	5	1	3.188	2	V										
362	6	1	3.513	1	H	ROPB									
362	7	1	3.515	2	V	ROSK									
362	8	1	3.041	2	H	SURB									
362	9	1	2.883	1	V										
362	10	1	3.603	2	H										
362	11	1	3.124	1	V										
362	12	1	3.404	1	H										
362	13	1	3.381	2	V	ROSK	ROST								
362	14	1	3.323	1	H										
362	15	1	3.066	2	V	ROSK									
362	16	1	3.267	2	H	ROST									
362	17	1	3.548	1	V										
362	18	1	3.020	2	H	ROST									
362	19	1	3.296	1	V										
363	1	1	3.794	1	V	DIUR									
363	2	1	3.744	1	H										
363	3	1	4.056	1	V										
363	4	4	.	.											
363	5	1	3.855	1	H										
363	6	2	.	.											
363	7	1	3.402	2	V										
363	8	2	.	.											
363	9	1	3.638	2	H										
363	10	1	3.594	1	V										
363	11	1	3.716	1	H										
363	12	1	3.876	1	V	DIUR	ROPB								
363	13	1	3.304	2	H										
363	14	1	3.786	1	V										
363	15	1	3.788	1	H	SURB									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

Inhalation Developmental Toxicity Study of Acetonitrile in Rodents: Raw Rat Fetal Data

TMT=1200 ppm Acetonitrile
(continued)

Matno	Site	Status	Fetal Wt(g)	Sex	Head or Visceral	Abn 1	Abn 2	Abn 3	Abn 4	Abn 5	Abn 6	Abn 7	Abn 8	Abn 9	Abn 10
363	16	1	3.433	2	V	DIUR									

Status: 1 = Live; 2 = Early Resorption; 4 = Late Resorption; 5 = Dead
Sex: Male = 1; Female = 2;

APPENDIX E

PROTOCOL AND CAGE MAPS

Study Protocol and Revisions
Cage Maps

Study Protocol and Revisions

STUDY PROTOCOL

Inhalation Developmental Toxicity Study of

Acetonitrile in Rats

Submitted to:

Dr. Bernard Schwetz
National Toxicology Program
National Institute Environmental Health Sciences
Research Triangle Park, NC

Submitted by:

Dr. Terryl J. Mast
Battelle - Pacific Northwest Laboratory
Richland, WA 99352

November 16, 1990

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INHALATION REPRODUCTIVE TOXICOLOGY STUDY PROTOCOL

ACETONITRILE

I. TITLE: Teratology Study of Acetonitrile in Rats

II. PURPOSE OF STUDY

Acetonitrile is used primarily as a solvent in extractive distillation and crystallization of pharmaceutical and agricultural products including vitamins, steroids, bactericides, insecticides, plant growth regulators and fungicides. It is used also as a catalyst in chemical reactions. Because acetonitrile is also produced commercially as a minor byproduct in the synthesis of acrylonitrile, its estimated annual production (80 million pounds) is much higher than the total U.S. market (<10 million pounds). The excess portion of acetonitrile is disposed of by the manufacturer. It is estimated that 26,000 workers may be exposed to acetonitrile. The volatile nature of acetonitrile dictates that the inhalation route will be the most likely route of human exposure in the workplace; dermal exposure may also be a significant route.

This study will determine the potential for inhaled acetonitrile to cause developmental toxicity in the CD (Sprague-Dawley) rat, and to more accurately assess the hazards of gestational exposure to acetonitrile.

Table 1. Summary of Physical and Chemical Properties of Acetonitrile.

Synonym:methyl cyanide, cyanomethane, and ethane nitrile		MW:	41.05
		CAS No.:	75-05-8
Molecular Formula:	CH_3CN	TLV:	40 ppm

III. SPONSOR AND SPONSOR'S REPRESENTATIVE

A. Sponsor:
National Institute of Environmental Health Sciences
National Toxicology Program (NTP)
P.O. Box 12233
Research Triangle Park, N.C. 27709

B. Sponsor's Representative:
Dr. Bernard Schwetz

IV. TESTING LABORATORY

A. Facility
Battelle - Pacific Northwest Laboratory (PNL)
P.O.Box 999; Richland, Washington 99352

B. Study Director:
Dr. Terryl J. Mast

V. PROPOSED SCHEDULE OF EVENTS **(This proposed schedule may be altered. All changes will be appended to the protocol.)**

[
A. Order animals: 9/28/90
B. Animals arrive week of: 10/22/90
C. Identification of females week of: 11/12/90
D. Health screen: 11/12/90
E. Prestart audit for GLP compliance: 11/21/90
F. Initiate breeding procedures: 11/26/90
G. Initiate exposure on dg 6: 12/3/90
H. Collect maternal blood week of: 12/3/90
I. Complete exposure: 12/19/90
J. Collect maternal blood week of: ~~12/15/90~~
I. Initiate sacrifice: ~~12/17/90~~
J. Complete fetal specimen evaluation: 2/18/91
K. Submit draft report: 5/8/91
L. Submit final report: 45 days after receipt of reviewers' comments

A. Order animals: 11/21/90
B. Animals arrive week of: 1/7/91
C. Identification of females week of: 1/22/91
D. Health screen: 1/28/91
E. Prestart audit for GLP compliance: 1/28/91
F. Initiate breeding procedures: 1/29/91 ⁶A
G. Initiate exposure on dg 6: 2/5/91
H. Collect maternal blood week of: 2/4/91
I. Complete exposure: 2/21/91
J. Collect maternal blood week of: 2/16/91
K. Initiate sacrifice: 2/19/91
L. Complete fetal specimen evaluation: 4/19/91
M. Submit draft report: 7/8/92
N. Submit final report: 45 days after receipt of reviewers' comments]

VI. TEST SYSTEM

A. Species: Rats

B. Strain: Sprague-Dawley [CrI:CD(SD)BR]

C. Number of Animals and Supplier:

Charles River Breeding Laboratories, Raleigh, NC.
90 males
360 females

D. Age of Animals Upon Arrival: 7-8 weeks

E. Experimental Animals (Females): The females will be mated by placing [~~at~~]⁻ᴬ 1 to 4 females with one male overnight in a breeding cage (ØB-DT-3BØD). Nine AM of the day that copulation is established will be designated as 0 dg (ØB-DT-3BØD). Forty non-pregnant female rats will be randomly selected and assigned to four dose groups (10/group) from the remaining female pool (ØB-DT-3BØB).

⁶A Changed 6/4/92 by Amendment A.
⁻A Deleted 6/4/92 by Amendment A.

F. Number of Animals in Study:

	Species		Sex		Animals		Treatment Groups		Total
Sperm-positive:									
Teratology	1	x	1	x	25-30	x	4	=	100-120
Blood Analyses	1	x	1	x	10	x	4	=	40
Non-pregnant	1	x	1	x	10	x	4	=	40
							Total	=	180-200

VII. EXPERIMENTAL DESIGN AND DOSE LEVELS

A. Experimental Design: Four groups of mated female rats will be exposed to the test chemical on 14 consecutive days (6-19 dg). The rats will be necropsied on 20 dg for maternal and fetal evaluations.

In addition, 10 non-pregnant females will be added to each exposure group for the purpose of comparing pregnant and non-pregnant animals. These animals will be exposed concurrently with the mated females and sacrificed immediately after the last exposure period.

In addition, 10 sperm-positive females in each exposure group will be used for blood acetonitrile and cyanide analyses (SOP# ØB-AC-3A3P). Blood will be collected by [~~eye bleeding~~ intracardiac puncture][6A] (SOP# ØB-CP-3EØ1) within 30 minutes postexposure on 8 and 18 dg (the 3rd and 13th days of exposure).

B. Exposure Regimen: Chamber atmospheric concentrations of acetonitrile will be 0 (filtered air), 100, 400 and 1200 ppm, 6 hr/day, 7 days/week. The exposure chamber doors will be closed throughout the exposure and non-exposure periods, except during animal care procedures. Exposure chamber temperatures will be maintained at 75±3°F and relative humidities at 55±15%. Airflow will be maintained at 15±3 cfm and the chamber pressure at approximately 1" water negative with respect to room pressure.

C. Selection of Atmospheric Concentrations: Exposure chamber concentrations are based on results from subchronic and chronic toxicology studies of acetonitrile in rats sponsored by NTP and conducted at Battelle, Pacific Northwest Laboratory. Selected concentrations were approved by the sponsor.

VIII. TEST SYSTEM HOUSING, HANDLING AND ENVIRONMENTAL CONDITIONS

A. Quarantine and Acclimatization (ØB-AR-3FØ3)

1. Animal shipping crates will be examined upon arrival for evidence of conditions likely to permit exposure to pathogens (soiled, wet or otherwise damaged).

2. The uncrating will be conducted at the door of the quarantine room after boxes have been wiped with [~~MicroQuat~~ Anafaside][6A]. While being removed from the crates the animals will be examined for evidence of shipping stress.

3. The animals will be quarantined and acclimatized in the LSL-II building for 3-4 weeks prior to the start of the study.

4. During the first 2-3 weeks of the quarantine/acclimatization period the animals will be housed by sex, approximately 5 rats per cage in wire cages on flush racks. During the last 2-3 weeks they will be individually caged on wire racks. The cage space will meet the requirements stated in the NIH "Guide for Care and Use of Laboratory Animals".

[6A] Changed 6/4/92 by Amendment A.

5. During the breeding period the animals will be housed in the quarantine room.

6. Sperm-positive rats will be acclimated from 0-5 dg in individual compartments of wire-mesh cages within exposure chambers (with chamber doors open). The 40 non-pregnant study females will be acclimated prior to exposure under the same conditions.

7. Room temperature during the acclimatization and exposure periods will be maintained at 75±3°F and relative humidity at 55±15%. These measurements will be recorded at least twice daily.

8. Twelve hours light and twelve hours dark will be maintained with light starting at 0600.

9. Five male and five female animals will be randomly selected for pre-exposure health screening (ØB-AR-3FØ2). They will be examined by gross necropsy, histopathology [~~and~~ ,]&A nasopharyngeal culture [and serologic testing]+A for evidence of disease and the presence of potentially pathogenic organisms.

10. The clinical veterinarian will make a visual inspection of the animals to be used in the study just prior to their release for the study (documented on the last quarantine/acclimatization record).

11. [~~As an added screen for viral infection~~ In order to confirm that viral infection did not occur during the study]&A, 5 animals from the control group and 5 animals from the highest dose group will be tested promptly after sacrifice at PNL for [antibodies to]+A viral pathogens (ØB-AR-3B1R).

12. Females not selected for the study or health screen and the males will be discarded during the first exposure week. The disposition of these females will be recorded on the Animal Disposition Record and retained in the study files (ØB-AR-3FØ3).

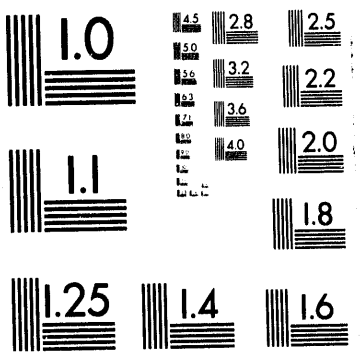
B. Inhalation Chamber Housing and Sanitation Procedures

The exposure chamber doors will be closed throughout the exposure and non-exposure periods, except during animal care procedures. Exposure chamber temperatures will be maintained at 75±3°F and relative humidities at 55±15%. Airflow will be maintained at 15±3 CFM and the chamber pressure at approximately 1" water negative with respect respect to room pressure.

1. NTP-approved untreated cageboard (Techsorb®, Shepherd Specialty Papers, Kalamazoo, MI) will be put in the excreta pans to reduce ammonia levels after the completion of exposure on each exposure day (ØB-AR-3FØA).

2. The soiled cageboard will be removed from the excreta pans during morning animal husbandry procedures and placed in plastic bags for proper disposal. The excreta pans will be hosed with water before being transported to the cage wash area for daily cleaning.

&A Changed 6/4/92 by Amendment A.
+A Added 6/4/92 by Amendment A.


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0

3 of 3

3. The automatic watering systems will be checked daily during animal care procedures to ensure they are functioning properly.

4. Chamber and cage units in use will be changed and washed every 7 days (ØB-AR-3BØ3).

5. Exposure chambers, with animal housing components (cage units, feeders, automatic watering lines and excreta pans), will be moved as a unit with the chamber doors closed to the wash area through the regulated (dirty) corridor.

6. The automatic water lines in the cage racks and chambers will be flushed with 180-190°F water for a minimum of 1 minute prior to being washed in the cage washer (ØB-AR-3GØ1).

7. The entire chamber unit will be washed in a cage washer.

8. The clean chamber unit will be stored in a clean holding area. The individual cage lixits will be checked before the chamber is moved back through the clean corridor to the exposure room.

C. Feed (ØB-AR-3FØ5)

1. NTP pre-approved NIH-07 Open Formula Diet (pellets) from Ziegler Bros., Inc., Gardner, PA will be used during the quarantine/acclimatization periods and throughout the duration of the experiment.

2. Feed will be provided *ad libitum* in slot feeders during the experiment, except during exposure hours.

C. Water

1. Fresh softened water (ion exchange softener, Illinois Water Treatment Company, Model 2R-2240, Rockford, IL) will be supplied *ad libitum* at all times. The hardness of the water will be checked approximately once every week. Records will be retained in the LSL-II building Engineer's office.

2. The automatic watering system (Edstrom Industries, Waterford, WI) will be used for the quarantine/acclimatization period and throughout the duration of the study.

3. A representative sample of animal drinking water from one of the NTP study rooms will be analyzed for contaminants at least once each calendar year.

D. Randomization: On the day of sperm detection (0 dg), the mated animals will be weighed and assigned to dose groups based on the body weights. Their weights will be ranked from lightest to heaviest and each animal randomly assigned to a treatment group by means of a computer-assisted randomization program which is based on a single blocking factor, body weight (ØB-DT-3BØB). On the last day of breeding, 40 non-pregnant females will be randomly chosen by tail tattoo number and then assigned to dose groups as above.

E. Identification (ØB-AR-3FØF)

1. All female animals will be individually identified by tail tattoo during the first weighing session.

2. Cage maps (ØB-DT-3BØ3) showing placement of individual animals in each cage unit of the exposure chamber will be prepared and updated as needed. Each exposure chamber will be identified by chamber number and exposure level. The proposed arrangement of the exposure chambers is included in Figure 1.

IX. TEST ARTICLE

A. Test Article:

1. Chemical name:	Acetonitrile
2. Formula:	CH_3CN
3. CAS No:	75-05-8
4. PNL Assigned Lot No.:	BNW53438-3 (Bottles 1-16)
5. Manufacturer:	J.T. Baker Chemical Co. Phillipsburg, NJ 08865
6. Vehicle Control:	Filtered air
7. Storage conditions:	The bulk test material will be stored in the chemical transfer and storage facility immediately adjacent to the Life Sciences Laboratory-II building. The bulk test material will be maintained at ~20°C in the original amber glass bottles. A nitrogen headspace will be maintained in each bottle.

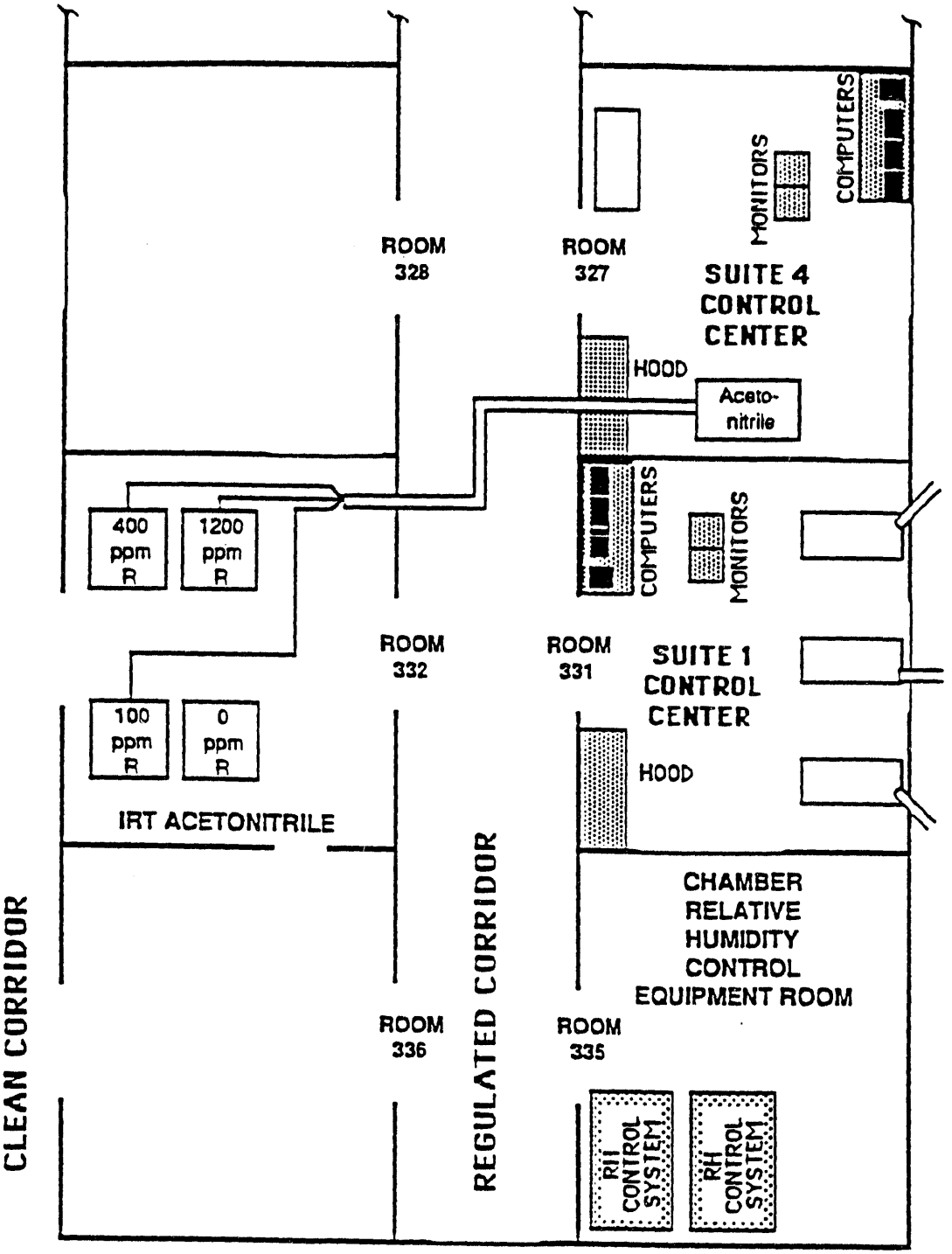


FIGURE 1. Schematic of the Acetonitrile Exposure Suite.

B. Bulk Assay Procedure (ØB-AC-3A11)

1. The manufacturer's assay results follow:

Manufacturer Lot#	D01103	D14109	D14082
BNW Assigned Lot#	53438-3-1 to 4	53438-3-5 to 8	53438-3-9 to 16
Manufacturer Analysis			
Assay Purity (by GC)	100.0%	100.0%	100.0%
Ultraviolet Absorbance (1.00 cm path vs Water)			
200 nm	0.01	0.03	0.04
220 nm	0.007	0.009	0.010
254 nm	<0.002	<0.002	<0.002
280 nm	<0.002	<0.002	<0.002
UV Cutoff, nm	189	189	189
Fluorescent Trace Impurities (as quinine base in ppb)			
Measured at 450 nm	0.1	0.1	0.1
Measured at Emission Maximum for Solvent Impurities	0.2	0.2	0.2
Titratable Acid (meq/g)	0.0003	0.0002	0.000003
Titratable Base (meq/g)	<0.00006	<0.0001	0.0002
Residue after Evaporation, ppm	0.2	0.3	0.8
Water (by Coulometry),	<0.003	0.01	0.01
Refractive Index	1.3435	1.3435	1.3434

2. PNL will confirm the identity by using infrared spectroscopy and determine the purity by using gas chromatography.

3. PNL Assay Conclusions: Infrared spectroscopy confirmed the identity of BNW# 53438-3 (bottles 1-16) as acetonitrile. Gas Chromatographic purity analyses are in progress at this time.

C. Analysis Schedule

1. The identity and purity of the test material will be determined upon receipt. The purity will be performed within one month of the start of animal exposures.

2. The stability of the test material in the generator will be characterized prior to the start of the study.

3. The stability of the test material in the highest and the lowest concentration exposure chambers will be characterized prior to the start of the study. This characterization will be repeated with occupied chambers following the initiation of animal exposures.

D. On-Line Gas Chromatographic (Hewlett-Packard 5840) Chamber
(ØB-AC-3B1K, ØB-AC-3CØS)

Detector:	Flame Ionization
Column:	1' x 1/8" OD Nickel
Packing:	Porapak Q, 80/100 mesh
Column and Valve Temperature:	100°C
Carrier:	Nitrogen
Carrier Flow:	20-30 ml/min
Sample Valve:	Hastelloy-C mounted in valve oven (Valco)
Sample Loop:	1-ml Nickel
Stream Select Valve:	12-port, Hastelloy-C mounted in column oven (Valco)
Chromatographic Run Time:	Approximately 1.5 min exclusive of report generation.
On-Line Standard:	Acetonitrile in nitrogen will be used to detect drift of the on-line monitor.
Calibration Frequency:	Daily check against on-line standard, additional checks against grab samples as indicated by the on-line standard. A full recalibration will be performed at least once during the study.
Control Range:	Target concentration ±10%
Critical Limits:	Target concentration ±20%, alarmed (will be automatically shut off if upper limit is reached).
Monitoring Frequency:	Each chamber and the room will be monitored approximately once every 30 minutes.

X. DESCRIPTION OF INHALATION EXPOSURE SYSTEM

The inhalation chambers will be located in room 332 of the LSL-II building. This room will be exclusively dedicated to the testing of acetonitrile. A detailed description of the inhalation exposure system to be used in this study is included in Attachment 2 of this protocol. The location of the exposure room and chamber layout are shown in Figure 1.

A. Environmental Monitoring

1. Air filtration: HEPA and charcoal filters will be used to condition intake air. A HEPA filter will be used to treat exhaust air. New intake and exhaust filters will be installed prior to the start of the study.

2. Temperatures will be monitored by resistance temperature detectors (RTDs) multiplexed to a digital thermometer with computer data acquisition recording values at ~4-hour cycles for 24 hours per day. The control range is 75±3° F with critical limits, <70 or >80° F. Any chamber temperature excursion beyond the critical limits will be recorded and alarmed automatically.

3. Relative humidity will be monitored by a single dew point hygrometer in conjunction with a multiplexed sampling system with computer data acquisition at ~4-hour cycles, 24 hours per day. The control range is 55±15% with critical limits of <35% or >75%. Any relative humidity excursion beyond the critical limits will be recorded and alarmed automatically.

4. Chamber airflow will be monitored at an exhaust orifice using a multiplexed Validyne pressure transducer system with computer data acquisition at approximately 4-hour cycles, 24 hours per day. The control range is 15±3 air changes/hour (~15±3 CFM) with critical limits of <10 CFM or >20 CFM. Any chamber flow excursion beyond critical limits will be recorded and alarmed automatically. Acetonitrile concentrations in the chambers will be primarily controlled by the automatic adjustment of chamber airflow.

5. Chamber vacuum will be monitored using a multiplexed Validyne pressure transducer system with computer data acquisition at ~4-hour cycles, 24 hours per day. The control range is -0.2 to -2.0 inches of water pressure with critical limits set at the same values. Any chamber vacuum excursion beyond the critical limits will be recorded and alarmed automatically. If chamber vacuum exceeds the limits of -0.2 inch of water, the chamber valve will be automatically turned off.

6. Uniformity of the concentration of the test chemical in each of the chambers (i.e., chamber balance) will be determined during the development of exposure generation without animals, at the during the first week of the study with the animals in the chambers. A between port and within port variability of ≤5% relative standard deviation (RSD) will be considered acceptable.

7. The exposure chambers test chemical buildup and decay times will be determined prior to start of the study and once during the study to determine T_{90} and T_{10} curves.

8. Persistence of acetonitrile in the high dose chamber after the end of an exposure day will be determined prior to the start of the study and at the beginning of the study with animals in the chamber. The time to decay to 1% of the initial concentration will be determined as well as an estimate of the time-weighted average concentration in the chamber for an 18-hour period following completion of animal care activities.

9. Prior to the start of the study, samples will be taken from all chambers using a Gardner condensation nuclei counter to assure that the vapor generation did not produce an aerosol of acetonitrile in the unoccupied chambers. During the first week of the study with animals, samples will be taken from all chambers and compared with the control chamber.

B. Effluent Treatment (ØB-AC-3A1L)

Chamber exhaust will be mixed with building air to comply with applicable State and Federal Regulations prior to release from the building exhaust stack. The building exhaust stack will be monitored once during the study to prove efficiency of the effluent treatment.

XI. EXPERIMENTAL OBSERVATIONS

A. Clinical Observations: The animals will be observed daily for mortality, morbidity, and signs of toxicity (ØB-DT-3BØ3). The date and time of death or euthanasia of moribund animals will be recorded and the animals will be necropsied according to (ØB-DT-3BØF).

B. Body Weights: All females will be weighed prior to mating. Sperm-positive rats will be weighed on 0, 6, 10, 14, 17, and 20 dg. Non-pregnant females will be weighed on the 1st, 5th, 10th day of exposure and at sacrifice.

C. Scheduled Necropsy: The rats are scheduled to be euthanitized with CO_2 on 20 dg. At necropsy (ØB-DT-3BØG) maternal animals will be weighed and examined for gross tissue abnormalities. To document the presence of lesions which may be due to chemical exposure, any organs or tissues with lesions will be preserved in neutral buffered formalin (NBF); in this case, comparable organs or tissues from approximately 20% of the control animals will be preserved in NBF; all other tissues will be discarded. The gravid uterus will be removed and weighed, and the number, position and status of implants will be recorded. The placentas will be examined. The identity of live fetuses (by study, dam number and uterine position) will be retained throughout all examinations and archiving. Live fetuses will be examined for gross defects and weighed. A complete visceral examination will be performed on 50% of all fetuses and on any fetuses with gross abnormalities (ØB-DT-3BØG). Sex will be determined on all fetuses by internal examination. All skeletons will be double-stained and examined for cartilage formation and centers of ossification (ØB-DT-3BØG). Approximately 50% of the fetal heads will be examined by razor-blade sectioning of fixed preparations (ØB-DT-3BØI). Records of morphologic lesions observed in gross and visceral examinations will include photographs of representative lesions.

D. Indices of Effects: The following parameters, expressed as mean ± SE, when appropriate, will be computed from data for inseminated animals and their litters in each treatment group and will be presented in the Final Report:

- Number of dead maternal animals, animals removed from the study and reason for removal
- Summary of maternal toxicity, including incidence of changes detected during clinical observations
- Number and percent pregnant
- Maternal body weights:
  Rats on 0, 6, 10, 14, 17, and 20 dg
- [• Nonpregnant female body weights:
  Rats on exposure days 1, 5, 10, and sacrifice][+A]
- Weight of gravid uterus
- Extragestational weight and weight gain
- Number of implantation sites/litter
- Number of litters with live fetuses
- Number and percent of live fetuses/litter
- Body weight of live fetuses/litter
- Body weight of male and female fetuses/litter
- Sex ratio of fetuses/litter
- Number and percent of early and late resorptions/litter
- Number and percent of non-live/litter (early and late resorptions and dead fetuses)
- Listing of malformations and variations observed in fetuses/litters
- Number and percent of malformed fetuses
- Number and percent of litters with malformed fetuses
- Maternal blood acetonitrile and cyanide levels within 30 minutes postexposure on 8 and 18 dg (exposure days 3 and 13)
- Maternal liver, kidneys, and adrenal weights
- [• Nonpregnant female liver, kidneys, and adrenal weights][+A]

XII. PROPOSED STATISTICAL METHODS

The methods proposed for the statistical analyses of representative maternal, reproductive and fetal indices of effects are: summary statistics, N, mean, standard deviation, with accompanying ANOVA based on multiple comparisons where appropriate. Arc sin transformations will be performed on data presented as percent incidence. Further statistical analyses may be performed at discretion of sponsor.

[+A] Added 6/4/92 by Amendment A.

XIII. **RECORDS RETENTION** (ØB-9A-3EØ6)

Records that accumulate during the study will be retained at PNL until requested and shipped to NTP archives. Some of these records may be

presented as part of the protocol or reports. These will include but not be limited to the following records:

A. Personnel Records

1. List of PNL personnel participating in the study.

2. Name, address, and function of any outside consultant(s)

3. Record of removal of any individual from direct contact of the test system due to illness.

B. Health and Safety Records Chemical specific records will be submitted with the study. Facility specific records will be submitted annually.

1. Medical records of all personnel participating in the study. These records will be retained by Hanford Environmental Health Foundation (HEHF), P.O. Box 100, Richland, WA 99352 for a minimum of 40 years. A letter verifying this arrangement will be retained for each test material file.

2. Records and results of any biological monitoring on laboratory personnel (if applicable).

3. NTP Health and Safety Package for Acetonitrile.

4. PNL Biosafety Protocols and PNL Health and Safety Plan.

5. Chemical specific health and safety training records.

6. Waste disposal records.

7. Respiratory protection program with documentation of user training (specific fit testing if needed) for each type of respirator.

8. Building ventilation system, hoods and exhausting system monitoring records (pertinent to NTP studies).

9. Hanford Environmental Health Foundation (HEHF) formaldehyde sampling results.

10. Health and Safety Section of the Monthly Progress Reports.

11. Accident/injury reports for personnel involved in this study.

12. NTP site visit reports, attention items and related correspondence on health and safety.

C. Protocols

1. Approved and dated PNL study protocol.

2. Protocol amendments including NTP technical contract modifications which affect the study.

3. Documentation of any deviation from the protocol.

4. Documentation of any unforeseen circumstances that may affect the integrity of the study and corrective actions taken.

D. Test Material Records

1. Test material identity records including manufacturer, quantity, lot number(s), purity grade and date(s), etc.
2. NTP analytical contractor characterization reports.
3. NTP analytical contractor bulk stability reports.
4. NTP analytical contractor shipment records (if available).
5. PNL test chemical receipt records.
6. PNL storage records including storage conditions.
7. PNL bulk analysis and degradation records.
8. PNL method development records.
9. Chemical exposure generation system description and procedures.
10. Chamber concentration monitoring records, including GC tracings.
11. Uniformity (chamber balance) records.
12. Gas chromatograph calibration records.
13. Generation and chamber degradation study records.
14. PNL test material inventory and usage records.
15. Records of shipment to NTP repository of any unused test material.

E. Animal Records - Pretest

1. Animal receiving records including supplier, species, strain, birth week, sex, number of animals for each sex, receiving date and receiving conditions (photocopy of a representative animal shipping crate label).
2. Quarantine and acclimatization records.
3. Pretest health screening records and animal health notebook.
4. Randomization records.
5. Animal identification records.
6. Written release records from clinical veterinarian.
7. Disposal of excess animals.
8. Bedding type.

F. Animal Records - On Test

1. Exposure room location and chamber layout records.
2. Chamber cage map.
3. Cage type, rack type and the rotation scheme during study.
4. Cageboard type.
5. Type of watering system.
6. Body weight records.
7. Daily observation records
8. Clinical signs of toxicity records.
9. Serology data and reports.
10. Fetal specimen inventory records.

G. Feed

1. Feed tags with manufacturer, lot numbers and milling dates.
2. Feed analysis records as provided by NTP analytical contract laboratory.

H. Water

1. Annual water analysis.
2. Weekly water hardness check (records will be maintained in building engineer and/or building manager's office).

I. Quarantine Room, Exposure Room, and Inhalation Exposure Chamber Records

1. Exposure chamber description.
2. Exposure suite control center description.
3. Temperature raw data and daily and monthly summation reports.
4. Relative humidity raw data and daily and monthly summation reports.
5. Airflow raw data and daily and monthly summation reports.
6. Chamber vacuum raw data and daily and monthly summation reports.
7. Exposure system monitors calibration and maintenance records.
8. Description of the lighting system and light/dark regimen.
9. Sanitation procedures and pest control program.

J. All Relevant Correspondence

K. Reports

1. Monthly Progress Report.
2. Special study reports if any.
3. Incident reports (if applicable).
4. Final Study Report.

M. Internal Computer Generated Forms and Tables

1. Developmental toxicology results and statistical analyses.
2. Analytical chemistry results.
3. Exposure suite control center computer printouts.

XIV. OTHER SPECIFICATIONS

A. This study will be performed in compliance with the FDA Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies (21 CFR 58) except where deviations are required by the NTP January, 1990 General Statement of Work and subsequent modifications.

B. This Protocol will be the controlling document in case of discrepancies between the Protocol and SOPs. If this occurs the Study Director is to be notified immediately for clarification.

C. A list of all relevant Standard Operating Procedures (SOPs) for this study are present in Attachment 1.

XV. HEALTH AND SAFETY (ØB-HS-3S1E)

PNL's Health and Safety Plan (ØB-HS-3S1C) has been approved by NTP. In addition, a respiratory program is instituted. This is supplemented by using supplied-air respirators (ØB-HS-3S19) which will be worn by personnel during periods of animal care while the chambers are open, and by having available self-contained breathing breathing apparatus (ØB-HS-3SØW) for use when entering a room under emergency conditions following a leak.

XVI. APPROVAL BY PNL

Study Director Date: 11/16/90

Quality Assurance Auditor Date: 11/16/90

XVII. APPROVAL BY NTP

Study Officer Date: 26 Nov 90

XVIII. AMMENDMENTS/REVISIONS

6/4/92 AMENDMENT A

Page 2, Section V. Change: Proposed Schedule of Events.

Reason: Reschedule study start date due to animal procurement problems and correction of alphabetical ordering in schedule of events.

Effective Date: 11/21/90

Page 2, Section VI.E. Delete: "at".

Reason: Correction of typing error.

Effective Date: 11/26/90

Page 3, Section VII.A. Change: "eye bleeding" to "intracardiac puncture".

Reason: Incorrect statement.

Effective Date: 11/26/90

Page 3, Section VIII.A.2. Change: "MicroQuat" to "Anafaside".

Reason: Incorrect statement.

Effective Date: 11/26/90

Page 4, 9. Change: "and" to ",".

Page 4, 9. Add: "and serologic testing".

Reason: Clarification.

Effective Date: 11/26/90

Page 4, 11. Change: "As an added....infection" to "In order....study".

Page 4, 11. Add: "antibodies to"

Reason: Clarification

Effective Date: 11/26/90

Page 12. Add: "Nonpregnant female body weights....sacrifice".

Page 12. Add: "Nonpregnant female liver....weights."

Reason: Information was inadvertantly omitted during protocol preparation.

Effective Date: 11/26/90

Terry D. Mast 2/15/94
[signature/date]

[signature/date]

V. PROPOSED SCHEDULE OF EVENTS **(This proposed schedule may be altered. All changes will be appended to the protocol.)**

A. Order animals:	9/28/90
B. Animals arrive week of:	10/22/90
C. Identification of females week of:	11/12/90
D. Health screen:	11/12/90
E. Prestart audit for GLP compliance:	11/21/90
F. Initiate breeding procedures:	11/26/90
G. Initiate exposure on dg 6:	12/3/90
H. Collect maternal blood week of:	12/3/90
I. Complete exposure:	12/19/90
J. Collect maternal blood week of:	12/15/90
I. Initiate sacrifice:	12/17/90
J. Complete fetal specimen evaluation:	2/18/91
K. Submit draft report:	5/8/91

L. Submit final report: 45 days after receipt of reviewers' comments

VI. TEST SYSTEM

A. Species: Rats

B. Strain: Sprague-Dawley [CrI:CD(SD)BR]

C. Number of Animals and Supplier:

Charles River Breeding Laboratories, Raleigh, NC.
90 males
360 females

D. Age of Animals Upon Arrival: 7-8 weeks

E. Experimental Animals (Females): The females will be mated by placing at 1 to 4 females with one male overnight in a breeding cage (ØB-DT-3BØD). Nine AM of the day that copulation is established will be designated as 0 dg (ØB-DT-3BØD). Forty non-pregnant female rats will be randomly selected and assigned to four dose groups (10/group) from the remaining female pool (ØB-DT-3BØB).

F. Number of Animals in Study:

	Species		Sex		Animals		Treatment Groups		Total
Sperm-positive:									
Teratology	1	x	1	x	25-30	x	4	=	100-120
Blood Analyses	1	x	1	x	10	x	4	=	40
Non-pregnant	1	x	1	x	10	x	4	=	40
							Total	=	180-200

VII. EXPERIMENTAL DESIGN AND DOSE LEVELS

A. Experimental Design: Four groups of mated female rats will be exposed to the test chemical on 14 consecutive days (6-19 dg). The rats will be necropsied on 20 dg for maternal and fetal evaluations.

In addition, 10 non-pregnant females will be added to each exposure group for the purpose of comparing pregnant and non-pregnant animals. These animals will be exposed concurrently with the mated females and sacrificed immediately after the last exposure period.

In addition, 10 sperm-positive females in each exposure group will be used for blood acetonitrile and cyanide analyses (SOP# ØB-AC-3A3P). Blood will be collected by eye bleeding (SOP# ØB-CP-3EØ1) within 30 minutes postexposure on 8 and 18 dg (the 3rd and 13th days of exposure).

B. Exposure Regimen: Chamber atmospheric concentrations of acetonitrile will be 0 (filtered air), 100, 400 and 1200 ppm, 6 hr/day, 7 days/week. The exposure chamber doors will be closed throughout the exposure and non-exposure periods, except during animal care procedures. Exposure chamber temperatures will be maintained at 75±3°F and relative humidities at 55±15%. Airflow will be maintained at 15±3 cfm and the chamber pressure at approximately 1" water negative with respect to room pressure.

C. Selection of Atmospheric Concentrations: Exposure chamber concentrations are based on results from subchronic and chronic toxicology studies of acetonitrile in rats sponsored by NTP and conducted at Battelle, Pacific Northwest Laboratory. Selected concentrations were approved by the sponsor.

VIII. TEST SYSTEM HOUSING, HANDLING AND ENVIRONMENTAL CONDITIONS

A. Quarantine and Acclimatization (ØB-AR-3FØ3)

1. Animal shipping crates will be examined upon arrival for evidence of conditions likely to permit exposure to pathogens (soiled, wet or otherwise damaged).

2. The uncrating will be conducted at the door of the quarantine room after boxes have been wiped with MicroQuat. While being removed from the crates the animals will be examined for evidence of shipping stress.

3. The animals will be quarantined and acclimatized in the LSL-II building for 3-4 weeks prior to the start of the study.

4. During the first 2-3 weeks of the quarantine/acclimatization period the animals will be housed by sex, approximately 5 rats per cage in wire cages on flush racks. During the last 2-3 weeks they will be individually caged on wire racks. The cage space will meet the requirements stated in the NIH "Guide for Care and Use of Laboratory Animals".

5. During the breeding period the animals will be housed in the quarantine room.

6. Sperm-positive rats will be acclimated from 0-5 dg in individual compartments of wire-mesh cages within exposure chambers (with chamber doors open). The 40 non-pregnant study females will be acclimated prior to exposure under the same conditions.

7. Room temperature during the acclimatization and exposure periods will be maintained at 75±3°F and relative humidity at 55±15%. These measurements will be recorded at least twice daily.

8. Twelve hours light and twelve hours dark will be maintained with light starting at 0600.

9. Five male and five female animals will be randomly selected for pre-exposure health screening (ØB-AR-3FØ2). They will be examined by gross necropsy, histopathology and nasopharyngeal culture for evidence of disease and the presence of potentially pathogenic organisms.

10. The clinical veterinarian will make a visual inspection of the animals to be used in the study just prior to their release for the study (documented on the last quarantine/acclimatization record).

11. As an added screen for viral infection, 5 animals from the control group and 5 animals from the highest dose group will be tested promptly after sacrifice at PNL for viral pathogens (ØB-AR-3B1R).

12. Females not selected for the study or health screen and the males will be discarded during the first exposure week. The disposition of these females will be recorded on the Animal Disposition Record and retained in the study files (ØB-AR-3FØ3).

B. Inhalation Chamber Housing and Sanitation Procedures

The exposure chamber doors will be closed throughout the exposure and non-exposure periods, except during animal care procedures. Exposure chamber temperatures will be maintained at 75±3°F and relative humidities at 55±15%. Airflow will be maintained at 15±3 CFM and the chamber pressure at approximately 1" water negative with respect respect to room pressure.

1. NTP-approved untreated cageboard (Techsorb®, Shepherd Specialty Papers, Kalamazoo, MI) will be put in the excreta pans to reduce ammonia levels after the completion of exposure on each exposure day (ØB-AR-3FØA).

2. The soiled cageboard will be removed from the excreta pans during morning animal husbandry procedures and placed in plastic bags for proper disposal. The excreta pans will be hosed with water before being transported to the cage wash area for daily cleaning.

3. The automatic watering systems will be checked daily during animal care procedures to ensure they are functioning properly.

4. Chamber and cage units in use will be changed and washed every 7 days (ØB-AR-3BØ3).

- Number of dead maternal animals, animals removed from the study and reason for removal
- Summary of maternal toxicity, including incidence of changes detected during clinical observations
- Number and percent pregnant
- Maternal body weights:
  Rats on 0, 6, 10, 14, 17, and 20 dg
- Weight of gravid uterus
- Extragestational weight and weight gain
- Number of implantation sites/litter
- Number of litters with live fetuses
- Number and percent of live fetuses/litter
- Body weight of live fetuses/litter
- Body weight of male and female fetuses/litter
- Sex ratio of fetuses/litter
- Number and percent of early and late resorptions/litter
- Number and percent of non-live/litter (early and late resorptions and dead fetuses)
- Listing of malformations and variations observed in fetuses/litters
- Number and percent of malformed fetuses
- Number and percent of litters with malformed fetuses
- Maternal blood acetonitrile and cyanide levels within 30 minutes postexposure on 8 and 18 dg (exposure days 3 and 13)
- Maternal liver, kidneys, and adrenal weights

XII. PROPOSED STATISTICAL METHODS

The methods proposed for the statistical analyses of representative maternal, reproductive and fetal indices of effects are: summary statistics, N, mean, standard deviation, with accompanying ANOVA based on multiple comparisons where appropriate. Arc sin transformations will be performed on data presented as percent incidence. Further statistical analyses may be performed at discretion of sponsor.

XIII. RECORDS RETENTION (ØB-9A-3EØ6)

Records that accumulate during the study will be retained at PNL until requested and shipped to NTP archives. Some of these records may be

XVIII. AMMENDMENTS/REVISIONS

STANDARD OPERATING PROCEDURES FOR DEVELOPMENTAL TOXICOLOGY STUDIES

DEVELOPMENTAL TOXICOLOGY

ØB-DT-3BØ3	Cage Location Maps and Daily Observations
ØB-DT-3BØB	Randomization of Animals
ØB-DT-3BØD	Rodent Mating Procedures
ØB-DT-3BØF	Necropsies of Dead or Moribund Animals
ØB-DT-3BØG	Fetal Evaluations for Developmental Toxicity Studies
ØB-DT-3BØI	Examination of Fetal Heads Fixed in Bouin's Solution
ØB-DT-3BØY	Double Staining and Examination of Fetal Skeletons
ØB-DT-3FØ2	Shipping Developmental Toxicology Materials
ØB-HI-3GØ9	Operation of Sealer
ØS-SI-3EØ3	Data Transfer from Macintosh to VAX Using MacTerminal
ØB-CP-3EØ1	Specimen Handling

ANIMAL FACILITIES

ØB-AR-3BØ3	Handling and Changing Out Exposure Chambers and Cage Units
ØB-AR-3BØ8	Handling Escaped Small Animals
ØB-AR-3BØG	Barrier Procedures for LSL-II Animal Facility
ØB-AR-3B1R	Pathogen Monitoring
ØB-AR-3B1S	Monitoring for Bacterial Contamination in Animal Drinking Water
ØB-AR-3FØ2	Pre-Study Health Screening of Rodents
ØB-AR-3FØ3	Quarantine of Animals
ØB-AR-3FØ5	Management of Animal Feed
ØB-AR-3FØA	Daily Care of Bioassay Animals and Cleaning of Exposure Rooms
ØB-AR-3FØF	Rodent Identification by Tail Tattooing
ØB-AR-3GØ1	Pre-Cleaning Equipment and Operation of Equipment Washers
ØB-AR-3GØH	Animal Weighing using Toledo 8142 Automatic System
ØB-AR-3HØ1	Bi-Weekly Deep Cleaning of Exposure Rooms and Occupied Animal Rooms

INHALATION EXPOSURE AND BIOENGINEERING

ØB-BE-3B1X	Relative Humidity Determination Via Use of Dewpoint Hygrometer
ØB-BE-3B24	Inhalation Exposure Chamber Balance
ØB-BE-3B3H	Build-up and Decay and Overnight Concentration Monitoring
ØB-BE-3B4B	Acetonitrile IRT Exposure System Daily Operating Procedure
ØB-BE-3CØJ	EG&G Hygrometer: Operation, Maintenance, and Calibration
ØB-BE-3CØL	RTD Thermometer Calibration
ØB-BE-3CØV	Calibration and Check of Chamber Airflow Using Digital Anemometer
ØB-BE-3DØ6	Chamber Leak Test
ØB-BE-3DØE	Exposure Suite QC, Maintenance, and Calibration
ØB-BE-3DØG	Acetonitrile Exposure System QC, Maintenance and Calibration
ØB-BE-3EØB	Exposure Suite Data Analysis Program Operation
ØB-BE-3EØE	Exposure Suite Data Editing Program Operation
ØB-BE-3GØ4	Exposure Suite Routine Computer Operation
ØB-BE-3DØT	Acetonitrile IRT Generator Reservoir Cleaning

ANALYTICAL CHEMISTRY

ØB-AC-3A11	Bulk Analysis of Acetonitrile
ØB-AC-3A1L	Acetonitrile Analysis of Building Exhaust
ØB-AC-3CØS	Acetonitrile Calibration of Chamber Monitor
ØB-AC-3B1K	Operation of HP 5840 GC for Monitoring Acetonitrile
ØB-AC-3A3P	Determination of Cyanide in Blood

SAFETY

ØB-HS-3S19	The 3M Brand W-2860 Hardcap, Continuous-Flow Air Line Respirator
ØB-HS-3SØW	Scott 4.5 Self-Contained Breathing Apparatus (SCBA)
ØB-HS-3S1B	Bioassay Studies: Respiratory Protection Program
ØB-HS-3S1C	Bioassay Studies: Health and Safety Plan
ØB-HS-3S1E	Biosafety Protocol - Acetonitrile

NTP PROJECT OFFICE

ØB-QA-3EØA	Filling Out Data Sheets
ØB-9A-3EØ6	Data Handling and Storage of NTP Study Documents and Materials

INHALATION EXPOSURE SYSTEM DESCRIPTION

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INHALATION EXPOSURE SYSTEM DESCRIPTION

I. ANIMAL EXPOSURE CHAMBER

The animals will be exposed and maintained in inhalation exposure chambers developed at BNW (U.S. Patent No. 4,216,741, August 12, 1980; Moss, 1980; Brown and Moss, 1981; Moss *et al.* 1982) and now commercially produced by the Harford Division of Lab Products, Inc., Aberdeen, MD. The chamber (Figure 1) facilitates multiple-tier exposures of various laboratory rodent species to aerosol- and vapor-laden atmospheres. The total volume of the chamber is 2.3 m^3 with an active mixing volume of 1.7 m^3, the remainder being the inlet and exhaust volumes where animals are not placed. There are three levels of caging, each level split into two tiers which are offset from each other and from the chamber walls. Drawer-like stainless steel cage units composed of individual animal compartments are suspended in the space above each tier. Stainless steel catch pans for the collection of urine and feces are suspended below each cage unit. Catch pans are left in position throughout the study. Cageboards are added to these catch pans during nonexposure periods to reduce ammonia concentrations.

The chamber was designed so that uniform aerosol or vapor concentrations can be maintained throughout the chamber when the catch pans are left in position. Incoming air containing a uniform mixture of test material is diverted so that it flows vertically along the inner surfaces of the chamber. Eddies are formed (Figure 1) at each tier as the aerosol or vapor flows past the catch pans. Stagnant zones that would normally exist above each pair of catch pans are cleared by exhaust flow through the space between the tiers. Aerosol or vapor reaching the lowest level is deflected across the bottom tiers by metal strips in the space between the catch pan and the wall. Tests have shown that aerosol or vapor concentration homogeneous to within 8% throughout the chamber can be obtained repeatedly provided the aerosol or vapor is uniformly mixed before passing through the chamber inlet (Griffis *et al.* 1981).

II. EXPOSURE SUITE SYSTEM DESCRIPTION

The exposures will be conducted using an automated data acquisition and control system in an exposure suite (Figure 2) consisting of three exposure rooms and a suite control center room (only one of the exposure rooms will be used for this study). A central computer monitors the basic chamber functions (i.e., test chemical concentration, airflow, vacuum, temperature, and relative humidity) in each of the three exposure rooms. The executive computer is a Hewlett-Packard (HP) Model 9816. All data acquisition and system control originates from this computer.

All experimental protocols related to the data acquisition and control system (such as data channel assignments, monitoring frequencies and alarm settings) reside in the executive computer and are entered into tables accessed by menus.

Data input to the executive computer is accomplished through several interface instruments. All on-line chemical monitor data are collected and preconditioned by an HP-85B computer.

Conditioned data are transferred to the executive computer for analysis, concentration control, data storage, and printing. Data from all monitoring equipment other than chemical monitors

are inputted through a Colorado Data Systems (CDS) Model 53A-IBX Intelligent Interface System (IIS).

System control is provided from the computer by means of control relays in the CDS IIS. These relays control such devices as valves, drive motors, audible alarms, indicator lamps, etc.

Data and comments from the exposure room are stored on a magnetic disk and are printed. Data are printed and stored immediately upon completion of the measurement to the "Daily Log". At the end of the day (24-hour period), the daily data are analyzed and a summary is printed. This summary includes the mean, standard deviation, % relative standard deviation, maximum, minimum, and number of measurements for each set of data for the 24-hour period. A second printout provides a table of outliers (i.e., all data points which are beyond the critical limits defined in the protocol). This outlier table allows rapid determination of problem areas in the data. A third printout provides a list of all comments generated by the computer and operators. This allows rapid inspection of generation start and stop times and any problem areas.

A complete description of the software for this system is contained in BNW document ØB-BE-5EØ1. Maintenance of this system is detailed in SOP # ØB-BE-3DØE. Routine operation of the system software and hardware are detailed in SOP # ØB-BE-3GØ4.

III. TEST ARTICLE GENERATION AND MONITORING

A. Test Article Generation System

A schematic diagram of the acetonitrile generation and delivery system is shown in Figure 3. The acetonitrile generator is housed in a vented cabinet located in the suite control center. The acetonitrile to be vaporized will be transferred from the original container in which it was shipped to a 5.6 liter stainless steel reservoir. A nitrogen cover is maintained at all times while transferring the acetonitrile and in the reservoir.

During exposure the acetonitrile is pumped from the stainless steel reservoir through an eductor tube and delivery tube to a vaporizer located in the fresh air duct leading directly to the vapor distribution manifold. The vaporizer is a stainless steel cylinder covered with a glass fiber wick from which the liquid is vaporized. The wick will be replaced prior to the start of exposures. An 80-watt heater and a temperature sensing element is incorporated within the cylinder and is connected to a remotely located temperature controller. A second temperature monitor is incorporated in the vaporizer allowing the operating temperature to be recorded by the automated data acquisition system.

A clear teflon® tube of measured volume preceded by a three-way valve is attached just upstream of the pump to allow measurement of the flow rate of the chemical. Measurement is accomplished by momentarily switching the three-way valve from the run position to the test position. A small bubble of nitrogen is pulled by the pump through the valve and into the clear tube. The progress of this bubble from one end to the other of the tube (calibrated volume) is timed with a stop watch. Flow rate is calculated by dividing the volume by the time. The concentration in the distribution line can be calculated from the flow measurements of liquid and dilution air. A three-way valve at the input of the vaporizer allow the liquid to be pumped either to the vaporizer or to a sample vial. In this way, samples could be taken from the reservoir for periodic purity assays, or for calibration of the monitoring equipment.

The vapor is mixed with charcoal-filtered and HEPA-filtered air from the building air handling system. This mixture is drawn into a stainless-steel distribution manifold by an Air-Vac® vacuum pump (Air-Vac Engineering Co, Milford, CT). From the manifold, the appropriate

amount of vapor/air mixture needed to reach the target concentration is carried to each exposure chamber by individual delivery lines . Withdrawal of vapor from the manifold is done by an AirVac pump at the chamber end of each delivery line. Chamber concentrations are adjusted by the adjustment of the compressed air pressure to the vacuum pumps. At the end of the delivery line, the vapor enters a pneumatic 3-way valve where it is directed to the exposure chamber or the chamber exhaust system as appropriate. These valves allow for a faster buildup of vapor concentration at start-up as well as more rapid diversion of chemical flow from chambers in the event rapid shutdown is necessary. At the start of a generation period, these valves are positioned to route the vapors to the chamber exhaust until the concentration in the distribution system is stabilized. When the system is stable and exposure is ready to proceed, computer-controlled start-up of the exposure is initiated by rotation of the 3-way valves to the exposure position.

Generation proceeds in stages: system stabilization, generation and shutdown. During the stabilization stage, the pneumatic 3-way valves are in the exhaust position as buildup of chemical in the delivery lines proceeds. After the system is stable, at the direction of the operator, computer-controlled start-up is initiated and the pneumatic valves are set to the exposure position. After stabilization of the chamber concentrations and possible adjustments of the vapor delivery flow rates, the generator is in its normal operating stage. During this stage, the system operats under computer and exposure-operator supervision. The exposure operator monitors the chamber concentrations and makes adjustments as necessary. In addition, the computer can initiate an interruption of chemical delivery to any chamber under certain conditions by rotating the appropriate 3-way valve.
Shutdown at the end of the exposure day begins with a computer-initiated rotation of the 3-way valves to the exhaust position, stopping exposures. The computer then stops the chemical delivery pump.

The method used for the generation and delivery of acetonitrile (ACN) is shown in Figure 3. Routine operation of the generation system is detailed in SOP# ØB-BE-3B4B. Maintenance and QC procedures are detailed in SOP# ØB-BE-3DØK.

Measurement of vapor concentration versus time (buildup and decays) will be obtained for all chambers before the start of the study and during the study. (SOP# OB-BE-3B3H)

B. Test Article Concentration Monitoring

An on-line Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector (FID) will be used to monitor the exposure chambers, the control chambers, the exposure room, and the output of a standard gas cylinder. Sampling from multiple positions will be accomplished by means of an oven-mounted, 12-port stream select valve. The sampling system (Figure 4) is incorporated into the relative humidity (RH) sampling system. Samples of the atmosphere from each sample location are continuously drawn by a vacuum pump through teflon sample lines to a location near the input to the 12-port sample valve. This assures fresh samples at the monitor. The sample lines, which continue from the point where they tee-off to the 12-port valve to the dewpoint monitor, are 1/16" teflon.

Sample values are accumulated and printed by an HP Model 85B computer until all required ports from the stream select valve have been sampled. These values are then sent to the executive computer for printing and storage. Each value accumulated by the HP85B is compared with limit values for that particular location. If the value is beyond the control limits, the HP85B will immediately send the information to the executive computer which will take the appropriate action as follows:

- Concentration ≥ non-critical low limit and ≤ non-critical high limit:
  No action
- Concentration < non-critical low limit but ≥ critical low limit:
  Indicate on daily printout
- Concentration < critical low limit:
  Indicate on daily printout and activate audible alarm
- Concentration > non-critical high limit but ≤ critical high limit:
  Indicate on daily printout
- Concentration > critical high limit:
  Indicate on daily printout, activate audible alarm, terminate generation of test material

The on-line gas chromatograph will be calibrated against quantitatively analyzed bubbler grab samples collected from the exposure chambers. Standards will be prepared gravimetrically. The on-line monitor calibration will be confirmed at least once during the animal exposure by grab sampling. The calibration check will be performed according to SOP #ØB-AC-3CØS. Instrument drift will be checked daily against an on-line standard.

A constant concentration stream of acetonitrile vapor, used only to detect instrument drift, is provided. The acetonitrile standard is monitored once per sampling cycle throughout the exposure period. The standard is also checked before the start of each exposure day. The measured concentration for the standard is required to be within ±10% of the assigned target value before any exposure begins without consultation with the exposure control task leader. During the course of the exposure, if the on-line standard is within ±5% of the target value, no change in calibration is required. If the on-line standard is beyond ±5% of its assigned target, the lead chemist is informed immediately by an exposure specialist. Additional grab sampling is initiated by the chemist on a case by case basis when drift in the on-line standard is between ±5-10%. A cumulative drift of 10% or greater requires immediate recalibration of the on-line monitor by grab sampling.

Daily operating procedures for the concentration monitoring system are contained in SOP #ØB-AC-3B1K. Routine maintenance of the GC is covered in SOP #ØB-AC-3DØ2.

The uniformity of the distribution of the test chemical in the chamber will be checked before the start of the study and during the first week of exposure. (SOP #ØB-BE-3B24)

IV. CHAMBER ENVIRONMENTAL CONTROL AND MONITORING

A. Temperature

Nearly all of the heat load contributed to the exposure chamber by the animals is dissipated from the chamber by radiation through the chamber wall (Bernstein and Drew, 1980). The temperature of the air supplied to the chamber has little effect on the temperature of the chamber. On the other hand, the temperature of the room housing the chamber has a great deal of effect. Consequently, chamber temperature is maintained in the proper range primarily by controlling the room temperature.

Temperatures of the exposure chambers and exposure room are measured by Resistance Temperature Detectors (RTDs). The RTDs are placed in a representative location in each chamber. Each RTD is connected to an Omega Model 412B Digital Thermometer by either a manual select switch or by computer controlled scanner relays. This allows the temperature to be read manually or to be recorded automatically by the exposure system executive computer. Temperatures are automatically recorded at regular intervals during each 24-hour day. All temperature measurement equipment except the RTDs is located in the exposure suite control

center. RTDs are calibrated to within 0.5°F of a certified mercury thermometer (SOP # ØB-BE-3CØL) before the start of the study and at least every two months thereafter.

B. Relative Humidity

Relative humidity (RH) in the exposure chambers is controlled by the system depicted in Figure 5. Equipment located in the RH Control Equipment Room (Room 335 of the LSL-II basement) provides separate ducts of dry and moist air to each exposure chamber. Filtered air with a maximum dewpoint of about 53°F is supplied to the RH Control Equipment by the building HVAC system. This air is evenly delivered to two ducts. Air from the first duct passes into a plenum where steam, generated from city tap water with no additional additives, is injected to bring the air to a dewpoint of about 60°F. This provides the moist air source for the chambers. The air from the second duct is passed through a refrigeration coil which reduces the moisture content of the air to a dewpoint of about 40°F. This provides the source of "dry" air for the chambers. A manually controlled mixing valve for each chamber mixes the proper proportions of the moist and dry air to maintain the proper RH in each chamber.

Relative humidity is measured using an EG&G Model 910 dewpoint hygrometer located in the exposure suite control center. Air from the exposure chambers is sampled from a representative location (a top port on the back side). A teflon filter is placed at the chamber end of the sample line if the test article is an aerosol. Samples of the air from each measurement location are continuously pulled through polytetrafluoroethylene (PTFE) tubing to a central location in the exposure suite control center. This assures a fresh sample at the dewpoint hygrometer. Sample air from a particular location is routed by a multiplexed valve system to either the exposure system exhaust or the dewpoint hygrometer for RH determination. The valves are controlled by either a manual switch or by a computer controlled relay, allowing RH to be measured manually or automatically by the exposure system executive computer. RH is automatically recorded at regular intervals during the 24-hour day.

Once the dewpoint has been determined by the hygrometer, the RH is automatically calculated by the exposure system executive computer using the dewpoint value and the drybulb temperature (measured simultaneously at the same location by the RTD system) by applying a form of the Antoine equation for determination of saturation vapor pressure of water at a given temperature.

Calibration of the dewpoint hygrometer is established prior to the start of the study and checked at least every two months following SOP # ØB-BE-3B1X. Initial calibration requires comparison at three RH levels (~30%, 50% and 70%) of the RH calculated by the monitor to measurements made by a calibrated portable hygrometer and RTD located near the chamber.

C. Airflow

Airflow in the chambers is maintained by the vacuum in the central chamber exhaust duct. This vacuum is created by the chamber exhaust flow fans located in the South Equipment Room of the LSL-II Building. There are two parallel exhaust fans, one operating with the other providing backup. Both fans can operate from emergency power.

Chamber airflow rate is controlled by a gate valve in each individual chamber exhaust duct. A drive motor attached to the stem of this valve allows the control of chamber flow either by computer or manually from the Exposure Control Center.

Fine control of exposure concentration can be accomplished by automatically or manually adjusting the valve position to control chamber dilution airflow within the allowable limits. Gross adjustment of concentration must be done manually by adjusting the generation system.

Chamber airflow is measured by a multiplexed orifice-meter system consisting of a calibrated orifice located in each chamber exhaust, a Validyne Model DP-45 pressure transducer, a Validyne Model CD-18 carrier demodulator, and a Validyne Model PM-12 digital voltmeter. The pressure transducer is multiplexed to each chamber's flow orifice by valves remotely controlled either manually or by means of the executive computer. This allows flow to be measured either manually or automatically. Flow is automatically recorded at regular intervals during the 24-hour day.

Calibrated flow orifice meters are located at both the inlet and exhaust to each chamber. By comparing the measured flow at the inlet and exhaust, leaks in the chamber can be detected. A leak check is automatically performed by the executive computer when each chamber is closed. If a leak is detected, the executive computer will notify the operator and will not allow exposures to proceed until the leak is repaired. This system is sensitive to very small leaks which may cause an imbalance of test article concentration within the chamber.

Calibration of the flow orifices will be done before the start of the study following SOP # ØB-BE-3CØV. Each flow orifice is calibrated to within ±0.5 CFM by comparison to a calibrated digital vane anemometer temporarily inserted in the chamber duct during non-exposure periods.

D. Chamber Vacuum

The chambers are maintained at a slight negative pressure compared to the room in order to reduce the possibility of escape of test article. This negative pressure is created by the pressure drop across the HEPA and charcoal filters at the inlet to each chamber.

The same Validyne pressure transducer system used to measure chamber flows is also used to measure chamber vacuum. Vacuum in the chamber is measured relative to atmospheric pressure in the suite control room. Vacuum is automatically recorded at regular intervals during the 24-hour day.

Vacuum is also continuously monitored by a mechanical pressure switch attached to each chamber. In the event of leak in the chamber, the pressure switch will immediately shut off the flow of test article to the chamber and activate an audible alarm.

V. ANIMAL FACILITY AIR HANDLING SYSTEM

Supply air enters the building through two identical parallel air handling systems (Figure 6). Each system consists of a pre-heat coil, a filter system, a heating coil, a chilling coil, and a supply fan. The pre-heat coil heats the air to a minimum of 45°F. The filter system, comprised of a roll filter, pre-filter, and a bag filter, rids the air of most particles. The heating and chilling coils maintain the temperature of the air exiting the air conditioning system at about 53°F. The chilling coils also dry the air to a dewpoint not greater than 53°F.

Although simultaneous operation of both of the parallel air supply systems is necessary to provide the 20 air changes per hour typically supplied to each animal room, only one of these systems, which can be operated from the emergency power system, is required to maintain the rooms within the temperature and flow specifications required by the protocol. Exposure of the animals to the test article can continue in the event of the failure of one of the air supply systems.

The air from the two parallel building air supply systems is mixed together by an air mixing unit and is divided into two ducts which feed the rooms on the East and West sides of the animal quarters. If necessary, steam is injected into the air in these ducts to maintain the relative humidity of all rooms in the basement at a minimum of 35%. In rooms where further room RH control is necessary, it is provided by individual steam generators located in the room. Prior to entering the animal room, the air is filtered through a HEPA filter.

Air for exposure chambers is supplied to a chamber relative humidity conditioning system from the building air supply systems. A single supply system is sufficient to supply air to the RH conditioning systems for all exposure chambers in the facility.

Exhaust from the animal room is filtered by a room HEPA filter and again through a bank of building exhaust HEPA filters assuring no escape of aerosol particles from the facility. Two parallel exhaust fans provide exhaust from the room. One of these fans is in operation with the second as a backup unit. One fan can be operated from the emergency power system in case of power failure.

VI. EXPOSURE SUITE ALARM SYSTEM

An extensive system of alarms has been incorporated into the exposure suite automated data acquisition and control system to provide safety for the system operators, protect the health of the animals and ensure the integrity of the study. There are actually two separate alarm systems; one provided by the computer and a separate "physical" alarm system which provides redundancy for some of the computer alarm functions.

Following each function measured by the computer, the value is compared to the alarm limit values (stored in the computer memory) for that function . There are four limit values for each function and location monitored by the computer; high and low non-critical and high and low critical. For example, chamber flows may have the following limits:

Critical low ..... 10 air changes per hour
Non-critical Low 12
Non-critical High..... 18
Critical High 20

The result of an alarm condition depends on the function measured and the measurement location. Each function and location has an alarm response assignment in the computer. Again

using chamber flow as an example, flows exceeding the non-critical limits but remaining within the critical limits will cause the computer to print a "beyond-non-critical-limits" symbol, "(" if low and ")" if high, next to the data on the daily log printed by the computer. A chamber flow which exceeds the critical limits will cause the computer to print a "beyond-critical-limits" symbol, ">" if high and "<" if low, on the daily log and to turn on the critical alarm audio alert. A critical low flow alarm will also shut off the flow of test article to the chamber. Although it is possible for the computer to make automatic corrections to air flow in the chamber, this is not done because to do so would affect concentration of the test article in the chamber. A critical low negative pressure in a chamber (which may be the result of a leak in the chamber) will also cause the computer to shut off the flow of test article to the chamber. Similar responses result from alarms arising from temperature and relative humidity measurements in the chambers, however these alarms have no affect on the operation of the test article generator.

The physical alarm system includes the following continuously monitoring devices:

- Chamber pressure (also detects critically low flow if it is the result of pump failure and not a clogged chamber inlet duct)
- Generator cabinet exhaust flow
- Chamber exhaust system flow
- Building exhaust system flow

In all cases an alarm condition from any one of these monitors is considered critical and results in the test article generator being shut off.

VII. CHAMBER EXHAUST WASTE TREATMENT

Chamber exhaust is mixed with the exhaust from the entire animal facility (~ 75,000 cfm) prior to its emission from the building stack. This method of waste treatment results in an acceptable stack concentration.

VIII. DATA RECORDING AND HANDLING

Data from the exposure room are stored in the exposure suite control center on a magnetic disk by Hewlett-Packard (HP) Model 9121 micro-floppy disc drive. Data and comments from the exposure room are printed by a thermal dot matrix printer (HP Model 2171G) or by an ink jet printer (HP Model 2225A). Data are printed and stored immediately upon completion of the measurement to a Daily Log (example, Figure 7). Both the Daily Log and the disk will be maintained in the study files. The Daily Log will be considered the raw data. The Daily Log includes the time of measurement, the measurement location (such as chamber), the measurement function (such as temperature), the value of the measurement, the percent of target, an alarm code, and a status code. See Figure 7 for an explanation of the alarm and status codes.

At the end of the day (24-hour period), the daily data are analyzed and three summaries are printed. The first (example, Figure 8) includes the mean, % of target, standard deviation, % relative standard deviation, maximum, minimum, and number of measurements for each function (such as temperature) and location (such as chamber) monitored over the 24-hour period. The second (example, Figure 9) provides a list of outliers; that is, all data points which were beyond the defined critical operating limits. This printout allows for quick review of data which are outside the operating limits. The final summary (example, Figure 10) is a printout of all comments made by the computer, exposure specialist, and exposure operator during the 24-hour period. This includes comments on startup time, exposure termination, new calibration

factors entered, and other information. This summary allows a quick review of events that occurred during the day.

Data handling and analysis procedures are described in SOPs ØB-BE-3EØB and ØB-BE-3EØE.

IX. EQUIPMENT OR POWER FAILURE PROTECTION SYSTEMS

Power is provided to the Battelle complex from two separate city substations through an automatic switching device. This significantly reduces the possibility of losing city power. Power from the city is routed to equipment in the LSL-II building through two types of motor control centers. One type can switch power from the LSL-II diesel generator. The other has access only to city power. The emergency-power-type motor control center has a low voltage detector on each leg of the three-phase input power. If the city supplied power should fail or "brown out", these detectors automatically start the emergency power diesel generator and route the emergency power to the equipment connected to the emergency-power-type motor control center.

All equipment critical to the well-being of the animals is connected to the emergency-power-type motor control center. This equipment includes:

- Emergency lighting and electrical outlets
- Building air conditioning chillers #1 and #2
- Building heating boilers and feedwater pump systems #1 and #2
- Air compressors #1 and #2
- Air supply fans #1 and #2
- Air exhaust fans #1 and #2

Note that there are two identical units of all equipment that is vital to the well-being of the animals (heating, cooling, supply air, exhaust air, and compressed air). Either of the two units has sufficient capacity to maintain the animal environment within a safe range. In all cases, the emergency power system will operate one of the two identical systems.

All building or chamber systems which are essential to the survival of the animals are alarmed. If a system malfunctions, an alarm is tripped in the Power Operator's office. A Power Operator is on duty 24 hours/day, 7 days/week. If the Power Operator is not authorized to correct the problem that caused the alarm, he immediately calls the appropriate personnel of the programs affected.

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Moss, O.R., Decker, J.R. and Cannon, W.C. (1982). "Aerosol Mixing in an Animal Exposure Chamber Having Three Levels of Caging with Excreta Pans", Am. Ind. Hyg. Assoc. J., 43, pp. 244-249.

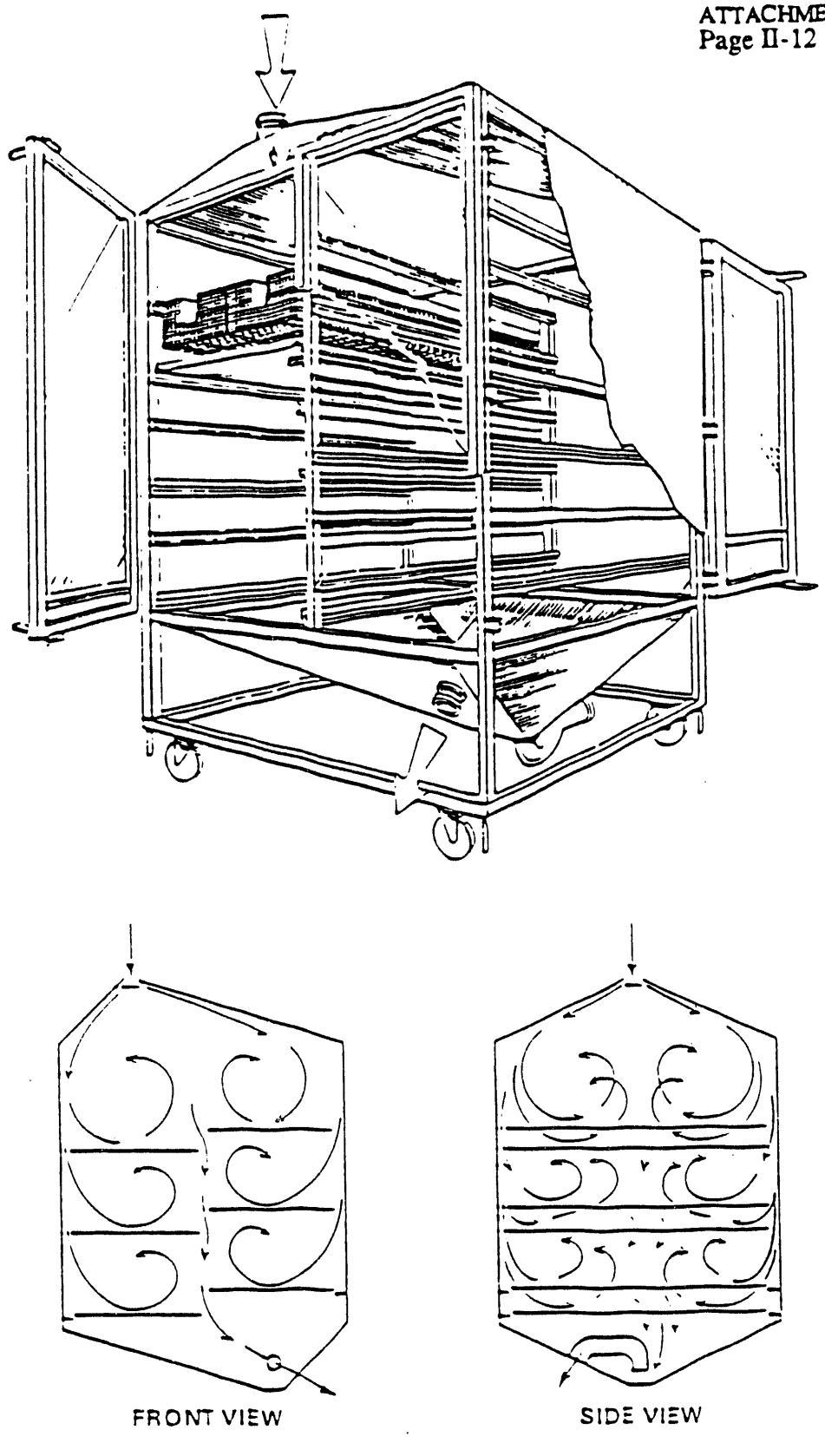

Figure 1. Inhalation Exposure Chamber Designed at BNW. Top: Oblique Cutaway View of the Chamber; Bottom: Airflow Patterns).

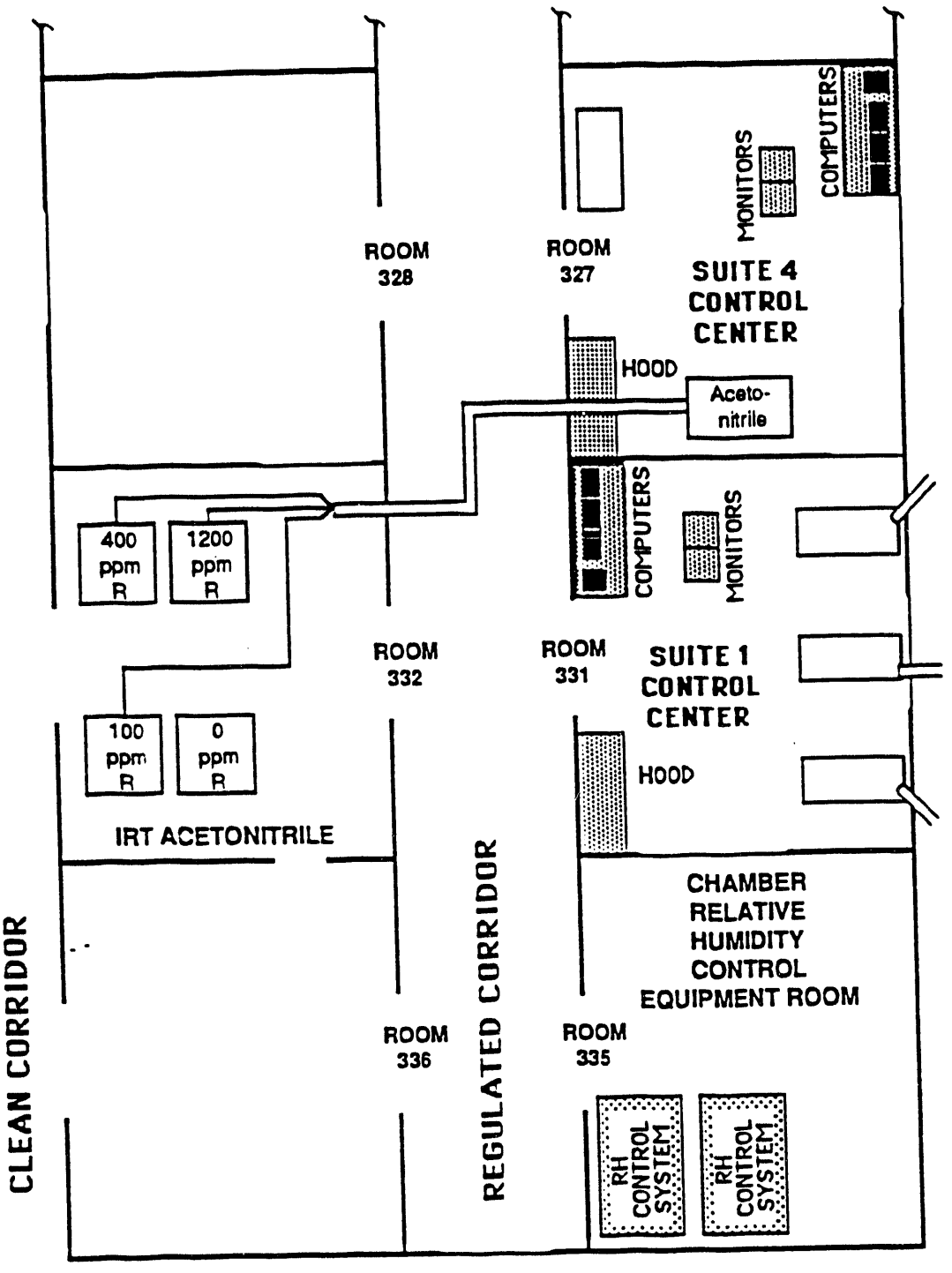

Figure 2. Schematic of the Acetonitrile Exposure Suite.

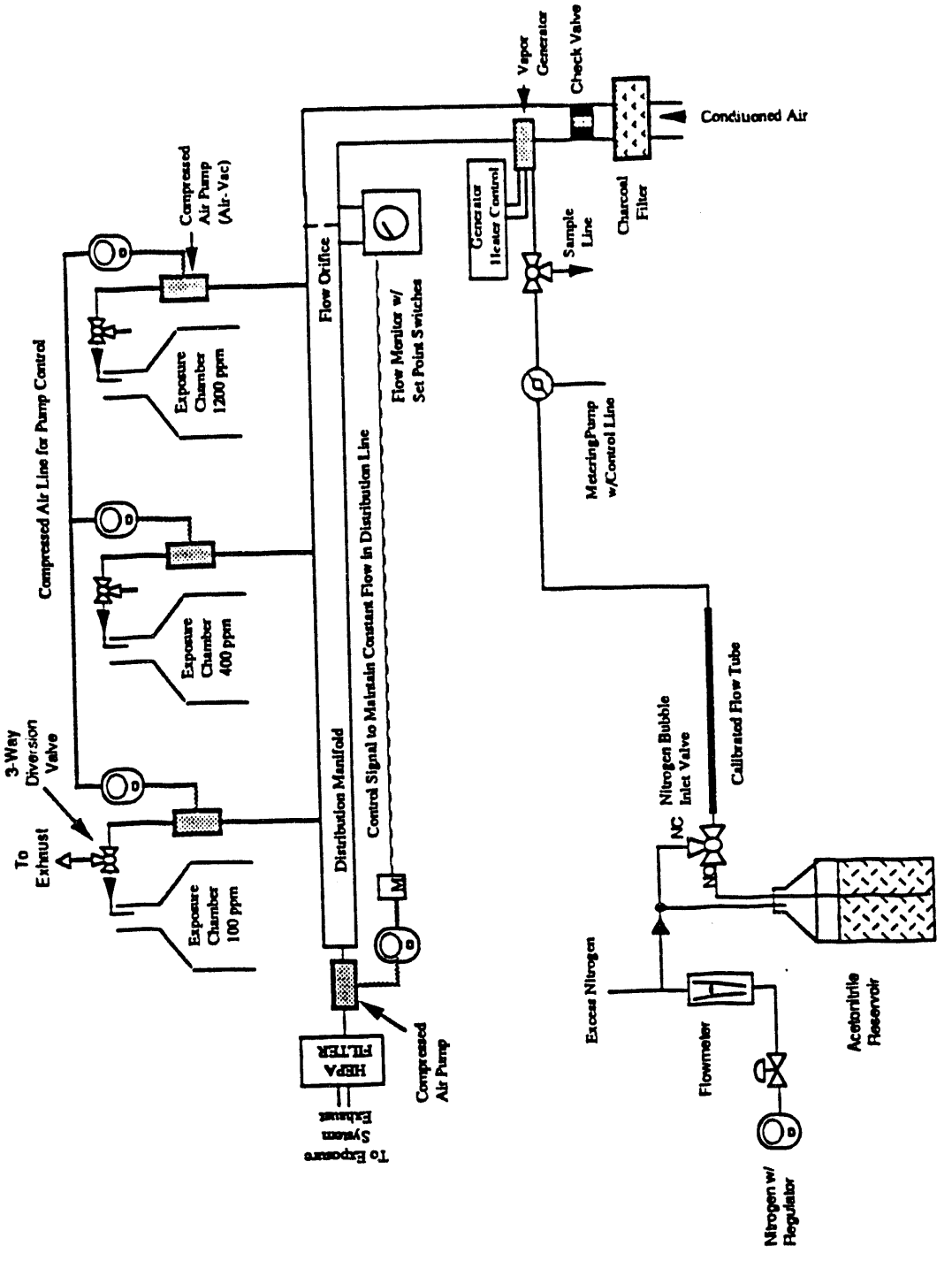

Figure 3. Schematic of Acetonitrile Vapor Generation and Distribution System.

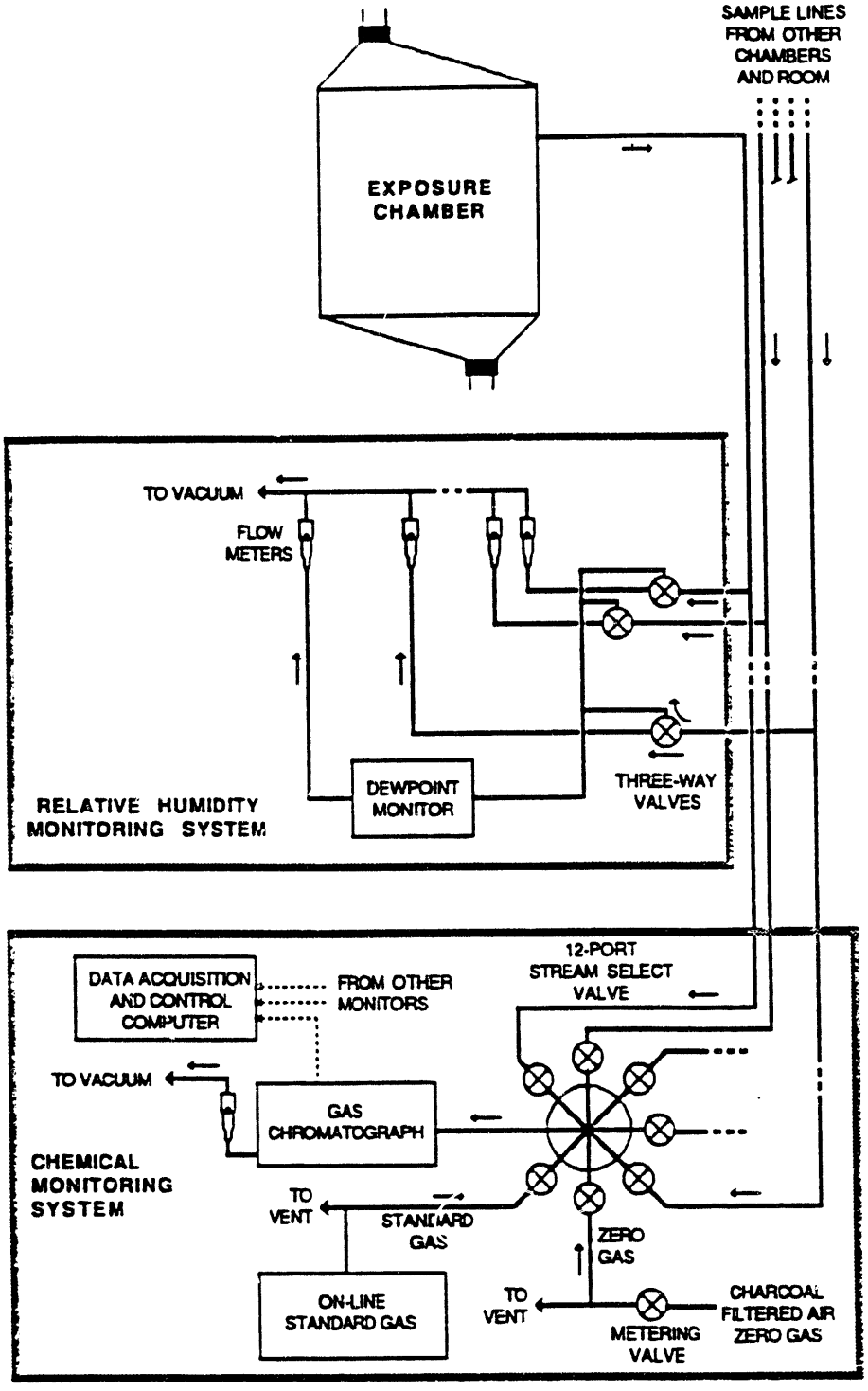

Figure 4. Schematic of the Dewpoint and Chemical Monitoring System.

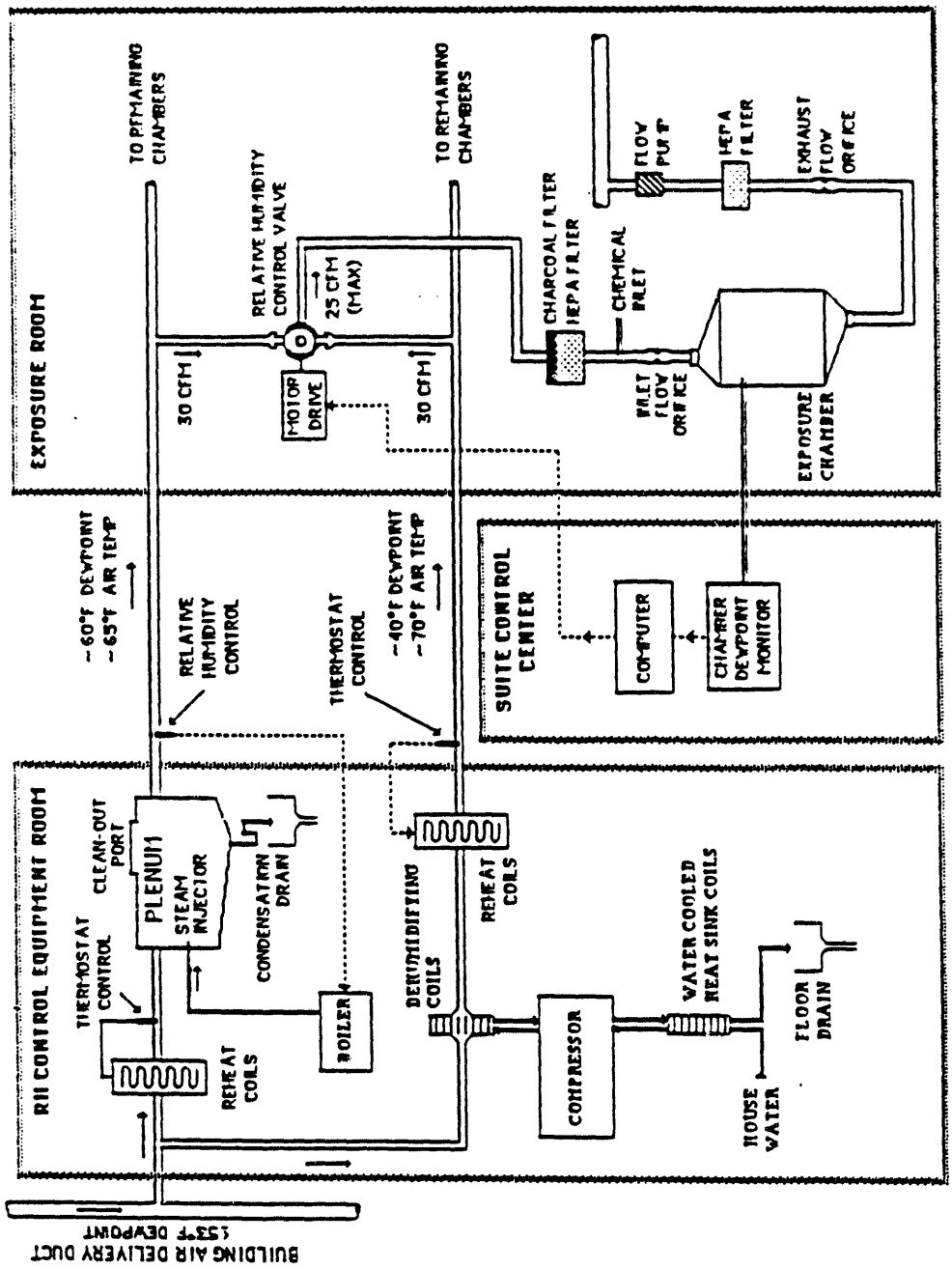

Figure 5. Schematic of Chamber Relative Humidity Control System.

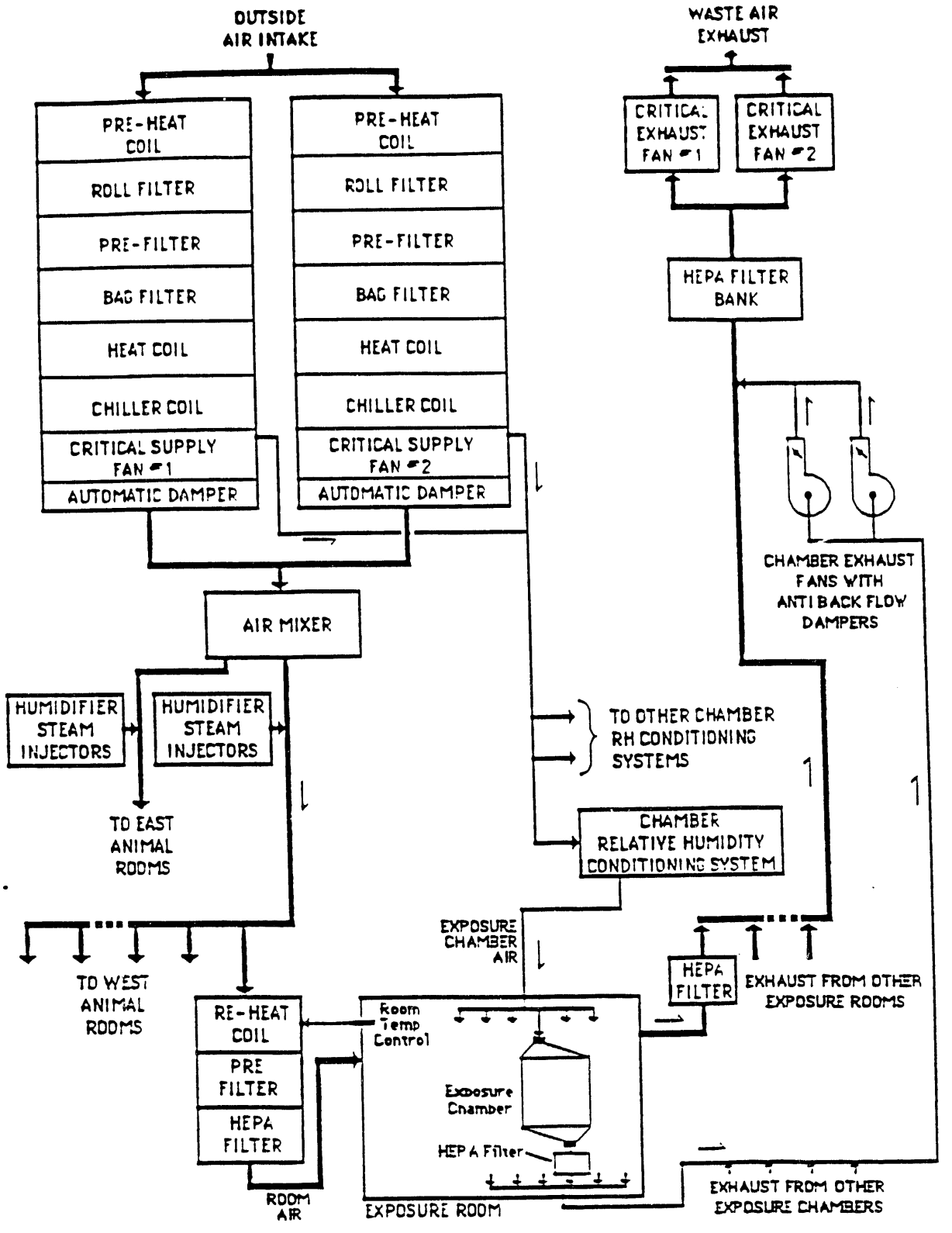

Figure 6. Air Handling System for Animal Rooms of Life Sciences II Building.

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Time	Data Origin	Function	Data	Targt
	[LR] TFE - 625 ppm -R/M -T90 Time Expired-Including Conc Data			
	[LR] TFE - 312 ppm -R/M -T90 Time Expired-Including Conc Data			
	[LR] TFE - 625 ppm -R -T90 Time Expired-Including Conc Data			
	[LR] TFE - 312 ppm -R -T90 Time Expired-Including Conc Data			
	[LR] TFE - 156 ppm -R -T90 Time Expired-Including Conc Data			
08:17	Hewlett Packard 85B (Access Level: Specialist)			
	TFE - 1250 ppm -R/M-Conc Data Excluded-Data Time<T90 & Data<Target			
08:19	Hewlett Packard 85B (Access Level: Specialist)			
	TFE - 62[F] ppm -R/M-Conc Data Excluded-Data Time<T90 & Data<Target			
08:21	Hewlett Packard 85B (Access Level: Specialist)			
	TFE - 312 ppm -R/M-Conc Data Excluded-Data Time<T90 & Data<Target			
08:29	TFE - 625 ppm -R	Temperature	OKI 74.3 F	99%
08:30	TFE - 1250 ppm -R/M	Temperature	OKI 74.0 F	99%
08:32	TFE - (332) Room	Temperature	OKI 69.3 F	96%
08:34	TFE - (332) Room	Relative Humidity	OKI 53.0 %	106%
08:37	TFE - 0 ppm -R	Relative Humidity	OKI 46.0 %	84%
08:39	TFE - 0 ppm -R/M	Relative Humidity	OKI 47.0 %	85%
08:42	TFE - 156 ppm -R	Relative Humidity	OKI 48.0 %	87%
08:44	TFE - 312 ppm -R/M	Relative Humidity	OKI 49.0 %	89%
08:17	TFE - 1250 ppm -R/M	GC#34-809569	OKI 6.760E+2 ppm	55%
08:19	TFE - 625 ppm -R/M	GC#34-809569	OKI 3.445E+2 ppm	56%
08:21	TFE - 312 ppm -R/M	GC#34-809569	OKI 2.507E+2 ppm	81%
08:24	TFE - 0 ppm -R/M	GC#34-809569	OKI 0.000E+0 ppm	0%
08:26	TFE - (332) Room	GC#34-809569	OKI 0.000E+0 ppm	0%
08:29	TFE - 625 ppm -R	GC#34-809569	OKI 5.874E+2 ppm	94%
08:31	TFE - 312 ppm -R	GC#34-809569	OKI 2.854E+2 ppm	92%
08:34	TFE - 156 ppm -R	GC#34-809569	OKI 1.521E+2 ppm	98%
08:36	TFE - 0 ppm -R	GC#34-809569	OKI 0.000E+0 ppm	0%
08:40	TFE - Standard Gas-L	GC#34-809569	OKI 2.880E+1 ppm	20%
08:43	TFE - Standard Gas-H	GC#34-809569	OKI 4.993E+2 ppm	100%
08:47	TFE - 312 ppm -R	Relative Humidity	OKI 49.0 %	89%
08:49	TFE - 625 ppm -R/M	Relative Humidity	OKI 48.0 %	87%
08:52	TFE - 625 ppm -R	Relative Humidity	OKI 47.0 %	85%
08:54	TFE - 1250 ppm -R/M	Relative Humidity	OKI 53.0 %	96%
08:54	TFE - 0 ppm -R	Exhaust Air Flow	OKI 15.3 CFM	102%
08:55	TFE - 0 ppm -R/M	Exhaust Air Flow	OKI 15.0 CFM	100%
08:55	TFE - 156 ppm -R	Exhaust Air Flow	OKI 14.6 CFM	97%
08:55	TFE - 312 ppm -R/M	Exhaust Air Flow	OKI 14.6 CFM	97%
08:56	TFE - 312 ppm -R	Exhaust Air Flow	OKI 14.6 CFM	97%
08:56	TFE - 625 ppm -R/M	Exhaust Air Flow	OKI 14.9 CFM	99%
08:56	TFE - 625 ppm -R	Exhaust Air Flow	OKI 15.3 CFM	102%
08:56	TFE - 1250 ppm -R/M	Exhaust Air Flow	OKI 14.8 CFM	99%
08:45	TFE - 1250 ppm -R/M	GC#34-809569	OKI 1.212E+3 ppm	97%
08:48	TFE - 625 ppm -R/M	GC#34-809569	(OKI 5.594E+2 ppm	90%
08:50	TFE - 312 ppm -R/M	GC#34-809569	OKI 2.923E+2 ppm	94%
08:53	TFE - 0 ppm -R/M	GC#34-809569	OKI 0.000E+0 ppm	0%

Figure 7. Example of 24-Hour "Daily Log" Printout from Data Acquisition and Control Computer. See Following Page for Explanation of Columns.

Figure 7. (continued)

DESCRIPTION OF COMPUTER "DAILY LOG" OUTPUT

The date, exposure name, program version and page number will be printed at the top of each page of the daily log

Column 1: Time -- time that measurement was taken
Column 2: Location -- location of measurement (for example, chamber)
Column 3: Function -- measurement function (for example, temperature)
Column 4: Data –

Alarm Code --	"("	Indicates data < non-critical low but ≥ critical low alarm value
	")"	Indicates data > non-critical high but ≤ critical high alarm value
	"<"	Indicates data < critical low alarm value
	">"	Indicates data > critical high alarm value
	"["	Indicates data < super-critical low alarm value
	"]"	Indicates data > super-critical high alarm value
Status Code --	"OK"	Indicates monitoring instrument is functioning properly and is calibrated
	"BS"	Indicates service time of monitoring instrument has expired. (Usually indicates that instrument calibration should be checked. Does not necessarily mean that data is not valid)
	"I"	Indicates data will be included in summary
	"E"	Indicates data will be excluded from summary
Data Value --		Data may be expressed in scientific notation (x.xxxEyy)
Units Label --		Units of measurement (e.g., ppm, °F, mg/m^3)

Column 5: Target --% of target concentration for the measurement

Summation for the File: Nov_03_88 Exposure: Tetrafluoroethylene-Chronic

Exhaust Air Flow	Mean	% Targ	Std Dev	% RSD	Maximum	Minimum	Num Xs
TFE - 0 ppm -R	15.5	103%	.09	1%	15.6	15.3	8.
TFE - 0 ppm -R/M	15.1	100%	.05	0%	15.1	15.0	8.
TFE - 156 ppm -R	14.6	98%	.03	0%	14.7	14.6	8.
TFE - 312 ppm -R/M	14.6	98%	.03	0%	14.7	14.6	8.
TFE - 312 ppm -R	14.7	98%	.05	0%	14.7	14.6	8.
TFE - 625 ppm -R/M	14.9	100%	.05	0%	15.0	14.9	8.
TFE - 625 ppm -R	15.4	103%	.06	0%	15.5	15.3	8.
TFE - 1250 ppm -R/M	14.9	99%	.08	1%	15.0	14.8	8.

Vacuum	Mean	% Targ	Std Dev	% RSD	Maximum	Minimum	Num Xs
TFE - 0 ppm -R	.9	94%	.04	5%	1.0	.9	9.
TFE - 0 ppm -R/M	1.0	98%	.02	2%	1.0	1.0	9.
TFE - 156 ppm -R	.9	89%	.01	1%	.9	.9	9.
TFE - 312 ppm -R/M	.9	95%	.01	1%	1.0	.9	9.
TFE - 312 ppm -R	.8	79%	.03	4%	.8	.8	9.
TFE - 625 ppm -R/M	.9	90%	.01	1%	.9	.9	9.
TFE - 625 ppm -R	.9	95%	.03	3%	1.0	.9	9.
TFE - 1250 ppm -R/M	1.1	108%	.03	3%	1.1	1.0	9.

Relative Humidity	Mean	% Targ	Std Dev	% RSD	Maximum	Minimum	Num Xs
TFE - 0 ppm -R	49.1	89%	3.09	6%	53.0	45.0	8.
TFE - 0 ppm -R/M	54.6	99%	8.53	16%	65.0	44.0	8.
TFE - 156 ppm -R	51.2	93%	3.28	6%	56.0	47.0	8.
TFE - 312 ppm -R/M	52.9	96%	6.83	13%	60.0	43.0	8.
TFE - 312 ppm -R	55.7	101%	4.68	8%	63.0	49.0	8.
TFE - 625 ppm -R/M	54.5	99%	7.23	13%	62.0	43.0	8.
TFE - 625 ppm -R	52.4	95%	4.69	9%	59.0	46.0	8.
TFE - 1250 ppm -R/M	59.5	108%	8.25	14%	69.0	48.0	8.

Temperature	Mean	% Targ	Std Dev	% RSD	Maximum	Minimum	Num Xs
TFE - (336) Room	69.9	97%	.58	1%	70.6	68.9	9.
TFE - 0 ppm -R	75.5	101%	.72	1%	76.6	74.7	9.
TFE - 0 ppm -R/M	75.0	100%	.76	1%	76.5	73.9	9.
TFE - 156 ppm -R	75.5	101%	.61	1%	76.3	74.7	9.
TFE - 312 ppm -R/M	75.2	100%	.36	0%	76.0	74.7	9.
TFE - 312 ppm -R	75.6	101%	.65	1%	76.4	74.6	9.
TFE - 625 ppm -R/M	76.2	102%	.57	1%	77.0	75.3	9.
TFE - 625 ppm -R	75.1	100%	.67	1%	76.0	74.3	8.
TFE - 1250 ppm -R/M	74.6	100%	.42	1%	75.2	74.0	8.
TFE - (332) Room	69.5	96%	.33	0%	69.9	69.0	8.

GC#34-809569	Mean	% Targ	Std Dev	% RSD	Maximum	Minimum	Num Xs
TFE - (332) Room	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	15.
TFE - 0 ppm -R	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	15.
TFE - 0 ppm -R/M	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	15.
TFE - 156 ppm -R	1.53E+2	98%	2.010E+0	1%	1.56E+2	1.49E+2	13.
TFE - 312 ppm -R/M	3.07E+2	98%	5.478E+0	2%	3.13E+2	2.92E+2	12.
TFE - 312 ppm -R	3.09E+2	99%	1.068E+1	3%	3.21E+2	2.85E+2	13.

Figure 8. Example of 24-Hour "Data Summation" Printout from Data Acquisition and Control Computer.

Outlier Table for the File: Nov_21_88 Exposure: Acetonitrile

Origin	Instrument	Time	Date	Lower	Target	Higher
Aceto - 0 ppm-M	Relative Humidity	10:14	33.0	35.0	55.0	75.0

Figure 9. Example of 24-Hour "Data Outlier Table" Printout from Data Acquisition and Control Computer. Table shows data which were beyond the defined Critical Limits.

Daily Comments Tetrafluoroethylene-Chronic File: Nov_03_88

Time	Operator	Comment
07:51		Chamber Leak Check for TFE - 156 ppm -R
07:51		Exhaust Flow= 14.6 Inlet Flow= 15.1 (-3.4% leak) [Acceptable]
07:52		Chamber Leak Check for TFE - 0 ppm -R
07:52		Exhaust Flow= 15.2 Inlet Flow= 15.6 (-2.6% leak) [Acceptable]
07:52		All Chambers have been found ACCEPTABLE.
08:01	Hewlett Packard 858	TFE - Standard Gas-H-Conc Data Excluded-Exposure Not Running
08:04		HP858 found Parameters Okay & Ready for Exposure Start.
08:14	Hewlett Packard 9816	Exposure Timing started. [Time=T(0)]
08:15		TFE Chronic - Main Distribution Valve - Valve Opened
08:15		[LR] TFE Chronic - 1250 ppm Chamber Rats/Mice - Valve Opened
08:15		[LR] TFE - 1250 ppm -R/M -ON Exposure-Enable Environ Data Collection
08:15		[LR] TFE Chronic - 625 ppm Chamber Rats/Mice - Valve Opened
08:15		[LR] TFE - 625 ppm -R/M -ON Exposure-Enable Environ Data Collection
08:15		[LR] TFE Chronic - 312 ppm Chamber Rats/Mice - Valve Opened
08:15		[LR] TFE - 312 ppm -R/M -ON Exposure-Enable Environ Data Collection
08:15		[LR] TFE Chronic - 625 ppm Chamber Rats - Valve Opened
08:15		[LR] TFE - 625 ppm -R -ON Exposure-Enable Environ Data Collection
08:15		[LR] TFE Chronic - 312 ppm Chamber Rats - Valve Opened
08:15		[LR] TFE - 312 ppm -R -ON Exposure-Enable Environ Data Collection
08:15		[LR] TFE Chronic - 156 ppm Chamber Rats - Valve Opened
08:15		[LR] TFE - 156 ppm -R -ON Exposure-Enable Environ Data Collection
08:15		[LR] TFE Chronic - Standard Gas Valve - Valve Opened
08:27		[LR] TFE - 1250 ppm -R/M -T90 Time Expired-Including Conc Data
08:27		[LR] TFE - 625 ppm -R/M -T90 Time Expired-Including Conc Data
08:27		[LR] TFE - 312 ppm -R/M -T90 Time Expired-Including Conc Data
08:27		[LR] TFE - 625 ppm -R -T90 Time Expired-Including Conc Data
08:28		[LR] TFE - 312 ppm -R -T90 Time Expired-Including Conc Data
08:28		[LR] TFE - 156 ppm -R -T90 Time Expired-Including Conc Data
08:17	Hewlett Packard 858	TFE - 1250 ppm -R/M-Conc Data Excluded-Data Time<T90 & Data<Target
08:19		TFE - 625 ppm -R/M-Conc Data Excluded-Data Time<T90 & Data<Target
08:21		TFE - 312 ppm -R/M-Conc Data Excluded-Data Time<T90 & Data<Target
10:25	Gary R. Ell	Service status updated on GC concentration from data collected
10:25		11-02-88 gas bag samples.No correction needed,as per Mr.
10:25		Rossignol.
10:26		Concentration Monitor-Service/Status
10:26		[1, 1]TFE - 1250 ppm -R/M Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1, 2]TFE - 625 ppm -R/M Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1, 3]TFE - 312 ppm -R/M Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1, 4]TFE - 0 ppm -R/M Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1, 5]TFE - (332) Room Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1, 6]TFE - 625 ppm -R Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1, 7]TFE - 312 ppm -R Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1, 8]TFE - 156 ppm -R Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1, 9]TFE - 0 ppm -R Srv Date was: 27 Oct 1988 is: 3 Nov 1988
10:26		[1,12]TFE - Standard Gas-H Srv Date was: 27 Oct 1988 is: 3 Nov 1988
14:27	Hewlett Packard 9816	[LR] TFE Chronic - Main Distribution Valve - Valve Closed
14:27		Exposure Terminated. Exposure timers stopped.
14:27		Generator valve already OFF.
14:27		[LR] TFE Chronic - 1250 ppm Chamber Rats/Mice - Valve Closed
14:27		[LR] TFE - 1250 ppm -R/M -OFF Exposure-Enable Environ Data Collection

Figure 10. Example of 24-Hour "Comment Summary" Printout from Data Acquisition and Control Computer. Table shows a summary of all comments recorded on "Daily Log" printout.

Cage Maps

Exposure Chamber Cage Maps

Project: NTP/IRT
Study: ACETONITRILE
Species: RAT
Chamber. 1200 ppm
Room: 332

Study Director: MAST
Date: 2/19/91 TUESDAY
Verified By: D Bayl

Level 1

Level 2

Level 3

	355
	350
	339
362	218
361	169
358	157
311	151
263	~~94~~ (1)
85	64
25	21

ges grp A | ges grp B

Level 4

179 **	
68 **	
41 **	340
4 **	170
363	106
344	87
282	60
247	53
110	34
109	23
31	xxxxxx
14	206 **

ges grp C | ges grp C & D

Level 5

364	
347	
261	
242	
145	
(1) ~~88~~	
69	
67	
39	
5	

NON-PREG

(1) moribund sac 2-15-91 RLR

Level 6

Comments:

** rats for blood collection

see calendar for weigh schedule

PRE-EXPOSURE- REMOVE GRP A RATS FOR SACRIFICE

POST-EXPOSURE- REMOVE GRPC **RATS FOR BLOOD COLLECTION

Exposure Chamber Cage Maps

Project: NTP/IRT
Study: ACETONITRILE
Species: RAT
Chamber: 400 ppm
Room: 332

Study Director: MAST
Date: 2/19/91 TUESDAY
Verified By: D Bruyn

Level 2

Level 1

Level 4

233 **	
94 **	
50 **	270
10 **	235
264	223
210	205
188	140
133	135
123	52
93	17
47	xxxxx
36	298 **
ges grp C	ges grp C & D

Level 3

	349
356	342
320	315
286	~~231~~ Died 2-14-91 RLR
198	165
174	72
156	46
137	30
73	28
ges grp A	ges grp B

Level 6

Level 5

249	
199	
168	
161	
153	
146	
112	
102	
27	
12	
NON-PREG	

Comments:
** rats for blood collection

see calendar for weigh schedule
PRE-EXPOSURE- REMOVE GRPA RATS FOR SACRIFICE
POST-EXPOSURE- REMOVE GRPC ** RATS FOR BLOOD COLLECTION

Exposure Chamber Cage Maps

Project: NTP/IRT
Study: ACETONITRILE
Species: RAT
Chamber: 100 ppm
Room: 332

Study Director: MAST
Date: 2/19/91 TUESDAY
Verified By: D Bruno

Level 2

Level 1

Level 4

305 **	
257 **	
246 **	
245 **	296
193 **	290
343	288
291	275
281	271
224	176
190	171
162	92
122	42
ges grp C	ges grp D

Level 3

	368
	321
	276
353	267
348	258
326	239
274	221
273	208
126	86
85	1
ges grp A	ges grp B

Level 6

Level 5

352	
334	
324	
250	
200	
149	
138	
105	
98	
13	
NON-PREG	

Comments:

** rats for blood collection

see calendar for weigh schedule
PRE-EXPOSURE- REMOVE GRP A FOR SACRIFICE
POST-EXPOSURE- REMOVE GRP C ** RATS FOR BLOOD COLLECTION

Exposure Chamber Cage Maps

Project: NTP/IRT
Study: ACETONITRILE
Species: RAT
Chamber: 0 ppm
Room: 332

Study Director: MAST
Date: 2/19/91 TUESDAY
Verified By: _____

Level 2

Level 1

Level 4

345 **	
304 **	
248 **	
62 **	373
24 **	323
319	23E
312	181
243	125
203	115
158	95
108	89
32	16
ges grp C	ges grp D

Level 3

	336
	314
	292
293	278
289	219
185	139
184	99
113	48
66	38
11	3
ges grp A	ges grp B

Level 6

Level 5

369	
253	
238	
212	
175	
12F	
127	
96	
79	
40	
NON-PREG	

Comments:
** rats for blood collection
see calendar for weigh schedule
PRE-EXPOSURE- REMOVE GRP A FOR SACRIFICE
POST-EXPOSURE- REMOVE GRP C ** RATS FOR BLOOD COLLECTION

APPENDIX F

QUALITY ASSURANCE

Quality Assurance Statement

Quality Assurance Statement

Teratology Study of Acetonitrile in Rats

Quality Assurance Statement

Listed below are the phases and/or procedures which were reviewed by the Quality Assurance Unit during the period, 1/1/91 - 3/31/91, and the dates the reviews were performed and findings reported to management. (All findings were reported to the study director or his designee at the time of the review.)

Phase/Procedure Reviewed	Review Date	Date Findings Submitted in Writing to Study Director/Management
Animal Receipt	1/3/91	1/9/91
Data	1/14 & 4/3-4/91	4/4/91
Animal Identification	1/22/91	1/23/91
Health Screen	1/28/91	1/31/91
Dosing	2/12/91	2/12/91
Body Weights	2/14/91	2/15/91
Teratology Examinations	2/22/91	2/22/91
Necropsy	2/22/91	2/22/91
Data	7/2-3, 8-9/91	7/16/91
Final Report	11/15-16/92 & 2/15/94	2/15/94

Deana Redetzke — Quality Assurance Specialist — 2/16/94 — Date

R A Gelmens — Quality Assurance Specialist — 2/16/94 — Date

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