ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)
FOR
ACRYLONITRILE
(CAS Reg. No. 107-13-1)

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PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Three levels - AEGL-1, AEGL-2 and AEGL-3 — are developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

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EXECUTIVE SUMMARY

Acrylonitrile (CAS Reg. No. 107-13-1) is a monomer used in the manufacture of acrylic fibers, synthetic rubber, resins, plastics, adhesives, and acrylamide. Acrylonitrile (AN) has a sharp onion-garlic odor. World-wide production is estimated at 4 to 4.5 million metric tons. A concentration range of 1.6 - 36.3 ppm has been reported as AN odor thresholds for humans.

Nonlethal effects of occupational exposure to AN include headache, nasal and ocular irritation, thoracic discomfort, nervousness and irritability. Available information indicates that these effects occur at 12-15 ppm regardless of exposure duration and resolve following removal from exposure. No signs or symptoms were reported for informed male volunteer subjects following exposure up to 4.6 ppm for 8 hours (Jakubowksi et al., 1987). Lethality following acute inhalation exposure to AN has been reported but no exposure terms are available. Limited information suggest that children may be more susceptible to the effects of acute inhalation exposure than adults.

Acute exposure data are available for several laboratory species (monkey, rat, dog, rabbit, guinea pig, cat) and demonstrate qualitatively similar responses ranging from mild irritation (redness of exposed skin, lacrimation, nasal discharge) and mild effects on ventilation and cardiovascular responses to severe respiratory effects, convulsions, and death. Four-hour exposure to concentrations ranging from 30 to 100 ppm produced little or no effect in most species tested but dogs appeared to be notably more sensitive exhibiting severe effects at the 100-ppm exposure level. Results of a recent nose-only exposure study in rats showed that concentrations up to 50 ppm for 6 hours or 225 ppm for 1.75 hours produced only minor transient effects on blood pressure. Lethality in rats appears to occur at cumulative exposure of 1800-1900 ppm•hrs for 0.5 to 6-hour exposure durations, although for nose-only exposures this is notably higher (~3800 ppm•hrs). Analysis of exposure concentration-duration data suggest a near linear relationship (i.e., $n \approx 1.1$ for $C^n \times t = k$). Results of studies in animals showed that lethality may be delayed especially at the lower limits of lethal exposures. One study provided evidence for teratogenic effects in rats following gestational exposure of dams to 80 ppm but not at 40 ppm AN. Another study showed an exposure-related decrease in fetal weight following gestational exposure of dams to 25, 50, or 100 ppm AN; no other reproductive/developmental effects were detected. Results of *in vitro* testing suggest that AN is weakly mutagenic. Results of *in vivo* mammalian cell assays measuring various endpoints were generally negative. Results of long-term inhalation exposure cancer bioassays have shown that AN is carcinogenic in rats. The brain, spinal cord, Zymbal's gland, tongue, nonglandular stomach, small intestine, and mammary gland have all been identified as targets.

AN toxicity appears to be directly related to its metabolism. Two major metabolism pathways have been described; conjugation with glutathione and epoxidation by microsomal cytochrome P4502E1 which forms 2-cyanoethylene oxide (CEO). Metabolites from both pathways are subject to additional biotransformation. The glutathione conjugate may form a mercapturic acid which is excreted in urine. CEO is further metabolized via conjugation with glutathione (catalysis with cytosolic GST or nonenzymatically) resulting in additional conjugates and via hydrolysis by microsomal epoxide hydrolase (EH). The secondary metabolites of CEO may also be further metabolized. Cyanide may be generated via the EH pathway and by one of the GSH conjugation products. Cyanide, in turn, is detoxified to thiocyanate via rhodanese-mediated reactions with thiosulfate.

Generally, the toxic effects following acute inhalation exposure to AN appear to be irritation of the respiratory tract and the metabolism of AN to cyanide. Acrylonitrile-induced neurological effects in laboratory animals appear to involve the parent compound and the cyanide metabolite. The pivotal role of cyanide has been clearly demonstrated. AN-induced convulsions, are likely the result of cyanide resulting from AN metabolism although recent work suggests that only the early seizures are cyanide-mediated and that severe clonic convulsions preceding death may be due to parent compound.

The AEGL-1 values were based on the absence of effects in informed human volunteer subjects (6 males) exposed for 8 hours to 4.6 ppm AN (Jakubowski et al., 1987). Industry reports noted that exposure to 12-15 ppm caused ocular irritation and headaches regardless of exposure duration. A 3-fold reduction (an appropriate adjustment for mild irritation effects) of the lower limit of this range is equivalent to the 4.6 ppm no-effect concentration reported by Jakubowski et al. (1987). Therefore, the 4.6 ppm value is recommended for all AEGL-1 exposure durations. In light of results of studies showing only mild effects (headache, nervousness, fatigue, nausea, and insomnia) following subchronic occupational exposure to AN levels possibly as high as 20 ppm, further reduction of the AEGL-1 values is not warranted.

The AEGL-2 values were based upon slight transient effects in rats exposed to 305 ppm AN for 2 hours (Dudley and Neal, 1942). The effects resolved within 12 hours post exposure. Analysis of occupational exposure effects indicated that routine exposure to 10-20 ppm (up to 2-higher than the 8-hr AEGL-2) resulted in complaints of headache, fatigue, nausea, and insomnia which were neither irreversible nor escape-impairing effects. Therefore, the critical effect upon which the AEGL-2 values are based is appropriate. The interspecies uncertainty factor was limited to 3 because PB-PK modeling has shown that predicted concentrations of AN and the metabolite CEO in blood and brain were similar in rats and humans exposed by inhalation. The intraspecies uncertainty factor was limited to 3 because the effects associated with acute irritation effects of AN are not likely to vary greatly among individuals and because metabolism may play only a limited role in the critical effects used as the basis for AEGL-2 derivation. Time scaling for developing AEGL-2 values from the 2-hour experimental POD to AEGL-specific exposure durations was performed using $C^n \times t = k$, where $n = 1.1$.

The AEGL-3 values were derived using 30-minute, 1-, 4-, and 8-hour $BMCL_{05}$ estimates of lethality threshold. Data for several AEGL-specific exposure periods were available from the reports by Apple et al. (1981) and Dudley and Neal (1942). A 30-minute $BMCL_{05}$ of 1748 ppm was calculated from the Appel et al. (1981a) data. The 1-hr, 2-hr, 4-hr, and 8-hr $BMCL_{05}$ values derived from lethality data published by Dudley and Neal (1942) are 1024.4, 491.3, 179.5 and 185.8 ppm, respectively, for rats exposed to various concentrations of AN for 1, 2, 4, or 8 hours. With the exception of the 4-hour value, the resulting $BMCL_{05}$ values are relationally consistent across time and the 30-minute, 1-hour, and 8-hour estimates were used to derive corresponding AEGL-3 values. Because the 4-hr value was not used due to the relational inconsistency, the 4-hour AEGL-3 value was derived by time-scaling the 8-hour $BMCL_{05}$ of 185.9 ppm. Although the dog appeared to be the most sensitive species, the overall database for rats is more robust thereby justifying use of the rat data. Further justification for limiting the interspecies uncertainty factor to 3 comes from PBPK models demonstrating that predicted concentrations of AN and the metabolite CEO in blood and brain were similar in rats and humans exposed by inhalation. The PBPK model for AN and CEO disposition in humans utilized human *in vitro* data and scaling from a rat model (Kedderis and Fennell, 1996) that incorporated major biotransformation and reactivity pathways. These included metabolism of AN to glutathione

conjugates and CEO, reaction rates of AN and CEO with glutathione and tissue components, and the metabolism of CEO by hydrolysis and glutathione conjugation. For effects resulting from a single acute exposure, an intraspecies uncertainty factor of 3 may be considered sufficient for accounting for variability in metabolism-mediated effects. Additional uncertainty factor application would result in incompatibility between AEGL-3 and AEGL-2 values.

Various inhalation unit risk values have been developed for acrylonitrile. IARC downgraded AN from category 2a to category 2b noting that data relative to human carcinogenicity are inadequate and that no causal association exists. Current data are sufficient for considering AN to be carcinogenic in animals following long-term inhalation exposure. That AN would induce a carcinogenic response in humans following a single, once-in-a-lifetime acute exposure is remote.

The AEGL values for acrylonitrile are summarized in the following table.

S 1. Summary of AEGL Values for Acrylonitrile (AN)

Classification	10-min	30-min	1-h	4-h	8-h	Endpoint (Reference)
AEGL-1 (Nondisabling)	4.6 ppm (10 mg/m^3)	4.6 ppm (10 mg/m^3)	4.6 ppm (10 mg/m^3)	4.6 ppm (10 mg/m^3)	4.6 ppm (10 mg/m^3)	No effect in volunteer human subjects exposed to 4.6 ppm for 8 hrs; UF=1x1 (Jakubowski et al., 1987)
AEGL-2 (Disabling)	290 ppm (630 mg/m^3)	110 ppm (240 mg/m^3)	57 ppm (120 mg/m^3)	16 ppm (35 mg/m^3)	8.6 ppm (19 mg/m^3)	Slight transient effects in rats exposed for 2 hrs to 3-5 ppm; UF=3x3; n=1.1 (Dudley and Neal, 1942)
AEGL-3 (Lethality)	480 ppm (1000 mg/m^3)	180 ppm (390 mg/m^3)	100 ppm (217 mg/m^3)	35 ppm (76 mg/m^3)	19 ppm (41 mg/m^3)	30-min, 1-hr, and 8-hr, $BMCL_{05}$ lethality threshold estimates in rats; UF=3x3; n=1.1 (Appel et al., 1981a; Dudley and Neal, 1942)

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1. INTRODUCTION

Acrylonitrile (CAS Reg. No. 107-13-1) is a monomer used in the manufacture of acrylic fibers, synthetic rubber, resins, plastics, adhesives, and acrylamide. Acrylonitrile (AN) has a sharp onion-garlic odor. Recent world-wide production has been estimated at 4 to 4.5 million metric tons (Collins et al., 2003; NPI, 2007). Production of acrylonitrile in the United States was 3.4 million pounds in 1996 (NTP, 2006).

TABLE 1. Chemical and Physical Data for Acrylonitrile (AN)

Parameter	Value	Reference
Synonyms	2-propenenitrile; vinyl cyanide; acrylonitrile monomer, cyanoethylene	O'Neil et al., 2001
Chemical formula	C_3H_3N	O'Neil et al., 2001
Molecular weight	53.06	O'Neil et al., 2001
CAS Registry No.	107-13-1	O'Neil et al., 2001
Physical state	Liquid	O'Neil et al., 2001
Solubility in water	73 g/L at 20 °C	American Cyanamid, 1959
Vapor pressure	100 torr @ 23EC 116 hPa	ACGIH, 1991 BASF AG, 1994
Specific gravity	0.8 @ 23EC	O'Neil et al., 2001
Melting point/boiling point	-83.55 EC @ 0EC/ 77.3EC @ 760 mm	O'Neil et al., 2001
Conversion factors in air	1 ppm = 2.17 mg/m^3 1 mg/m^3 = 0.46 ppm	

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

A child exposed overnight in a room fumigated with AN died. Vomiting, lacrimation, convulsions, respiratory difficulty, cyanosis, and tachyacardia were present. Five adults also in the room experienced little or no effect (see Section 2.2.) (Grunske, 1949). No exposure concentration-duration information was reported.

Loss of consciousness, convulsions, and respiratory arrest have been reported as outcomes of severe acute inhalation exposure to AN (Buchter and Peter, 1984). However, no exposure terms were available.

The death of a worker cleaning an AN-containing wagon at a train depot was attributed to exposure to the chemical (Bader and Wrbitzky, 2006). No exposure terms were available although liquid AN was present on the clothing of the individual. Cause of death was reportedly "blood circulation collapse".

2.2. Nonlethal Toxicity

The AIHA (1989) lists an odor threshold range of 1.6-22 ppm for AN while Ruth (1986) reported a range of 3.7-36.3 ppm.

Wilson et al. (1948) reported that exposure of workers handling "polymerizers" at concentrations of 16-100 ppm for 20-45 minutes experienced dull headaches, nasal and ocular irritation, discomfort in the chest, nervousness and irritability. Workers with notable poisoning

experienced nausea, vomiting, and weakness. Some developed mild jaundice, low-grade anemia, and leukocytosis. No exposure terms were provide for the workers with these more serious effects but all recovered upon removal from exposure. Additional reports (NAC/AEGL, pers. communication) affirmed that occupational exposure to 12-15 ppm resulted in ocular irritation and headache.

Five adults who spent the night in the room in which a child died of AN poisoning (see Section 2.1.), had no signs of poisoning or complained only of eye irritation (Grunske, 1949). No exposure concentration-duration information was reported.

Subchronic (-3 years) occupational exposure AN at concentrations ranging from 0.6 to 6.0 mg/m^3 (-0.3 to 3 ppm) produced headaches, insomnia, general weakness, decreased working capacity, and irritability (Babanov et al., 1959).

Lacrimation and visual disturbance were reported for some non-fatal exposures to AN (Davis et al., 1973). Although exposure concentrations were not reported, these effects were likely associated with very high AN concentrations.

In a report by Sakurai and Kusumoto (1972), the health records of 576 workers working in five AN fiber plants over a 10 year period were examined. Workers exposed to AN concentrations of 11 mg/m^3 (5 ppm) complained of headache, fatigue, nausea, and insomnia. There was a positive correlation with exposure time but not with the exposure concentration or age of workers. The report analyzed 4439 examinations acquired over 10 years prior to 1970. Two cohorts, one exposed to concentrations of AN of below 11 mg/m^3 (5 ppm) and the other exposed to less than 45 mg/m^3 (20 ppm) were considered. In a later report, however, Sakurai et al. (1978) stated that the study lacked adequate epidemiological design, the findings were based on routine health examinations, and the "exposure levels were not reliably reported" and may have been much higher. In this later appraisal it was noted that many of the symptoms were associated with exposures well in excess of 5 ppm. Sakurai et al. (1978) stated that their findings were not contradictory to those of Wilson et al. (1948) reflecting the older and less controlled workplace environment where levels could be up to 20 ppm.

Ocular irritation was a primary effect in a 24-year old man whose face, eyes and body were sprayed by AN (no exposure concentration data) explosively released from a defective valve (Vogel and Kirkendall, 1984). Mild conjunctivitis with no corneal clouding was reported. Results of fundascopic examination were normal.

A study was conducted to evaluate the metabolism and excretion of AN in human informed volunteer subjects (Jakubowski et al., 1987). The six volunteers (including the investigators) were all males aged 28-45. Being toxicologists, they were all aware of the toxic properties of AN. The subjects were exposed for 8 hours to AN vapors generated by a saturator immersed in a thermostat-controlled water bath and diluted with carrier air to produce the desired AN concentrations (5 or 10 mg/m^3; equivalent to 2.3 and 4.6 ppm, respectively). Airflow in the 11.7 m^3 chamber was approximately 200 m^3/hr. There were three 10-minute breaks from the exposure at 2, 4, and 6 hours. Gas chromatography was used to monitor the AN concentration every 15 minutes. No symptoms were reported by any of the subjects.

The World Health Organization (WHO, 1983) summarized various workplace studies (Zotova, 1975; Delivanova et al., 1978; Enikeeva et al., 1976; Ivanov, 1983).

Blepharoconjunctivitis was reported following exposure to 5 ppm AN. Other non-ocular symptoms were also reported.

Ginceva et al. (1977) reported no changes in the health status for a group of 23 men occupationally exposed to 1.9 to3.3 ppm AN for three to five years.

2.3. Developmental/Reproductive Effects

Developmental/reproductive toxicity of AN in humans is very limited. A reported decreased testosterone level in AN factory workers (Ivanescu et al., 1990) was confounded by concurrent exposure to other chemicals. No adverse effect was detected for gynecological health of 410 women occupationally exposed to AN (no exposure terms) compared to 436 unexposed women (Dorodnova, 1976). Czeizel et al. (1999) reported on the rate and type of congenital abnormalities in 46,326 infants born to mothers living within a 25 km radius of an AN factory in Hungary. Significant clusters of pectus excavatum (depressed sternum), undescended testes, and clubfoot were noted. The authors, however, reported that the overall results supported the null hypothesis for AN-induced effects in people living in the vicinity of the AN factory.

2.4. Genotoxicity

2.4.1. *In Vitro* Studies

In experiments with human lymphocytes, Perocco et al. (1982) showed that exposure of human lymphocytes to 0.5 mM AN (26.5 µg/ml) resulted in a significant increase in SCE. Obe et al. (1985), however, was unable to demonstrate SCE-induction by AN in human lymphocytes exposed for 24 hours to AN at concentrations of 1 or 10 µg/ml in the absence of S9 and for one hour in the presence of S9 from Arochlor-induced rat livers.

Rizzi et al. (1984) examined the incorporation of [$_3$H]TdR into DNA in HeLa cells. The test groups inlcuded a control and AN-treated cells without hydroxyurea (-HU), and control and treated cells treated with hydroxyurea (+HU). The -HU/+HU relationship between treated and control cells and the value of +HU between treated and control cells were statistically significant at AN dose levels of 0.18 ($p < 0.01$) and 0.036 mM ($p < 0.09$). It was concluded that AN is mutagenic and genotoxic at very low concentrations. Contrary to this, Martin and Campbell (1985) failed to demonstrate unscheduled DNA repair in HeLa cells.

AN produced positive results in tests with human lymphoblasts (TK6, *TK* locus) both with and without metabolic activation (Crespi et al.,1985). Tests were conducted at AN concentrations of 5 - 50 µg/ml for three hours in the presence of S9 (from Arochlor-induced rat livers) or for 20 hours without S9. There was a 3.5-fold increase in mutational frequency in the presence of S9 at 40 and 50 µg/ml. In the absence of S9, mutational frequency was increased 2-fold at 15µg/ml and 1.3-fold at 20 µg/ml (compared to controls).

Crespi et al. (1985) also conducted tests using the AHH-1 cell line (HGPRT locus). Concentrations of AN were 5 - 25 µg/ml for 28 hours. Test were conducted with metabolic activation and an expression period of 6 days. An approximate 4.5-fold increase in mutation frequency at 25 µg/ml was detected relative to controls which was similar to the response obtained with the benzo(a)pyrene (3.1 µg/ml) positive control.

The mutagenic potential of both AN and its metabolite 2-CEO (2-cyanoethylene oxide) was examined using the TK human lymphoblast cell line (with and without S9) with heterozygous thymidine kinase (*tk*) locus as the marker (Recio et al.,1989). Cells were exposed for two hours with an expression period of 6-8 days. AN was not mutagenic in the absence of S9 (less than a 2-fold increase in mutation frequency) over a concentration range of 0.4 to 1.5 mM (21 to 80 μg/ml). With S9, there was a statistically significant ($p<0.05$) 4-fold mutagenic response with the highest exposure concentration 1.5 mM (74 μg/ml). Survival was only 10% at a concentration of 1.5 mM. The metabolite peroduced a 17-fold increase in mutation frequency without S9 at 100 μM. The results indicated AN to be weakly mutagenic in mammalian cells, while the mutagenic response induced by CEO suggests that it may be the primary mutagenic metabolite of AN. In a follow-up study (Recio et al., 1990), human TK6 lymphoblasts were treated with CEO (150 uM for 2h). Base-pair substitution mutations and frameshift mutations were observed.

Sister chromatid exchange (SCE) and the induction of DNA single breaks was examined using adult human bronchial epithelial cells (Chang *et al.*, 1990). The cultures were exposed for 20 hours to 150, 300, 500, or 600 μg/ml An and assessed for SCE and DNA strand breaks. Notable cytotoxicity was observed at 600 μg/ml, but not at the lower concentrations. SCEs were significantly increased ($p < 0.01$) at 150 and 300 μg/ml; incidence of SCE per cell was 6.6 and 10.7 respectively (3.7 in unexposed controls). The extent of DNA single strand breaks appeared to be positively correlated with AN concentrations.

A human mammary epithelial cell (HMEC) DNA repair assay in secondary cultures of HMEC was reported by by Eldridge et al. (1992). The cultures of normal HMEC were derived from mammoplasties of five healthy women. Although CEO was cytotoxic to HMEC, a positive UDS response was produced thereby confirming its genotoxicity. AN exhibited considerable cytotoxicity but no genotoxicity was observed in the HMEC DNA repair assay.

2.4.2. *In Vivo* Studies

Chromosomal damage in peripheral lymphocytes of 18 workers exposed to AN for an average of 15.4 years was studied by Thiess and Fleig (1978). The workers were also exposed to styrene, ethylbenzene, butadiene, and butylacrylate. The actual AN exposure was not reported. Air concentrations of AN over approximately 10 years averaged 5 ppm and were reportedly representative of normal operating conditions. During the actual conduct of the study workplace AN levels were about 1.5 ppm. The frequency of chromosomal aberrations in peripheral lymphocytes of the workers was not increased compared to the unexposed controls.

Borba et al. (1996) reported chromosomal aberrations and SCEs in 14 workers employed in the polymerization area and in 12 maintenance workers of an acrylic fiber plant. A control group consisted of 20 unexposed workers in administration jobs. No AN exposure concentration or exposure duration terms were provided. No difference in SCEs was detected when the exposed groups and the controls were compared.

2.5. Carcinogenicity

Numerous studies have been conducted to assess the potential carcinogenicity of AN. These have been previously reviewed (Felter and Dollarhide,1997; Sapphire Group, Inc., 2004). Because a carcinogenic response is unlikely following a single acute once-in-a-lifetime exposure, an extensive review of the available information on this subject is considered beyond the scope of this document.

Following extensive analysis of the epidemiology studies (occupational cohort studies, supporting cohort and case-control studies), it has been concluded that many of the older studies had methodological or design weaknesses (e.g., insufficient sample size, insufficient or incomplete follow-up, inadequate exposure assessment, confounding factors such as simultaneous exposures and smoking habits for which there were no controls) and that the results of the studies did not provide adequate evidence that AN is carcinogenic in humans at current occupational exposure levels or at lower levels that would be characteristic of environmental exposures (Sapphire Group, Inc., 2004). In the evaluation it was also noted that results of more recent studies (Benn and Osborne, 1988; Blair et al., 1988; Wood et al., 1988; Swaen et al., 1998, 2004) supported this conclusion and that meta-analysis (Rothman, 1994; Collins and Acquavella 1998, EU, 2001) affirmed that cancer risk associated with AN exposure is extremely low.

Felter and Dollarhide (1997) concluded that the human weight of evidence for the carcinogenicity of AN is insufficient. Evaluations of recent literature indicate that the weight of evidence from human studies does not support the conclusion that there is a causal association between exposure to humans and lung cancer. A $1x10^{-4}$ risk specific concentration of 9 Fg/m^3 was derived based upon the LED_{10}.

The disparity between findings from laboratory animal bioassays and human epidemiological findings was evaluated by Ward and Starr (1993). According to the US EPA estimates derived from animal studies (based on USEPA's potency estimates from their 1983 assessment), lifetime exposure to 1 µg/m^3 AN translates into an increased cancer risk of 1 in 6,700 people ($6.7 H 10^{-3}$) and into an increased risk of brain cancer of 1 in 12,000 people ($1.2 H 10^{-4}$). Assuming that workers in older studies were exposed to an average level of 2 to 5 ppm AN during their working lifetime, they determined the statistical power of the AN epidemiological studies was high enough (>80%) to reliably detect the USEPA predicted increases of cancer due to occupational AN exposure. However, these predicted increases were not found in any of the epidemiological studies. The authors concluded that the upper bound estimate of the AN inhalation cancer potency as estimated by the USEPA was too high to be consistent with the human experience in occupational exposure situations.

IARC downgraded AN from a category 2a to a category 2b (IARC, 1999). This status change was based upon the lack of carcinogenic evidence from the more recent epidemiological studies. The data regarding potential carcinogenicity of AN in humans is considered to be inadequate and no evidence of a causal association exists. This decision supports the conclusion that AN is probably not carcinogenic to man.

2.6. Summary

A concentration range of 1.6 - 36.3 ppm has been reported as AN odor thresholds for humans. Nonlethal effects of occupational exposure to AN include headache, nasal and ocular irritation, thoracic discomfort, nervousness and irritability but definitive exposure-response data are lacking. Available information indicates that such effects resolve following removal from exposure. No signs or symptoms were reported for male volunteer subjects following exposure up to 4.6 ppm for 8 hours. Lethality following acute inhalation exposure to AN has been reported. Although no exposure terms are available and information is limited, children appeared to be more susceptible than adults in the same exposure conditions.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Monkey

Rhesus monkeys (4.2-4.8 kg) were exposed to 65 ppm AN (2 males and 2 females) or to 90 ppm (2 females) for 4 hours (Dudley and Neal, 1942). The AN test atmosphere was generated by bubbling air through AN (purity determined through repeated fractional distillations free of cyanide and with a boiling point of 76-77EC) and mixing this AN-saturated air stream with a main air stream. Air flow through the exposure chamber was 260 L/min ("2%). The concentration of the AN was varied by adjusting the volume of air passing through the bubbler. The concentration of AN in the chamber was determined by the change in weight of the AN in the bubbler, air flows and start/stop times. Even at the highest exposure (90 ppm), the rhesus monkeys (all individuals in this exposure group) exhibited only slight redness of the face and genitals, and a slight increased in respiratory rate upon initial exposure.

Dudley et al. (1942), exposed four rhesus monkeys to 56 ppm (average concentration) of AN 4 hours/day, 5days/week for four weeks. All four monkeys survived and showed no evidence of toxicity during the four week exposure period.

3.1.2. Rat

Dudley and Neal (1942) conducted single exposure experiments in which groups of 16 Osborne-Mendel rats (-295 g, gender not specified) were exposed for 0.5, 1, 2, 4, or 8 hours to various concentrations of acrylonitrile (Table 2). Details regarding generation of the test atmospheres are provided in the preceding paragraph (Section 3.1.1.). Responses included initial stimulation of respiration followed by rapid shallow respiration. Above 300 ppm, rats started exhibiting signs of ocular and nasal irritation. Rats exposed to any concentration of AN exhibited flushing (reddening) of the skin, nose, ears, and feet. Prior to death, the rats were gasping and convulsing. Gross pathology findings of dead rats revealed bright red lungs of “normal consistency” and dark red blood. Rats which survived any acute exposure to AN exhibited no residual effects. Results of the experiments are summarized in Table 2.

TABLE 2. Toxicity of AN Vapor In Rats Exposed for 0.5 to 8 Hours.

Exposure Time (hrs)	Exposure Conc. (ppm)	Mortality (%) During Exposure	Total Mortality (%)	Effects[a]
0.5	2445	0	0	Marked; slight residual effects to 24 hrs
	1490	0	0	Marked; no residual effects in 24 hrs
	1270	0	0	Marked; no residual effects in 24 hrs
	665	0	0	Moderate transitory effects
1	2445	0	81	Deaths in 4 hrs; slight effects at 24 hrs in survivors
	1490	0	25	Deaths in 4 hrs; slight effects at 24 hrs in survivors
	1270	0	0	Marked effects; slight effects at 24 hrs; normal at 48 hrs
	665	0	0	Marked transitory effects
2	1260	0	100	Fatal; deaths within 4 hrs
	595	0	6	Marked transitory effects
	305	0	0	Slight transitory effects
4	635	50	100	Fatal
	315	25	31	Marked; no effects in survivors at 24 hrs
	130	0	0	slight transitory effects
8	320	94	94	Fatal
	270	44	44	Marked; no effects in survivors at 24 hrs
	210	6	6	Marked transitory effects
	135	0	0	Moderate transitory effects
	90	0	0	Slight discomfort

[a]Nonlethal effects included initial rapid respiration followed by rapid shallow breathing; prior to death animals exhibited slow, gasping respiration, convulsions, followed by coma.
Dudley and Neal, 1942.

In another phase of the study by Dudley and Neal (1942), rats (16/group) were exposed for 4 hours to 635, 315, 130, or 100 ppm (Table 3). Exposure to 130 ppm slight transient effects and no lethality. Effects were similar to those described in the preceding paragraph. Exposure to 315 ppm resulted in 31% mortality and exposure to 635 ppm produced 100% mortality.

TABLE 3. Toxicity of AN Vapor in Rats Exposed For 4 Hours.

Exposure Conc. (ppm)	Mortality (%) During Exposure	Total Mortality (%)	Effects
635	50	100	Death occurred in 2-6 hrs
315	25	31	Marked effects; no residual effects in survivors
130	0	0	Slight transitory effects
100	0	0	Slight transitory effects

Dudley and Neal, 1942

In a lethality study conducted at Haskell Laboratory (du Pont & Co., 1968), groups of adult male ChR-CD rats (248-268 g) were exposed to AN for 4 hours. The test chamber atmosphere was analyzed at least every half hour by gas chromatography. Test animals were observed for 14 days. During exposure the rats exhibited irregular respiration, hyperemia, lacrimation, tremors, convulsions. Deaths occurring during exposure occurred within 2-4 hours after the start of the exposure. Deaths occurring after exposure occurred between 7 minutes and 18 hours. A 4-hr LC_{50} of 333 ppm (275-405 ppm 95% confidence interval) was reported. Rats surviving the exposure exhibited mild to severe, dose-related weight loss the first day of observation followed by normal weight gain.

Appel et al. (1981a) provided lethality data for groups of 3 to 6 male Wistar rats exposed to AN for 30-180 minutes with exposure concentration varying with exposure duration (Table 4). In this study (designed to assess potential antidotes for acute AN toxicity), AN vapor was generated by evaporating AN (99.5% purity) in a halothane vaporator and adjusting the AN vapor concentration with clean filtered air. Vapor concentration was determined by gas chromatography.

TABLE 4. Lethal Response of Rats Exposed to AN at Various Exposure Concentration/Durations.

Exposure conc. (ppm)	Exposure duration (min)	Mortality ratio
650	180	1/3
950	120	1/3
1100	120	3/3
1600	30	0/3
2600	30	1/3
3000	30	6/6
2400	10	0/3

Appel et al. (1981a).

In a rat study reported by Vernon et al. (1990), a group of 10 adult Sprague-Dawley rats (5/gender) was exposed for 1 hour to 1,080 ppm AN. None of the rats died. Clinical signs reported included rapid shallow breathing, decreased activity, nasal discharge, salivation, lacrimation and coma (in 3 of 10 animals). The extremities of all animals were red at 37 minutes into the exposure. All rats recovered within five minutes of exposure termination.

A GLP-OECD guideline study sponsored by the Shanghai SECCO Petrochemical Company, Ltd. examined the acute toxicity of AN in rats (WIL Research Laboratories, 2005). In this study, groups of 5 male and 5 female Crl:CD®(SD) rats (8-12 weeks old; 242-297 g) were exposed (nose-only) for 4 hours to 539, 775, 871, 1006, or 1181 ppm AN (99.9 % purity). The rats were acclimated for 7 days prior to exposure and observed for 14 days after exposure. Exposure was in a two-tiered conventional nose-only exposure system where exposure atmosphere conditions (temperature, oxygen, humidity, etc.) were monitored every 20-30 minutes. The AN test atmosphere was generated by passing compressed nitrogen through the test material to create a vapor which was diluted with compressed air prior to being delivered to the exposure system. Actual AN concentrations were determined by gas chromatography. Mortality data are summarized in Table 5. The report provided 4-hour LC_{50} values of 964 ppm (857-1085 95% c.i) for males, 920 ppm (807-1050 95% c.i) for females, and 946 ppm (866-1032 95% c.i.) combined (determined by the method of Litchfield and Wilcoxon, 1949).

TABLE 5. Lethality in rats following nose-only inhalation exposure to AN for 4 hours

Exposure Conc. (ppm)	Mortality During Exposure		Total Mortality		Comments
	M	F	M	F	
539	0	0	0	0	
775	0	0	0	0	
871	0	0	1	3	Deaths at 0 to 1 day postexposure
1006	1	1	3	4	2 ( ), 3 ( ) at 0 to 1 day postexposure
1181	4	3	5	4	1 ( ), 1 ( ) at 0 to 1 day postexposure

WIL Research Laboratories, 2005

Clinical observations immediately following exposure included tremors, ataxia, labored respiration, hypoactivity, decreased defecation, and gasping but there was no apparent exposure concentration-effect relationship. Necropsy findings in dead rats included the presence of a distended, gas-filled jejunum in one female of the 871-ppm group, distended gas-filled stomach in three females in the 871-ppm and 1006-ppm groups, and dark, discoloration of the lungs in one male and one female in the 1181-ppm group. No other findings were noted for rats that died. At scheduled sacrifice, the only finding was dark discoloration of the lungs in one male of the 871-ppm group.

3.1.3. Dog

In their assessment of AN lethality in multiple species, Dudley and Neal (1942) also exposed groups of 2-4 male and female dogs (5.5-12.0 kg; strain not specified) to various AN concentrations for 4 hours (Table 6). The investigators found dogs to be more sensitive to inhaled AN; exposures producing only minor effects in other species caused coma and death in the dogs.

TABLE 6. Toxicity of AN Vapor In Dogs Exposed for 4 Hours.

Exposure Conc. (ppm)	Gender	Effects
30	F	Slight salivation by end of exposure period; no other effects
	F	Slight salivation by end of exposure period; no other effects
	F	Slight salivation by end of exposure period; no other effects
	F	Slight salivation by end of exposure period; no other effects
65	F	Severe salivation; weak by end of exposure
	F	Coma by end of exposure; died at 8 hrs
100	M	Severe salivation during exposure; full recovery within 24 hrs
	F	Convulsions at 2.5 hrs; coma by end of exposure; partial paralysis of hind legs for 3 days
	F	Convulsions at 2.5 hrs; coma by end of exposure; full recovery within 48 hrs
110	F	Coma at end of exposure; dead at 4.5 hrs
	M	Coma at end of exposure; dead at 3 days
	F	Coma at end of exposure; food refusal for 10 days; slowly recovered
165	F	Convulsions at 2 hrs; dead at 3 hrs of exposure
	M	Coma from end of exposure to death at 4 hrs.

Dudley and Neal, 1942

Results of a 4-week repeat exposure experiment using two dogs exposed to an average concentration of 56 ppm AN for 4 hours/day was reported by Dudley et al. (1942). After the first four hour exposure, one dog died in convulsions while the second dog developed a transient paralysis of the hind legs after the 5th, 13th and 14th exposure. Subsequent exposures were well tolerated.

3.1.4. Guinea pig

Results of 4-hour exposure experiments with guinea pigs (8-16 per group; - 695 g) are shown in Table 7 (Dudley and Neal, 1942). Neither redness of the skin nor eyes was observed in guinea pigs as it was in other species. Exposure to AN did cause watering of the eyes, nasal discharge, and coughing. As exposure increased, coughing was accompanied by moist breath

sounds. Exposures that were lethal in dogs had very little effect on guinea pigs. Delayed death (3-6 days post exposure) was attributed to pulmonary edema.

TABLE 7. Toxicity of AN Vapor in Guinea Pigs Exposed for 4 hours

Exposure Conc. (ppm)	Mortality (%) During Exposure	Total Mortality (%)	Effects
100	0	0	Slight to no effect
265	0	0	Slight transitory effect; reduced feed consumption for 4 days
575	25	63	Ocular and nasal irritation during exposure; delayed death (3-6 days) probably from pulmonary edema
1160	13	100	5 Dead within 1.5 hrs post exposure; 2 dead at 18 hrs

Dudley and Neal, 1942

3.1.5. Cat

In the study by Dudley and Neal (1942), groups of 2-4 cats (gender not specified; -3.6 kg) were exposed to AN for 4 hours. Exposure to 100 ppm produced only salivation and slight transient effects (redness of the skin and mucosae) while exposure to 275 ppm resulted in more severe effects (marked salivation, signs of pain) but no deaths. At 600 ppm, 100% mortality (preceded by convulsions) occurred within 1.5 hour following exposure.

Four cats were exposed to 56 ppm AN (average concentration) 4 hours/day, 5 days/week for 8 weeks (Dudley et al., 1942). The cats occasionally vomited, were lethargic, and lost weight. One cat developed a transitory weakness of the hind legs after the 3rd exposure and died after the 11th exposure. The remaining three cats survived the entire exposure period with minimal effects.

3.1.6. Rabbit

In the Dudley and Neal (1942) report, groups of 2-3 albino rabbits (gender not specified; -4.5 kg) were exposed to AN for 4 hours. Signs of exposure were similar to those observed for rats but the rabbits appeared to be more susceptible to AN-induced lethality. Exposure to 100 or 135 ppm produced slight to marked transitory effects. Exposure to 260 ppm killed 1 of 2 rabbits during exposure while the second died within 4-5 hours. Exposure to 580 ppm resulted in a similar response with the second rabbit dead within 3-4 hours.

In an 8-week repeat exposure study, three rabbits were exposed for 4 hours/day, 5 days/week to 100 ppm AN (average concentration) (Dudley et al., 1942). The rabbits survived for the full exposure duration, but were drowsy and listless during exposure and gained no weight gain. No additional effects were observed.

3.2. Nonlethal Toxicity

3.2.1. Monkey

No evidence of toxicity was observed in rhesus monkeys (four per group; gender not specified) exposed to 56 ppm AN (126 mg/m^3) four hours/day, five days/week for 4-weeks

(Dudley et al., 1942). A slight increase in respiration upon initial exposure was the only effect reported for 2 male and 2 female monkeys exposed for 4 hours to 65 ppm AN (Dudley and Neal, 1942). In the same study, two female monkeys exposed to 90 ppm AN for 4 hours exhibited slight weakness, redness of the face and genitals, and a slight increase in respiratory rate. These effects resolved within 12 hours post exposure. Details regarding generation of the test atmospheres are provided in Section 3.1.1.

3.2.2. Dog

In a preliminary investigation into the toxicity of AN (Haskell Laboratory, 1942), exposure of 3 dogs (strain, gender, age, weight not specified) for 6 hours to 25 ppm AN caused a rise in body temperature of at least 2EF. Exposure to 50 ppm resulted in a drop in body temperature of as much as 1.6EF. Three dogs were exposed for 1.75 hours to 225 ppm AN. Two of three dogs exhibited an initial marked increase in pulse rate followed by a decrease. Blood pressure increased in 2 of 3 dogs and decreased in a third dog. Overt signs of exposure included ocular and nasal irritation, vomiting, incoordination, and "noisy" respiration. All dogs recovered within 24 hours.

Four dogs exposed to 30 ppm for 4 hours showed only slight salivation (Dudley and Neal, 1942). Severity of effects increased with increasing concentration. Exposure to 65 ppm produced weakness in one dog and coma in another while exposure to100 ppm resulted in convulsions in two of three dogs (see Table 6, Section 3.1.4). All of the dogs in these exposure groups fully recovered within 48 hours or less. Details regarding generation of the test atmospheres for these experiments are described in Section 3.1.1.

3.2.3. Cat

In the study by Dudley and Neal (1942), groups of 2-4 cats (gender not specified; -3.6 kg) were exposed to 100 ppm AN for 4 hours exhibited only salivation and slight transient effects (redness of the skin and mucosae) while exposure to 275 ppm resulted in more severe effects (marked salivation, signs of pain) but no deaths.

3.2.4. Rat

Dudley et al. (1942) exposed 16 rats to an average concentration of 100 ppm AN 5 days/week for 8 weeks. Slight lethargy during exposure was the only adverse effect observed. During the test period, 3 of the 7 females gave birth and raised normal litters.

Results of a study by Bhooma et al. (1992) study demonstrated fibrin network formation in the lung of 6 male Wistar rats exposed to 100 ppm AN 5 hours/day for 5 days and observed for 28 days. Alveolar macrophage activity was elevated from post exposure day 1 to day 14 and returned to normal by day 28. Procoagulant activity in lavage fluid was unaltered for the first 5 days but elevated when assessed at days 14 and 28.

In the Dow Chemical study (Quast et al., 1980) study, rats exposed 6 hours/day, 5 days/week to 80 ppm AN exhibited "minimal changes microscopically in the respiratory epithelium of the nasal turbinates of 80 ppm rats suggestive of slight degree of irritation" at the 6- month interim sacrifice interval. There was no mention of adverse effects associated with the 20-ppm exposure.

In the study by WIL Research Laboratories (2005), vocalization upon handling was reported for rats exposed (nose-only) to 539 ppm for 4 hours. Some rats exposed to 775 ppm exhibited ataxia, labored breathing, hyperactivity, and decreased urination and defecation during or after exposure. The rats in both groups were normal within 2 days (539-ppm group) or 8 days (775-ppm group) after exposure.

3.2.5. Rabbit

In the Dudley and Neal (1942) report, groups of 2-3 albino rabbits (gender not specified; -4.5 kg) exposed to 100 or 135 ppm AN for 4 hours produced slight to marked transitory effects in respiratory pattern and signs of irritation.

3.2.6. Guinea pig

Dudley et al. (1942) also exposed 16 guinea pigs to an average concentration of 100 ppm AN days/week for 8 weeks. The guinea pigs gained weight moderately and exhibited slight lethargy during the exposure but no other adverse signs were observed.

3.3. Developmental/Reproductive Effects

In a developmental toxicity study conducted by Murray et al. (1978), groups of 30 pregnant Sprague-Dawley rats were exposed to 0, 40, or 80 ppm AN (>99 purity) 6 hrs/day on gestation days 6 through 15. The exposure levels were selected based upon the TLV (20 ppm) and preliminary results of a long-term inhalation toxicity study. Clinical signs (daily), maternal body weight, feed consumption were monitored and gross necropsies were performed. Standard developmental parameters were assessed. Gender and body weight, external abnormalities and skeletal and soft-tissue anomalies of fetuses were evaluated. The rats were exposed in stainless steel and glass Rochester type chambers (4.3 m^3) with dynamic airflow conditions. The AN vapor was generated by metering AN into an airstream. The test atmosphere was analyzed by gas-liquid chromatography three times per day. Time-weighted mean concentrations of AN were 40"2 and 77"8 ppm (mean " s.d.)

Results of the Murray et al. (1978) study are summarized in Tables 8, 9, and 10. No treatment-related signs of toxicity were observed during the exposure period. Mean body weight and maternal body weight gain was significantly decreased during treatment in both dose groups. Relative to controls, food consumption was decreased during gestation days 15-17 but increased on days 18-20. Maternal liver weight was unaffected by AN exposure. Pregnancy incidence, mean litter size, incidence of resorptions and average fetal body measurements were unaffected by exposure to AN. A significant ($p<0.06$) increased incidence of total malformations was detected for litters from the 80-ppm group. Specific malformations included short tail, short trunk, missing ribs, delayed ossification of skull bones, omphalocele and hemivertebrae; observed only in the 80 ppm treatment group. Although the incidence of malformations was not statistically increased compared to the control group, these high-dose effects were considered to be exposure-related, because of similar findings in a gavage study by Murray et al. (1976). The investigators concluded that the data suggested a teratogenic effect of AN at 80 ppm but that there was no evidence of teratogenicity or embryotoxicity in rats exposed to 40 ppm.

TABLE 8. Maternal Toxicity Among Rats Exposed by Inhalation To Acrylonitrile (AN)			
Parameter	**Exposure concentration**		
	0 ppm	**40 ppm**	**80 ppm**
No. deaths/no. females	0/40	0/38	0/40
% pregnant (no.)	88 (35)	97 (37)	90 (36)
Additional pregnancies (detected by stain)	0	0	0
Body weight gain of dams	19"5	1"6*	-5"10*
g.d. 6-9	43"8	32"14*	31"17*
g.d. 10-15	82"12	84"22	92"15
g.d. 16-20			
Liver weight (g.d. 21)	16.0"1.8	15.9"1.8	15.3"1.6
Abs. (g)	38.6"2.9	41.3"3.1	40.3"4.3
Rel. to b.w. (g/kg)			

* $p<0.05$

Murray et al., 1978

TABLE 9. Litter Data for Pregnant Rats Exposed to Acrylonitrile (AN) Vapor			
Parameter	**Exposure concentration**		
	0 ppm	**40 ppm**	**80 ppm**
No. of litters	33	36	35
Implantations/dam	13"2	13"2	12"3
Live fetuses/litter	13"2	12"2	12"3
Resorptions/litter	0.6"0.7	0.7"1.1	0.5"0.6
Fetal b.w. (g)	5.79"0.33	5.72"0.42	5.90"0.25
Fetal crown-rumplength (mm)	43.9"2.1	43.5"2.2	43.7"2.2

Murray et al., 1978

TABLE 10. Incidence of Fetal Malformations Among Litters of Rats Exposed To Acrylonitrile (AN) Vapor			
Parameter	**Exposure concentration**		
	0 ppm	**40 ppm**	**80 ppm**
No. fetuses/No. litters examined			
External & skeletal malformations	421/33	441/36	406/35
Visceral malformations	140/33	148/36	136/35
No. fetuses (litters) affected			
External malformations			
Short tail	0(0)	0(0)	2(2)
Short trunk	0(0)	0(0)	1(1)
Imperforate anus	0(0)	0(0)	0(0)
Omphalocele	0(0)	1(1)	1(1)
Visceral malformations			
Right-sided aortic arch	0(0)	0(0)	0(0)
Missing kidney, unilateral	0(0)	0(0)	0(0)
Anteriorly-displaced ovaries	0(0)	0(0)	1(1)
Skeletal malformations			
Missing vertebrae (associated with short tail)	0(0)	2(1)	2(2)
Missing two vertebrae and a pair of ribs	8(1)	2(1)	7(2)
Hemivertebra	0(0)	0(0)	1(1)
Total malformed	8(1)	3(2)	11(6)*

*$p<0.06$

Murray et al., 1978

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In a comparative study of the relative reproductive/developmental toxicities of aliphatic mononitriles, Saillenfait et al. (1993a) exposed groups of 20-23 pregnant Sprague-Dawley rats to 0, 12, 25, 50 or 100 ppm AN (>99% purity) by inhalation for 6 hrs/day on gestation days 6 through 20, and euthanized on day 21. Clinical signs of toxicity, maternal body weight, and feed consumption were monitored, and gross necropsies were performed. Fetal examinations included gender ratio and body weight, external abnormalities and skeletal and soft-tissue anomalies. The rats were exposed to the test article in 200 liter stainless steel chambers (23 EC, 50% rel. humidity) with dynamic and adjustable laminar air flow (10-20 m^3/hr). The AN vapor was generated by bubbling air through a flask containing AN, the concentration in the chamber being calculated from the ratio of the amount of AN vaporized to the total chamber air flow during the test period. Concentration of AN was determined analytically by hourly sampling and gas-liquid chromatography.

There were no maternal deaths, but a concentration-dependent decreased absolute body weight gain was observed (significant at $p<0.01$ at three highest dose groups; 25.1g, 16.1 g, -0.1 g, -7.8 g, and -24.3 g, respectively for the 0, 12, 25, 50, and 100 ppm groups). There was no adverse effect on pregnancy rate, average number of implantations or number of live fetuses, incidences of non-surviving implants and resorptions, or fetal sex ratio (Table 11). A statistically significant ($p<0.01$ to 0.005; see Table 11) exposure-related reduction in fetal weights was observed at 25 ppm and higher concentrations. Evaluation of external, visceral and skeletal variations in the fetuses revealed no AN-related effects. The NOAEL for maternal and developmental toxicity was 12 ppm based on the absence of fetal body weight effect.

TABLE 11. Reproductive parameters in rats exposed to acrylonitrile (AN) vaporon gestation days 6-20.

Parameter	0 ppm	12 pm	25 ppm	50 ppm	100 ppm
No. deaths of treated females	0/20	0/21	0/21	0/20	0/21
% Pregnant at euthanization	100.0	95.2	95.2	90.0	90.5
No. examined litters	20	20	20	18	19
Implantations sites[a]	13.65"2.81	14.80"1.99	14.40"3.38	15.11"2.00	14.37"2.17
Live fetuses/litter[a]	12.30"4.09	14.00"2.18	13.85"3.26	14.50"1.89	13.63"2.22
% Non-surviving implants/litter[a]	10.40"22.75	5.44"7.38	3.49"6.10	3.89"5.37	4.94"8.33
% Resorption sites/litter[a]	10.40"22.75	5.11"6.46	3.49"6.10	3.89"5.37	4.94"8.33
Fetal sex ratio (M:F) %	1.05	0.96	1.23	1.10	0.96
Fetal b.w **%** **&**	5.95"0.28 5.66"0.36	5.79"0.28 5.51"0.27	5.64"0.36** 5.37"0.28*	5.54"0.24** 5.18"0.25**	5.04"0.36** 4.90"0.49**

[a] Mean " s.d.
* $p<0.05$; **$p<0.01$
Saillenfait et al., 1993a

3.4. Genotoxicity

The gentotoxicity of AN in animal test systems has been extensively reviewed (Sapphire Group, 2004). AN has been shown to be weakly mutagenic in *Salmonella typhimurium* but metabolic activation (S9) appears to be required. In *Escherichia coli* and in rodent test systems, metabolic activation is not required, but tends to enhance a weak AN-induced response. Results from most *in vivo* mammalian cell assays (chromosome aberration induction in mouse and rat

bone marrow micronucleus, and sister chromatid exchange induction in mice, and induction of dominant lethal mutations in rat and mouse sperm) were negative. However, positive results were detected in a variety of *in vitro* assays although *in vivo* clastogenicity was not demonstrated. In the Sapphire Group assessment, it was suggested that variability in results between *in vitro* and *in vivo* tests for AN-induced chromosomal damage may be a function of the metabolism of AN in intact animals versus that in cultured cells. Specifically, metabolism of 2-cyanoethylene oxide may be less efficient in animals, or there may be rapid detoxification and elimination of 2-cyanoethylene oxide, thereby limiting the interaction with DNA. Considering the positive results for genotoxicity from *in vitro* studies and the generally negative results from *in vivo* studies, the evidence of AN-induced genotoxicity animals is limited.

3.5. Carcinogenicity

A 12-month cancer bioassay was conducted by Maltoni et al. (1977). In this study groups of 30 male and 30 female rats were exposed by inhalation to 5, 10, 20, or 40 ppm of AN for 4 hours/day, 5 days/week. A group of rats exposed to clean air served as the control group. The rats were observed until death. Body weight was unaffected by the AN exposure. There was statistically significant increase in the percentage of animals with benign and malignant tumors ($P < 0.01$) and malignant tumors alone ($P < 0.01$). The total malignant tumors per 100 animals was noted for several treated groups, but lacked a definitive dose-response relationship. There was no increase in Zymbal's gland tumors, extrahepatic angiosarcomas, or hepatomas. Encephalic glioma incidence was increased in rats exposed to 20 ppm (3.3%; 3/60) and 40 ppm (5%; 3/60). Although not statistically significant, this response was considered by the investigators to be of possible biological relevance because the brain was shown to be a target organ in the oral administration part of the study.

Maltoni et al (1988) also conducted experiments in which groups of 54 breeder female rats (Group I) were exposed to 60 ppm AN 4 hours/day, 5 days/week for 7 weeks followed by 7 hours/day, 5 days/week for 97 weeks. A group of 54 untreated breeder female rats served as controls (Group II). Following transplacental exposure in the pregnant rats in the aforementioned group, inhalation exposure of offspring continued at 4 hours/day, 7 days/week for 7 weeks followed by 7 hour/day, 5 days/ week for 97 weeks (Group Ia), or 4 hours/day, 5 days/week for 7 weeks followed by 7 hours/day, 5 days/week for 8 weeks (Group Ib). Offspring group size was 67 males and 54 females in the former exposure protocol and 60 of each gender in the latter protocol. The control offspring group (Group IIa) included 158 males and 149 females. Percent of animals with malignant tumors was 37% (20/54) in Group I and 15% (9/60) in the Group II (control). For the offspring in group Ia, the percent of animals (males + females) was 54.5%; 66/121) and for Group Ib 33.3% (40/120). For control group II, percent of animals with malignant tumors was 16.7% (10/60) and for Group IIb was 17.9% (55/307).

In the long-term inhalation study of Quast et al. (1980), Sprague-Dawley (Spartan substrain) rats (100/sex/concentration) were exposed by inhalation to 0 (control), 20 ppm, and 80 ppm AN for 6 hours/day, 5 days/week for two years (analytical concentrations were 20.1"2.1 and 79.5"7.3 ppm' respectively, at the 6-month sacrifice). A control group was exposed to clean air. The groups also included animals for interim sacrifices at 6 months (7/gender/dose) and 12 months (13/gender/dose). Hematology, urinalysis, and clinical chemistry assessments were performed at specific intervals. Clinical observations included body weight, mortality, clinical appearance, onset of tumors, and frequency observed palpable tumors._All rats, regardless of time of death, were subjected to gross pathology examinations.

Alterations in the aforementioned clinical observations occurred earliest and with highest frequency in the high dose (80 ppm) group. Non-neoplastic effects for both exposure groups included exposure concentration-related inflammation and degeneration of tissue in the nasal turbinates (mucosa suppurative rhinitis, hyperplasia, focal erosions, and squamous metaplasia of the respiratory epithelium, with hyperplasia of the mucous secreting cells). Mortality rate was significantly increased ($p < 0.05$) during the first year in both male and female rats of the 80 ppm group and for females of the 20 ppm group during the last 10 weeks of the study. The increased mortality for the 20 ppm females was the result of early sacrifice due to benign mammary gland tumors. Although these tumors are known to occur spontaneously and at a high rate in Sprague-Dawley rats, they were observed earlier and at higher frequency in AN-exposed animals._Focal perivascular cuffing and gliosis was reported in the brain of male rats at 20 (2/99; $p<0.05$) and 80 ppm 7/99 ($p<0.05$), and 2/100 and 8/100 ($p<0.05$), respectively, for females in the 20-ppm and 80-ppm groups. There was an increased incidence of brain tumors ($p<0.05$ for both male and females at the 80 ppm exposure level compared to the controls) identified histopathologically as focal or multifocal glial cell tumors (astrocytomas). Proliferative glial cell lesion incidence was significantly increased in the 80 ppm males only.

Deaths of rats in the Quast et al. (1980) study were often attributable to severe ulceration of the Zymbal's gland or mammary tissue tumors, and suppurative pneumonia (80-ppm group only) resulting from AN-induced pulmonary irritation. The frequency of Zymbal's gland tumors was significantly increased (11/100; $p<0.05$) in both male and female animals in the 80 ppm group; in females the highest incidence occurred during the 13 to 18 month interval. An incidence of 3/100 was observed in males exposed to 20 ppm (1/100 for controls). No Zymbal's gland tumors were seen in 20-ppm females. Tumor type and incidence data are summarized in Table 12.

TABLE 12. Tumor Type And Incidence Data For Rats Exposed to Acrylonitrile (AN) Vapor

Exposure Concentration (ppm)	Zymbals Gland Carcinoma	Tongue Papilloma/ Carcinoma	Mammary Gland Fibroadenoma	Small Intestine Cystadeno-carcinoma	Brain astrocytoma
Males					
0	1/100	1/96	-	2/99	0/100
20	3/100	0/14	-	2/20	4/99
80	11/100*	7/89*	-	14/98*	15/99*
Females					
0	0/100	-	79/100	-	0/100
20	0/100	-	95/100*	-	4/100*
80	10/100*	-	75/100	-	17/100*

*Significantly different from control group ($p<0.05$)
Quast et al., 1980

3.6. Summary

Acute exposure data from tests with various laboratory species (monkey, rat, dog, rabbit, guinea pig, cat) revealed qualitatively similar responses ranging from mild irritation (redness of exposed skin, lacrimation, nasal discharge) and mild effects on ventilation and cardiovascular responses to severe respiratory effects, convulsions, and death. Four-hour exposure to concentrations ranging from 30 to 100 ppm produced little or no effect in all species except dogs

which exhibited severe effects at 100 ppm. Results of a recent nose-only exposure study in rats showed that concentrations up to 50 ppm for 6 hours or 225 ppm for 1.75 hours produced only minor transient effects on blood pressure. Lethality in rats appears to occur at cumulative exposure of 1800-1900 ppm·hrs for 0.5 to 6-hour exposure durations, although for nose-only exposures this is notably higher (~3800 ppm·hrs). Lethality data for various exposure durations and exposure concentrations suggest a near linear relationship (i.e., $n \approx 1.1$ for $C^n \times t = k$). Death may be delayed especially at the lower limits of lethal exposures. One study provided evidence for teratogenic effects in rats following gestational exposure of dams to 80 ppm but not 40 ppm AN. Another study showed an exposure-related decrease in fetal weight following gestational exposure of dams to 25, 50, or 100 ppm AN; no other reproductive/developmental effects were detected. Results of *in vitro* testing suggest that AN is weakly mutagenic. Results of *in vivo* mammalian cell assays measuring various endpoints were generally negative Results of inhalation exposure cancer bioassays have shown that AN is carcinogenic in rats. The brain, spinal cord, Zymbal's gland, tongue, nonglandular stomach, small intestine, and mammary gland have all been identified as targets.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Following inhalation exposure, AN will undergo rapid absorption by passive diffusion. Data from 6 human male volunteers exposed to AN (5 or 22 ppm) for 8 hours indicated that about 52% of the inhaled AN was retained (Jakubowski et al., 1987). Approximately 91.5% retention was reported for rats exposed 1800 ppm (3,900 mg/m^3) AN with absorption exhibiting a biphasic pattern (Peter and Bolt, 1984). These investigators also reported that rhesus monkeys absorbed nearly all AN after 6 hours.

Absorbed AN is readily distributed throughout the body. Kedderis et al.(1996) reported detection of AN and 2-cyanoethylene oxide (CEO) in blood, brain, and liver of Fisher F-344 rat three hours after exposure to 186, 254, or 291 ppm. Concentrations of AN and CEO tended to be greatest in the brain than in liver, and decreased rapidly following cessation of exposure. GSH depletion was shown to enhance tissue uptake of AN into brain, stomach, liver, kidney, and blood of GSH-depleted (phorone/buthionine sulfoximine treatment) F-344 rats (Pilon et al., 1988b). GSH depletion, however, resulted in a decrease in total radioactivity recovered in brain, stomach, liver, kidney, and blood and a decrease in the nondialyzable radioactivity (AN-derived) in the same organs. Control rats showed an accumulation of radiolabel which was greatest in brain RNA; no radioactivity was detected in DNA of any organ examined. In the GSH-depleted rats, radiolabel was greater in brain RNA than in that of the liver or stomach, but was only about half that observed in brain RNA of control rats.

Excretion of AN and its metabolites is primarily via the urine, with feces and exhaled air being minor routes of excretion. AN and its metabolites have been detected in the urine of exposed workers. Perbellini et al. (1998) reported that levels of AN in urine of pre- and post-shift workers were greater than in non-exposed controls.

At 24 hours after inhalation exposure of male Sprague-Dawley rats to 0, 4, 20, or 100 ppm AN for 6 hours, 2-cyanoethylmercapturic acid, 2-hydroxyethylmercapturic acid, and thiocyanate were measured in the urine (Tardif et al, 1987). The relationship between total urinary metabolites and exposure appeared to be linear. A dose-dependent excretion profile was reported for male Wistar rats following inhalation exposure to 1, 5, 10, 50, or 100 ppm AN for 8

hours (Müller et al.1987). Cyanoethyl mercaturic acid, S-carboxymethyl cysteine, hydroxyethyl mercapturic acid, and thioglycolic acid were detected as urinary metabolites. The investigators concluded that urinary metabolite profiles may be useful for biological monitoring of industrial exposure. Specifically, unmetabolized AN and the metabolites, cyanoethyl mercapturic acid and thioglycolic acid, were considered important.

AN toxicity appears to be directly related to its metabolism. Two major metabolism pathways have been described (Dahl and Waruszewski, 1989; Fennell et al., 1991; Kedderis et al., 1993; Burka et al., 1994; Gargas et al., 1995, Sumner et al., 1999). One pathway is conjugation with glutathione and the second is epoxidation by microsomal cytochrome P4502E1 which forms CEO. Metabolites from both pathways are subject to additional biotransformation. The glutathione conjugate may form a mercapturic acid which is excreted in urine. CEO is further metabolized via conjugation with glutathione (catalysis with cytosolic GST or nonenzymatically) resulting in additional conjugates and via hydrolysis by microsomal epoxide hydrolase (EH). The secondary metabolites of CEO may also be further metabolized. Cyanide may be generated via the EH pathway and by one of the GSH conjugation products. Cyanide, in turn, is detoxified to thiocyanate via rhodanese-mediated reactions with thiosulfate. Thiocyanate has been detected in the blood and urine of volunteer subjects following exposure to AN (21-51 ppm for 30 minutes) (Wilson and McCormick, 1949).

Vodi…ka et al. (1990) provided data showing that rats exposed for 6 hours to 75, 150, or 300 mg AN/m^3 (equivalent to 35, 69, and 138 ppm AN, respectively) excreted as thioethers 35.0%, 22.7%, and 18.1%, respectively, of the dose within 24 hours. About one-third to one-half of the excretion occurred during the 6-hour exposure.

Benz and Nerland (2005) reported on the effect of cytochrome P450 inhibitors and anticonvulsants on the toxicity of AN in male Sprague-Dawley rats. Treatment of rats with 1-benzylimidazole and ethanol effectively reduced blood cyanide levels and early seizures in rats given an LD_{90} subcutaneous dose of AN but did not affect the clonic convulsions that precede death or AN-induced mortality, thereby suggesting that AN is acutely toxic even in the absence of cyanide.

4.2. Mechanism of Toxicity

The mechanism by which AN causes irritation is unknown. Nasal tissue damage in rats may be related to metabolism of AN by this tissue (Dahl and Waruszewski, 1989). Hematologic effects may be due to AN and CEO hemoglobin adducts (Bergmark, 1997; Fennell et al., 2000) while GSH depletion in red blood cells may result in the oxidation of hemoglobin to methemoglobin (Farooqui and Ahmed, 1983a).

Generally, the toxic effects following acute inhalation exposure to AN appear to be irritation of the respiratory tract and the metabolism of AN to cyanide. Acrylonitrile-induced neurological effects in laboratory animals appear to involve the parent compound and the cyanide metabolite. The pivotal role cyanide in the acute toxicity of a series of aliphatic nitriles has been clearly demonstrated (Willhite and Smith, 1981). AN-induced convulsions, are likely the result of cyanide resulting from AN metabolism (Ghanayem et al., 1991; Nerland et al., 1989) although results of metabolism studies by Benz and Nerland (2005) suggest that only the early seizures are cyanide-mediated and that severe clonic convulsions preceding death may be due to parent compound as previously described in Section 4.1. Other possible modes of action

include inhibition of glyceraldehyde-3-phosphate dehydrogenase, by binding to critical cysteine residues (Campian et al., 2002) and ATP production by cyanide with respect to CNS effects. Additionally, it has been hypothesized that AN-induced oxidative stress may be related to some neurological effects (Fechter et al., 2003).

Cyanide formation by dams may be responsible, in part, for the developmental toxicity of AN in animals. may be associated with the release of cyanide during maternal metabolism of AN. Saillenfait and Sabate (2000) reported that a series of aliphatic nitriles produced embryotoxicity similar to that observed for sodium cyanide. Saillenfait et al. (1993b) suggested that glutathione depletion may be involved in the embryotoxicity of inhaled AN in rats.

4.3. Structure-Activity Relationships

Willhite and Smith (1981) demonstrated the importance of the AN metabolite, cyanide, in the lethal response of CD-1 mice following intraperitoneal injections of acetonitrile, proprionitrile, acrylonitrile, *n*-butyronitrile, malonitrile, or succinonitrile. In studies on the effects of P450 inhibitors and anticonvulsants, Benz and Nerland (2005) reported that AN appears to have inherent acute toxicity even in the absence of cyanide. With the data available for AN and considering the apparent complexity of AN acute toxicity compared to other nitriles, structure-activity was not used in the derivation of AEGL values.

4.4. Species Variability

The effects of acute inhalation exposure to AN are qualitatively similar among several animal species (monkey, dog, cat, rat, rabbit, guinea pig). Nerland et al. (1989) categorized the clinical signs of acute inhalation exposure to AN into four stages: 1) an excitatory phase characterized by lacrimation and agitation, 2) a tranquil phase in which cholinergic responses (salivation, lachrymation, urination, defecation) occur, 3) a convulsive stage characterized by clonic seizures, and 4) a terminal stage characterized by paralysis and death. At least some the variability in the toxic response to acrylonitrile may be a function of the cyanide metabolite and activity levels of rhodanese. Drawbaugh et al. (1987) reported dogs to have relatively lower levels of rhodanese and that rats had relatively high levels; overall species variability was about 3-fold. Results of experiments by Dudley and Neal (1942) also indicated that the dog was the most sensitive species.

Species differences in metabolism of AN are notable. Both rats and mice appear to form CEO at a greater rate (1.5-fold and 4-fold, respectively) than humans (Roberts et al., 1991). Although the rate of CEO formation was greater in mice, levels of CEO were only a third that found in rats (Roberts et al., 1991) suggesting difference between these rodent species. The conjugation rate for CEO with GSH is reportedly faster in humans (1.5-fold) than in mice or rats (Kedderis et al., 1995). The hydrolysis of CEO by EH is notably higher in humans and virtually absent in mice and rats (Kedderis et al., 1995). Based upon spectral analysis of AN interaction with microsomal preparations from rats, mice, and humans, Appel et al. (1981b) conclude that rats resemble humans more closely than do mice.

4.5. Susceptible populations

Due to the pivotal role of oxidative metabolism of AN in the formation of cyanide,

alterations in oxidative metabolism capacity (e.g., induction or inhibition of CYP2E1) may affect cyanide production rate (induction resulting in greater cyanide formation). Because cyanide detoxification may be affected by variances in sulfane sulfur as a source for thiocyanate formation via rodanese, individuals with lower circulating levels of sulfane sulfur (e.g., low cysteine content diets) may have lowered capacity for cyanide detoxification. It is the net difference between the capacities of these processes that will ultimately determine the overall cyanide-induced toxicity.

Results of a study examining the relationship between cigarette smoking, AN-derived hemoglobin adducts (N-(2-cyanoethyl)valine), and null genotypes for glutathione transferase (GSTM1 and GSTT1) were reported by Fennell et al. (2000). Analysis of the GST genotypes (by blood analysis) from 16 nonsmokers and 32 smokers (one to two packs/day) showed that hemoglobin adduct levels increased with increased cigarette smoking dose. Because the GSTM1 and GSTT1 genotypes had little effect on adduct levels concentrations, the results suggest that GST polymorphism may not be relevant to assessing susceptibility to AN toxicity.

4.6. Concurrent Exposure Issues

Concurrent exposure to agents capable of altering CYP2E1 function or glutathione levels may affect the biotransformation of AN and, therefore, its potential toxicity. Data are unavailable to allow for a quantitative adjustment of AEGL values due to potential concurrent exposure issues.

5. DATA ANALYSIS FOR AEGL-1

5.1. Human Data Relevant to AEGL-1

Occupational exposure of 16-100 ppm for 20-45 minutes produced headache, nasal and ocular irritation, discomfort of the chest, nervousness and irritability (Wilson, et al. 1948). Such effects are of greater severity than the AEGL-1 tier definition. Occupational exposure to 0.3 to 3 ppm for approximately 3 three years produced similar effects (Babanov et al., 1959). Sakurai et al. (1978) reported that workers routinely exposed to approximately 5 ppm AN in an acrylic fiber factory experienced initial conjunctival irritation to which some degree of accommodation occurred. Six informed male volunteer subjects (including the investigators) exposed to 2.3 and 4.6 ppm AN for 8 hours reported no symptoms of exposure (Jakubowski et al.,1987). A report of occupational exposures indicated that exposure to AN at 10 ppm or less was without effects while exposure to 12-15 ppm produced ocular irritation and headache regardless of exposure duration (NAC/AEGL pers. communication).

5.2. Animal Data Relevant to AEGL-1

Dudley et al. (1942) reported that rhesus monkeys exposed to 65 ppm AN for 4 hours exhibited no adverse effects. Nonlethal responses in rats included slight to marked transitory effects upon exposure to 665 ppm for 0.5 or 1 hour, 305 ppm for 2 hours, 130 ppm for 4 hours, and 90 ppm for 8 hours. Four-hour exposure of dogs to 30 ppm, and guinea pigs, cats, and rabbits to 100 ppm resulted in slight to moderate transitory effects. The WIL Research Laboratories report (2005) reported only vocalization upon handling for rats exposed (nose-only) to 539 ppm for 4 hours. Some rats exposed to 775 ppm exhibited ataxia , hyperactivity, and decreased urination and defecation. Other lethality bioassay reports simply indicated some exposures as nonlethal with no details regarding the presence or absence of nonlethal effects.

5.3. Derivation of AEGL-1 Values

The most relevant data for AEGL-1 derivation is the human response data reported by Jakubowski et al. (1987) regarding the absence of effects in volunteer subjects exposed for 8 hours to 4.6 ppm AN. This is consistent with the report by Sakurai et al. (1978) in which workers routinely exposed to approximately 5 ppm AN experienced initial conjunctival irritation and for which there was some accommodation. It is also consistent with more recent occupational exposure data indicating that exposure to 10 ppm was without effect while exposure to 12-15 ppm produced ocular irritation and headache regardless of exposure duration (NAC/AEGL pers. communication). It is reasonable to assume that for AEGL-1 severity effects, individual variability in the response to AN would vary no more than 3-fold, thereby indicating a point-of-departure (POD) of about 5 ppm (3-fold reduction of the 15 ppm concentration from occupational exposure data). Therefore, measured 4.6 ppm reported by Jakubowski et (1987) was considered an appropriate POD for AEGL-1 derivation. This is slightly lower than the no-effect level of 10 ppm noted in the occupational exposure findings but is appropriate for the general public who may not be accustomed to acrylonitrile exposure as would workers. Because occupational exposure data indicated the occurrence and severity of minor effects (ocular irritation and headache) to be independent of exposure duration, the AEGL-1 values were held constant at 4.6 ppm across all exposure durations. In light of results of studies showing only mild effects (headache, nervousness, fatigue, nausea, and insomnia) following subchronic occupational exposure to AN levels possibly as high 10-20 ppm (Wilson et al., 1948; Sakurai et al., 1978), further reduction of the AEGL-1 value by increased uncertainty factor application does not appear warranted._AEGL-1 values are shown in Table 13 and their derivation shown in Appendix A.

TABLE 13. AEGL-1 Values for Acrylonitrile

Classification	10-min	30-min	1-hr	4-hr	8-hr
AEGL-1	4.6 ppm	4.6 ppm	4.6 ppm	24.6 ppm	4.6 ppm

6. DATA ANALYSIS FOR AEGL-2

6.1. Human Data Relevant to AEGL-2

There are no quantitative exposure-response data regarding AEGL-2 type effects in humans. Occupational exposure studies reported varying levels of irritation and reversible effects.

6.2. Animal Data Relevant to AEGL-2

Results of studies with laboratory species show that AEGL-2 type effects in animals include changes in respiratory patterns, tremors, and convulsions, the severity of which appear to increase immediately prior to death. The onset of most of the more severe effects are usually preceded by varying signs of irritation (salivation, redness of skin, lacrimation). Post exposure observation in multiple species showed qualitatively similar effects and that that even severe effects are often reversible upon cessation of exposure.

The report by Dudley and Neal (1942) provides data for six species (monkey, rat, dog, guinea pig, rabbit, and cat). For rats 0.5-, 1-, 2-, 4-, or 8-hour exposure to 2445, 1270, 305, and 135 ppm AN, respectively, produced reversible effects. Apple et al (1981) provided data for rats showing that 10-minute exposure to 2400 ppm or 30-minute exposure to 1600 ppm was not lethal to rats. Dogs were more sensitive to the effects of AN as demonstrated by convulsions and coma at exposures as low as 65 ppm for 4 hours (Dudley and Neal, 1942). Results of a nose-only experiment with rats showed that 4-hour exposure to 775 ppm was not lethal but details were lacking regarding the attribution of observed effects (tremors, ataxia, labored breathing, hypoactivity, and gasping) to these exposures. For rabbits, 4-hour exposure up to 135 ppm AN produced slight to marked, but reversible, effects (Dudley and Neal, 1942).

6.3. Derivation of AEGL-2 Values

The AEGL-2 values are based upon data from rats (16/group) showing slight transient effects (ocular and nasal irritation) following a 2-hour exposure to 305 ppm (Dudley and Neal, 1942). All effects resolved within 12 hours post exposure. The interspecies uncertainty factor was limited to 3 because a non-human primate is considered a more relevant model than rodents, dogs or cats. The intraspecies uncertainty factor was limited to 3 because the effects associated with acute irritation effects of AN are not likely to vary greatly among individuals and because metabolism may be of limited relevance regarding such effects. Additional uncertainty factor application would also result in AEGL-2 values unacceptably similar to AEGL-1 values that are based upon human exposure data. Time scaling for AEGL-2 specific durations from the 4-hour experimental POD was performed using $C^n \times t = k$, where $n = 1.1$ (ten Berge et al., 1986). Occupational exposure data showed that routine exposure to 10-20 ppm (up to ~2-fold higher than the 8-hr AEGL-2) resulted in complaints of headache, fatigue, nausea, and insomnia (Wilson et al. (1948; Sakurai et al. (1978) which are neither irreversible nor escape-impairing effects. The AEGL-2 values for acrylonitrile are shown in Table 14 and their derivation summarized in Appendix A.

TABLE 14. AEGL-2 Values for Acrylonitrile

Classification	10-min	30-min	1-h	4-h	8-h
AEGL-2	290 ppm	110 ppm	57 ppm	16 ppm	8.6 ppm

7. DATA ANALYSIS FOR AEGL-3

7.1. Human Data Relevant to AEGL-3

Quantitative exposure-response data in humans regarding the lethality of AN are not available.

7.2. Animal Data Relevant to AEGL-3

Lethality data in multiple laboratory species (monkey, rat, dog, rabbit, guinea pig, cat) are available. Lethality in rats appears to occur at cumulative exposure of 1800-1900 ppm•hrs for 0.5 to 6-hour exposure durations, although for nose-only exposures this is notably higher (~3800 ppm•hrs). Lethal response data for monkeys were not available. Dogs were the most sensitive species with lethality in one of two dogs observed following a 4-hour exposure to 65 ppm. However, a 4-hour exposure of 4 dogs to 100 ppm resulted in no deaths while exposure to

110 ppm killed 2 of 3 dogs. Data for rats was most extensive. Dudley and Neal (1942) provided response data for rats exposed for 0.5, 1, 2. 4, or 8 hours. Thirty-minute exposure of rats to AN levels as high as 2445 ppm were without lethal effect. Exposure to 1270 ppm for 1 hour, 305 ppm for 2 hours, 130 ppm for 4 hours, or 135 ppm for 8 hours did not result in deaths of any rats (16/group). A four-hour LC_{50} of 333 ppm was reported for rats (du Pont & Co., 1968). At higher exposure rats died within 2 to 4 hours into the exposure period while deaths following exposure occurred between 7 minutes and 18 hours; there was a 14-day observation period. There were no deaths among 10 rats exposed to 1030 ppm AN for 1 hour (Vernon et al. 1990). A 33% mortality (1 of 3 rats) was reported for exposures of 650 ppm for 180 minutes, 950 ppm for 120 minutes, and 2600 ppm for 30 minutes and no deaths at exposures of 1600 ppm for 30 minutes or 2400 ppm for 10 minutes (Appel et al., 1981a).

7.3. Derivation of AEGL-3 Values

The AEGL-3 values were derived using $BMCL_{05}$ as estimates of lethality thresholds. Data for 30-minute, 1-, 4-, and 8-hour AEGL-specific exposure periods are available from the reports by Apple et al. (1981) and Dudley and Neal (1942). A 30-minute $BMCL_{05}$ of 1784 ppm was calculated from the Appel et al. (1981a) data. The 1-hr, 2-hr, 4-hr, and 8-hr $BMCL_{05}$ values derived from lethality data published by Dudley and Neal (1942) are 1024.4, 491.3, 179.5 and 185.8 ppm, respectively, for rats exposed to various concentrations of AN for 1, 2, 4, or 8 hours. With the exception of the 4-hour value, the resulting $BMCL_{05}$ values are relationally consistent across time and were used to derive corresponding AEGL-3 values. The 4-hr value was not used due to this inconsistency. Consequently, the 4-hour AEGL-3 was time-scaled using the 8-hour $BMCL_{05}$ of 185.9 ppm. Although the dog appeared to be the most sensitive species, the overall database for rats is more robust thereby justifying use of the rat data. Further justification for limiting the interspecies uncertainty factor to 3 comes from PBPK models demonstrating that predicted concentrations of AN and the metabolite CEO in blood and brain were similar in rats and humans exposed by inhalation. The PBPK model for AN and CEO disposition in humans utilized human *in vitro* data and scaling from a rat model (Kedderis and Fennell, 1996) that incorporated major biotransformation and reactivity pathways. These included metabolism of AN to glutathione conjugates and CEO, reaction rates of AN and CEO with glutathione and tissue components, and the metabolism of CEO by hydrolysis and glutathione conjugation. For effects resulting from a single acute exposure, an intraspecies uncertainty factor of 3 may be considered sufficient for accounting for variability in metabolism-mediated effects. Additional uncertainty factor application would result in incompatibility between AEGL-3 and AEGL-2 values. The resulting AEGL-3 values are shown in Table 15. and their derivation is summarized in Appendix A.

TABLE 15. AEGL-3 Values for Acrylonitrile

Classification	10-min	30-min	1-h	4-h	8-h
AEGL-3	480 ppm	180 ppm	100 ppm	35 ppm	19 ppm

8. SUMMARY OF AEGLs

8.1. AEGL Values and Toxicity Endpoints

A summary of AEGL values is shown in Table 16. The AEGL-1 values are based upon the absence of effects in male volunteer subjects in a controlled exposure study (Jakubowski et

al., 1987) and occupational exposure data showing ocular irritation and headache at 16-20 ppm. The AEGL-2 values are based upon slight transient effect (ocular and nasal irritation) in rats exposed to 305 ppm AN for 2 hours. The AEGL-3 values were derived based upon an estimated lethality threshold ($BMCL_{05}$) in rats, the species for which the most lethality data are available.

TABLE 16. AEGL Values for Acrylonitrile

Classification	10-min	30-min	1-h	4-h	8-h
AEGL-1 (Nondisabling)	4.6 ppm	4.6 ppm	4.6 ppm	4.6 ppm	4.6 ppm
AEGL-2 (Disabling)	290 ppm	110 ppm	57 ppm	16 ppm	8.6 ppm
AEGL-3 (Lethality)	480 ppm	180 ppm	100 ppm	35 ppm	19 ppm

8.2. Comparisons with Other Standards and Guidelines

The AEGL values and existing standards and guidelines for acrylonitrile are summarized in Table 17. The 30-minute AEGL-2 value is consistent with the NIOSH IDLH and the 1-hour AEGL values are consistent with the ERPG values developed by AIHA.

TABLE 17. Extant Standards and Guidelines for Acrylonitrile

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1	4.6ppm	4.6ppm	4.6 ppm	4.6 ppm	4.6 ppm
AEGL-2	290 ppm	110 ppm	57 ppm	16 ppm	8.6 ppm
AEGL-3	480 ppm	180 ppm	100 ppm	35 ppm	19 ppm
ERPG-1 (AIHA)[a]			10 ppm		
ERPG-2 (AIHA)			35 ppm		
ERPG-3 (AIHA)			75 ppm		
EEGL (NRC)[b]					
PEL-TWA (OSHA)[c]					2 ppm
PEL-STEL (OSHA)[d]					
IDLH (NIOSH)[e]		85 ppm			
REL-TWA (NIOSH)[f]	1 ppm 15 min ceiling				1 ppm
REL-STEL (NIOSH)[g]					
TLV-TWA (ACGIH)[h]					2 ppm*
TLV-STEL (ACGIH)[i]					
MAK (Germany)[j]					
MAK Spitzenbegrenzung (Germany)[k]					
Einsaztoleranzwert (Germany)[l]					
MAC-Peak Category (The Netherlands)[m]					4 ppm TWA 10 ppm STEL

[a] ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association) (AIHA, 1994)

The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor.
The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action.
The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects.

[b] EEGL (Emergency Exposure Guidance Levels, National Research Council) (NRC, 1985) is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace, but avoids death, other severe acute effects and long-term or chronic injury.

[c] OSHA PEL-TWA (Occupational Health and Safety Administration, Permissible Exposure Limits - Time Weighted Average) (OSHA, 1993) is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[d] OSHA PEL-STEL (Permissible Exposure Limits - Short Term Exposure Limit) (OSHA, 1993) is defined analogous to the ACGIH-TLV-STEL.

[e] IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health) (NIOSH, 1996) represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects.

[f] NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average) (NIOSH, 1994) is defined analogous to the ACGIH-TLV-TWA.

[g] NIOSH REL-STEL (Recommended Exposure Limits - Short Term Exposure Limit) (NIOSH, 1994) is defined analogous to the ACGIH-TLV-STEL.

[h] ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average) (ACGIH, 1997) is the time-weighted average concentration for a normal 8-hour workday and a 40-hour work week, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. * Acrylonitrile noted as confirmed animal carcinogen with unknown relevance to humans.

[i] ACGIH TLV-STEL (Threshold Limit Value - Short Term Exposure Limit) (ACGIH, 1997) is defined as a 15-minute TWA exposure which should not be exceeded at any time during the workday even if the 8-hour TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 minutes and should not occur more than 4 times per day. There should be at least 60 minutes between successive exposures in this range.

[j] MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration], Deutsche Forschungs-gemeinschaft [German Research Association], Germany) (DFG, 1999) is defined analogous to the ACGIH-TLV-TWA.

[k] MAK Spitzenbegrenzung (Kategorie II,2) [Peak Limit Category II,2] (DFG, 1999) constitutes the maximum average concentration to which workers can be exposed for a period up to 30 minutes, with no more than 2 exposure periods per work shift; total exposure may not exceed 8-hour MAK. Cat. III indicates possible significant contribution to cancer risk.

[l] Einsatztoleranzwert [Action Tolerance Levels] (Vereinigung zur Förderung des deutschen Brandschutzes e.V. [Federation for the Advancement of German Fire Prevention]) constitutes a concentration to which unprotected firemen and the general population can be exposed to for up to 4 hours without any health risks.

[m]MAC (Maximaal Aanvaaarde Concentratie [Maximal Accepted Concentration - Peak Category]) (SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands 2000) is defined analogous to the ACGIH-Ceiling.

8.3. Data Adequacy and Research Needs

Data were adequate for the development of justifiable AEGL values. Human data were used for deriving AEGL-1 values and data in monkeys were used for developing AEGL-2 values. A robust data set in rats allowed for derivation of AEGL-3 values.

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APPENDIX A: Derivation of AEGL Values

Derivation of AEGL-1 Values for Acrylonitrile

Key study: Jakubowski, M., Linhart, I., Pielas, G., Kopecky, J. 1987. 2-Cyanoethylmercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. Brit. J. Industr. Med. 44: 834-840.

Critical effect: Occupational exposure reports indicated no effects at ≤ 10 ppm and ocular irritation and headaches at 12-15 ppm regardless of exposure duration (NAC/AEGL, pers. communication). A 3-fold reduction of the 15-ppm is equivalent to the no-effect concentration reported by Jacubowski et al. (1987) for male human volunteers exposed to 4.6 ppm AN for 8 hours.

Time scaling: none applied;

Uncertainty factors: Total uncertainty factor adjustment was 1:

Interspecies: none; human subjects

Intraspecies: 1; effects associated with very low-level AN exposure are not likely to vary among individuals; metabolism is not likely to play a significant role in very minor effects resulting from low-level exposure. An intraspecies UF of 3 applied to the lower limit of the occupational exposure range associated with ocular irritation and headache results in an exposure concentration equivalent to the no-effect concentration (4.6 ppm) reported by Jakubowski et al. (1987).

Modifying factor: None

Occupational exposure data indicated that for low exposures, exposure duration was irrelevant. Therefore, an AEGL-1 value of 4.6 ppm is recommended for all durations.

Derivation of AEGL-2 Values for Acrylonitrile

Key study: Dudley, H.C. and Neal, P.A. 1942. Toxicology of acrylonitrile (vinyl cyanide). I. Study of the acute toxicity. J. Ind. Hyg. Toxicol., 24 (2): 27-36.

Critical effect: Slight transient effects in rats exposed for 2 hours to 305 ppm AN. All effects were transient and resolved within 12 hours post exposure.
Support: Sakurai et al. (1978) noted that many of the symptoms (headache, fatigue, nausea, and insomnia) upon initial exposure observed for occupational exposure to AN were associated with exposures in excess of 5 ppm, and that the findings were not contradictory to those of Wilson et al. (1948) who reported that occupational exposure to 16-100 ppm for 20-45 minutes produced transient dull headaches, nasal and ocular irritation, discomfort in the chest, nervousness and irritability. Murray et al. (1978) provided evidence of teratogenicity in rats following multiple exposure to 80 ppm AN on gestation days 6-20 but effects in dams were limited to only food consumption and body weight decrease.

Time scaling: $C^n \times t = k$, where $n = 1.1$, ten Berge et al., 1986

Uncertainty factors: Total uncertainty factor adjustment was 10, additional uncertainty adjustment would result in AEGL-2 values that are inconsistent with the overall data and that would be similar to AEGL-1 values.
Interspecies: 3; PB-PK modeling has shown that predicted concentrations of AN and the metabolite CEO in blood and brain were similar in rats and humans exposed by inhalation.
Intraspecies: 3; the effects associated with acute AN exposure are not likely to vary greatly among individuals; metabolism is not likely to be instrumental in initial minor effects resulting from low-level exposure.

Modifying factor: None

Calculation: $(305 \text{ ppm})^{1.1} \times 2 \text{ hrs} = 1080 \text{ ppm}^{1.1} \cdot \text{hrs}$

10-minute AEGL-2

$C^{1.1} \times 0.1667 \text{ hr} = 1080 \text{ ppm}^{1.1} \cdot \text{hrs}$
$C = 2917.4 \text{ ppm}$
10-min AEGL-2 = 2917.4 ppm/10 = 291.7 ppm (rounded to 290 ppm)

30-minute AEGL-2

$C^{1.1} \times 0.5 \text{ hr} = 1080 \text{ ppm}^{1.1} \cdot \text{hrs}$
$C = 1074 \text{ ppm}$
30-min AEGL-2 = 1074 ppm/10 = 107.4 ppm (rounded to 110 ppm)

1-hour AEGL-2

$C^{1.1}$ x 1 hr = 1080 $ppm^{1.1}$ @ hrs
C = 572.3 ppm
1-hr AEGL-2 = 572.3 ppm/10 = 57.2 ppm (rounded to 57 ppm)

4-hour AEGL-2

$C^{1.1}$ x 4 hrs = 1080 $ppm^{1.1}$ @ hrs
C = 162.30 ppm
4-hr AEGL-2 = 162.3 ppm/10 = 16.2 ppm (rounded to 16 ppm)

8-hour AEGL-2

$C^{1.1}$ x 8 hrs = 1080 $ppm^{1.1}$ @ hrs
C = 86.4 ppm
8-hr AEGL-2 = 86.4 ppm/10 = 8.6 ppm

Derivation of AEGL-3 Values for Acrylonitrile

Key studies: Dudley, H.C. and Neal, P.A. 1942. Toxicology of acrylonitrile (vinyl cyanide). I. Study of the acute toxicity. J. Ind. Hyg. Toxicol., 24 (2): 27-36.

Appel, K.E., Peter, H., Bolt, H.M. 1981a. Effect of potential antidotes on the acute toxicity of acrylonitrile. Int. Arch. Occup. Environ. Health 48: 157-163.

Critical effect: Estimated lethality threshold (30-minute, 1-hr, 2-hr,4-hr, and 8-hr $BMCL_{05}$ values are 1784.0, 1024.4, 491.3, 179.5 and 185.8 ppm, respectively) for rats exposed to various concentrations of AN for 30 minutes, 1, 2, 4, or 8 hours. The 4-hr value was not used due to inconsistency with values of the other durations. The 4-hour AEGL was time-scaled using the 8-hour $BMCL_{05}$.

Time scaling: $C^n \times t = k$, where $n = 1.1$, ten Berge et al. (1986); applied for derivation of 10-minute and 4-hour values only. The 30-minute, 1-hour and 8-hour AEGL-3 values were derived based upon their respective $BMCL_{05}$ values.

Uncertainty factors: Total uncertainty factor adjustment was 10.
Interspecies: 3; Although the dog appears to be the most sensitive species, the overall database for rats is more robust thereby justifying use of the rat data. PBPK model simulations (Kedderis and Fennell, 1996; Sweeney et al., 2003) indicated that predicted blood and brain concentrations of AN and the metabolite CEO (2-cyanoethylene oxide) were similar in rats and humans exposed to AN by inhalation. A factor of 3 is considered sufficient to account for possible toxicodynamic/metabolism differences
Intraspecies:3; For effects resulting from a single acute exposure, an intraspecies uncertainty factor of 3 would seem sufficient for accounting for variability in metabolism-mediated effects. Additional uncertainty factor application would result in incompatible AEGL-3 and AEGL-2 values.

Calculation: For the 30-minute, 1-hr and 8-hr AEGL-3 values the 1-hr and 8-hr rat $BMCL_{05}$ values were simply adjusted by the total uncertainty factor product of 10.
The 10-minute values were derived by time-scaling from the 30-minute rat $BMCL_{05}$:

$$(1784 \text{ ppm})^{1.1} \times 0.5 \text{ hr} = 1885.8 \text{ ppm}^{1.1} \text{ A hrs}$$

The 4-hr value was derived by scaling from the 8-hr rat $BMCL_{05}$ (the 8- hr $BMCL_{05}$ was considered more appropriate that the 2-hr value because it was derived from data for five dose groups rather than three):

$$(185.8 \text{ ppm})^{1.1} \times 8 \text{ hrs} = 2506.3 \text{ ppm}^{1.1} \text{ A hrs}$$

10-minute AEGL-3

$$C^{1.1} \times 0.1667 \text{ hr} = 1885.8 \text{ ppm}^{1.1} \text{ @ hrs}$$
$$C = 4842.4 \text{ ppm}$$
$$\text{10-min AEGL-3} = 4842.4 \text{ ppm}/10 = 484 \text{ ppm (rounded to 480 ppm)}$$

30-minute AEGL-3

30-minute $BMCL_{05}$ = 1784 ppm
1784 ppm/10 = 178 ppm (rounded to 180 ppm)

1-hour AEGL-3

1-hr $BMCL_{05}$ 1024.42 ppm
1024.42 ppm/10 = 102 ppm (round to 100 ppm)

4-hour AEGL-3

$C^{1.1}$ x 4 hrs = 2506.3 $ppm^{1.1}$ @ hrs
C = 348.9 ppm
4-hr AEGL-3 = 348.9 ppm/10 = 34.9 ppm (round to 35 ppm)

8-hour AEGL-3

8-hr $BMCL_{05}$ 185.8 ppm
185.8 ppm/10 = 18.6 ppm (round to 19 ppm)

APPENDIX B: Time Scaling Calculations

The relationship between dose and time for any given chemical is a function of the physical and chemical properties of the substance and the unique toxicological and pharmacological properties of the individual substance. Historically, the relationship according to Haber (1924), commonly called Haber's Law or Haber's Rule (i.e., $C \times t = k$, where C = exposure concentration, t = exposure duration, and k = a constant) has been used to relate exposure concentration and duration to effect (Rinehart and Hatch, 1964). This concept states that exposure concentration and exposure duration may be reciprocally adjusted to maintain a cumulative exposure constant (k) and that this cumulative exposure constant will always reflect a specific quantitative and qualitative response. This inverse relationship of concentration and time may be valid when the toxic response to a chemical is equally dependent upon the concentration and the exposure duration. However, an assessment by ten Berge et al. (1986) of LC_{50} data for certain chemicals revealed chemical-specific relationships between exposure concentration and exposure duration that were often exponential. This relationship can be expressed by the equation $C^n \times t = k$, where n represents a chemical specific, and even a toxic endpoint specific, exponent. The relationship described by this equation is basically the form of a linear regression analysis of the log-log transformation of a plot of C vs t. ten Berge et al. (1986) examined the airborne concentration (C) and short-term exposure duration (t) relationship relative to death for approximately 20 chemicals and found that the empirically derived value of n ranged from 0.8 to 3.5 among this group of chemicals. Hence, the value of the exponent (n) in the equation $C^n \times t = k$ quantitatively defines the relationship between exposure concentration and exposure duration for a given chemical and for a specific health effect endpoint. Haber's Rule is the special case where $n = 1$. As the value of n increases, the plot of concentration vs time yields a progressive decrease in the slope of the curve.

For acrylonitrile, analysis of available data by ten Berge et al. (1986) showed that the relationship between exposure concentration and exposure duration was near linear, where $n = 1.1$ for the relationship $C^n \times t = k$.

APPENDIX C: AEGL DERIVATION SUMMARY TABLES

ACUTE EXPOSURE GUIDELINE LEVELS FOR ACRYLONITRILE
DERIVATION SUMMARY

AEGL-1 VALUES FOR ACRYLONITRILE				
10 min	**30 min**	**1 h**	**4 h**	**8 h**
4.6 ppm	**4.6 ppm**	**4.6 ppm**	**4.6 ppm**	**4.6 ppm**

Reference: Jakubowski, M., Linhart, I., Pielas, G., Kopecky, J. 1987. 2-Cyanoethylmercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. Brit. J. Industr. Med. 44: 834-840.

Test Species/Strain/Number: six informed volunteer male humans subjects

Exposure Route/Concentrations/Durations: inhalation/ 2.3 or 4.6 ppm AN for 8 hours

Effects: no effects in any of the subjects at either exposure level

Endpoint/Concentration/Rationale: 4.6 ppm AN for 8 hours is considered a NOAEL

Uncertainty Factors/Rationale: Total uncertainty factor adjustment was 3:
Interspecies: None; human subjects
Intraspecies: 1; Effects associated with very low-level acute AN exposure are not likely to vary among individuals; metabolism will not be instrumental in initial, minor effects

Based upon occupational exposure data showing no effects at concentrations of 10 ppm AN, further reduction of the AEGL-1 values is not warranted.

Modifying Factor: None applied

Animal to Human Dosimetric Adjustment: no adjustments

Time Scaling: None applied; occupational data suggest that minor irritation effect occur immediately and are independent of exposure duration

Data Adequacy: AEGL-1 values for acrylonitrile are developed based upon results from a controlled experiment with human volunteer subjects with incorporation of occupational exposure data. The data effectively define a no-observed-adverse-effect level for acrylonitrile and for an AEGL-specific exposure duration.

1

AEGL-2 VALUES FOR ACRYLONITRILE				
10 minutes	**30 min**	**1 h**	**4 h**	**8 h**
290 ppm	**100 ppm**	**57 ppm**	**16 ppm**	**8.6 ppm**
Reference: Dudley, H.C. and Neal, P.A. 1942. Toxicology of acrylonitrile (vinyl cyanide). I. Study of the acute toxicity. J. Ind. Hyg. Toxicol., 24 (2): 27-36.				
Test Species/Strain/Sex/Number: Osborne-Mendel rat; 16/group				
Exposure Route/Concentrations/Durations: inhalation; 305 ppm for 2 hours				
Effects: slight initial alteration in respiratory rate, slight, transient nasal and ocular irritation; effects transient and resolved by 12 hours post exposure				
Endpoint/Concentration/Rationale: slight transient effects following 2-hour exposure to 305 ppm.				
Uncertainty Factors/Rationale: Total uncertainty factor adjustment was 10 Interspecies: 3; All species tested exhibited similar array of effects Intraspecies: 3; The effects associated with acute AN exposure are not likely to vary greatly among individuals; metabolism is not likely to be instrumental in minor effects resulting from low-level exposure.				
Modifying Factor: none				
Animal to Human Dosimetric Adjustment: Not applicable				
Time Scaling: $C^n \times t = k$, where $n = 1.1$ as reported by ten Berge et al. 1986				
Data Adequacy: The AEGL-2 value are based upon effects that are indicative of AN exposure but not yet demonstrating more severe toxicity (e.g., convulsions, extreme respiratory alterations) or irreversible effects. The test species is a nonhuman primate considered to be more appropriate than rodents or other test species.				

2

AEGL-3 VALUES FOR ACRYLONITIRLE

10 min	30 min	1 h	4 h	8 h
480 ppm	**180 ppm**	**100 ppm**	**35 ppm**	**19 ppm**

Reference: Dudley, H.C. and Neal, P.A. 1942. Toxicology of acrylonitrile (vinyl cyanide). I. Study of the acute toxicity. J. Ind. Hyg. Toxicol., 24 (2): 27-36.

Appel, K.E., Peter, H., Bolt, H.M. 1981. Effect of potential antidotes on the acute toxicity of acrylonitrile. Int. Arch. Occup. Environ. Health 48: 157-163.

Test Species/Strain/Sex/Number: 16 Osborne-Mendel rats (gender not specified) per exposure concentration (Dudley and Neal, 1942); 3-6 male Wistar rats (Appel et al., 1981)

Effects: Lethal response frequency (see Tables 2 & 4, Section 3.1.2 for details) for details.

Exposure duration (h)	Exposure concentration (ppm)	Mortality
0.5 (Appel et al., 1981)	1600	0/3
	2600	1/3
	3000	6/6
1 (Dudley and Neal, 1942)	665	0/16
	1270	0/16
	1490	4/16
	2445	13/16
2 (Dudley and Neal, 1942)	305	0/16
	595	1/16
	1260	16/16
4 (Dudley and Neal, 1942)	130	0/16
	315	2/16
	635	16/16
8 (Dudley and Neal, 1942)	90	0/16
	135	0/16
	210	1/16
	270	7/16
	320	15/16

Endpoint/Concentration/Rationale: Estimated lethality threshold (30-minute, 1-hr, 2-hr,4-hr, and 8-hr $BMCL_{05}$ values are 1784.0, 1024.4, 491.3, 179.5 and 185.8 ppm, respectively) for rats exposed to various concentrations of AN for 30 minutes, 1, 2, 4, or 8 hours. The 4-hr value was not used due to inconsistency with values of the other durations.

Uncertainty Factors/Rationale: Total uncertainty factor adjustment was 10

Interspecies: 3; Although the dog appears to be the most sensitive species, the overall database for rats is more robust thereby justifying use of the rat data. PBPK model simulations (Kedderis and Fennell, 1996; Sweeney et al., 2003) indicated that predicted blood and brain concentrations of AN and the metabolite CEO (2-cyanoethylene oxide) were similar in rats and humans exposed to AN by inhalation. A factor of 3 is considered sufficient to account for possible toxicodynamic/metabolism differences.

Intraspecies: For effects resulting from a single acute exposure, an intraspecies uncertainty factor of 3 would seem sufficient for accounting for variability in metabolism-mediated effects. Additional uncertainty factor application would result in incompatible AEGL-3 and AEGL-2 values.

Modifying Factor: None applied

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: For the 30-minute, 1-hr, and 8-hr AEGL-3 values, the corresponding rat $BMCL_{05}$ values were simply adjusted by the total uncertainty factor product of 10.

The 10-minute value was derived by time-scaling from the 3-min. rat $BMCL_{05}$:

$(1578\ ppm)^{1.1} \times 0.5\ hr = 1647.7\ ppm^{1.1} \cdot hrs$

The 4-hr values was derived by scaling from the 8-hr rat $BMCL_{05}$ (the 8- hr $BMCL_{05}$ was considered more appropriate that the 2-hr value because it was derived from data for five dose groups rather than three):

$(185.8\ ppm)^{1.1} \times 8\ hrs = 2506.3\ ppm^{1.1} \cdot hrs$

Data Adequacy: Although definitive exposure response data for lethality in humans are not available, data are available from acute and subchronic bioassays in multiple species. These data are sufficient for development of scientifically justified AEGL values.

APPENDIX D: BMC ANALYSIS FOR ACRYLONITRILE

$BMCL_{01}$ 30-minute exposure of rats to acrylonitrile (Appel et al., 1981)

```
 ===============================================================================
        Probit Model. (Version: 2.8;  Date: 02/20/2007)
        Input Data File: C:\BMDS\APPEL_30-MIN.(d)
        Gnuplot Plotting File:  C:\BMDS\APPEL_30-MIN.plt
                                                  Fri Jul 13 13:22:35 2007
 ===============================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:
   P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
   where CumNorm(.) is the cumulative normal distribution function

   Dependent variable = COLUMN3
   Independent variable = COLUMN1
   Slope parameter is not restricted

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model
              Default Initial (and Specified) Parameter Values
                background =          0
                 intercept =    -30.2755
                     slope =     3.91797

         Asymptotic Correlation Matrix of Parameter Estimates

         ( *** The model parameter(s)  -background    -slope
             have been estimated at a boundary point, or have been specified by the user,
             and do not appear in the correlation matrix )

                     intercept
 intercept               1

                          Parameter Estimates
                                        95.0% Wald Confidence Interval
      Variable       Estimate       Std. Err.    Lower Conf. Limit   Upper Conf. Limit
    background              0            NA
     intercept       -141.863      0.665192          -143.167          -140.559
         slope             18            NA
```

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

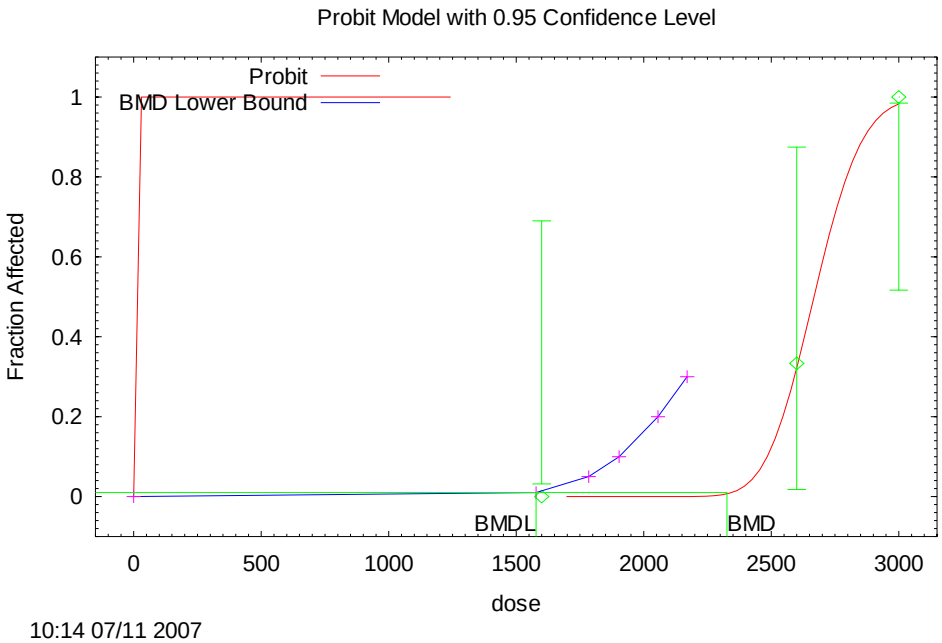
Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-1.90954	3			
Fitted model	-1.99323	1	0.167371	2	0.9197
Reduced model	-8.15032	1	12.4816	2	0.001948
AIC:	5.98646				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
1600.0000	0.0000	0.000	0	3	-0.000
2600.0000	0.3729	1.119	1	3	-0.142
3000.0000	0.9878	5.927	6	6	0.272

Chi^2 = 0.09 d.f. = 2 P-value = 0.9541

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMC = 2416.07
BMCL = 1784.1



1 10:14 07/11 2007

$BMCL_{05}$ 1-hr exposure of rats (Dudley and Neal, 1942)

```
 ================================================================================
          Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
          Input Data File: C:\BMDS\UNSAVED1.(d)
          Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                    Thu Mar 01 08:34:09 2007
 ================================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:
          P[response] = Background  + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
          where CumNorm(.) is the cumulative normal distribution function

  Dependent variable = COLUMN3
  Independent variable = COLUMN1
 Slope parameter is not restricted

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008

  User has chosen the log transformed model

  Default Initial (and Specified) Parameter Values
          background    =          0
          intercept     =    -16.2084
          slope         =     2.13067

  Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -background  have been estimated at a boundary point, or
          have been specified by the user, and do not appear in the correlation matrix )

                      intercept       slope
   intercept            1              -1
    slope              -1               1

                       Parameter Estimates

          Variable         Estimate          Std. Err.
          background          0                 NA
          intercept        -29.6647            6.43448
          slope              3.92636           0.860001

 NA - Indicates that this parameter has hit a bound  implied by some inequality constraint and
```

thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-16.7186			
Fitted model	-18.0178	2.5984	2	0.2728
Reduced model	-37.047	40.6567	3	<.0001
AIC:	40.0356			

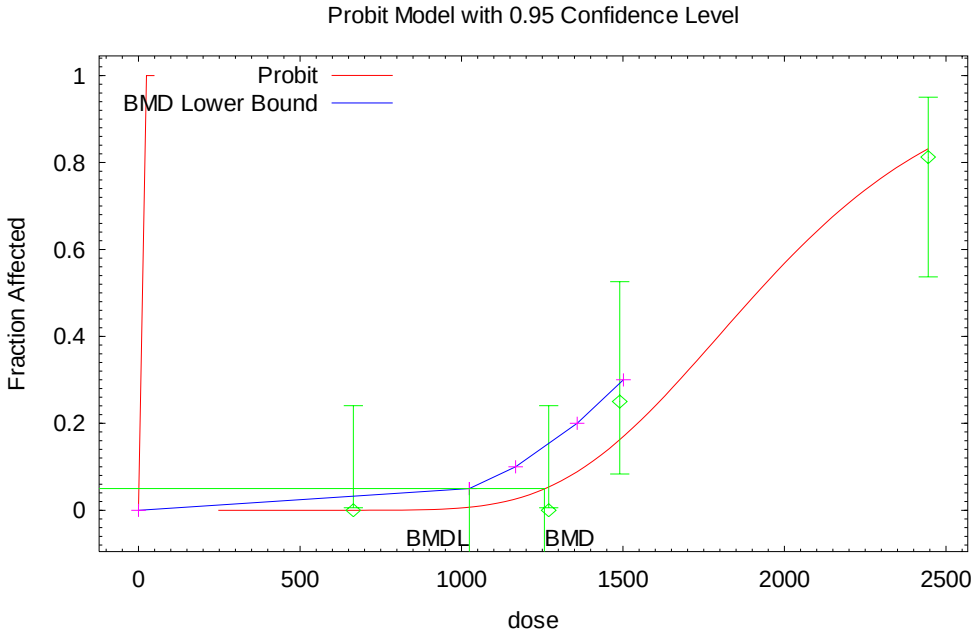
Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
665.0000	0.0000	0.000	0	16	-0.01652
1270.0000	0.0544	0.870	0	16	-0.9591
1490.0000	0.1644	2.630	4	16	0.9241
2445.0000	0.8335	13.336	13	16	-0.2251

Chi-square = 1.82 DF = 2 P-value = 0.4015

Benchmark Dose Computation

Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMC = 1256.83
BMCL = 1024.42



10:17 07/11 2007

$BMCL_{05}$ 2-hr exposure of rats (Dudley and Neal, 1942)

```
==========================================================================
        Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                Thu Mar 01 08:39:20 2007
==========================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
        P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
         where CumNorm(.) is the cumulative normal distribution function

 Dependent variable = COLUMN3
 Independent variable = COLUMN1
 Slope parameter is not restricted

 Total number of observations = 3
 Total number of records with missing values = 0
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

 User has chosen the log transformed model
            Default Initial (and Specified) Parameter Values
                background    =          0
                intercept     =    -17.8516
                slope         =     2.70268

Asymptotic Correlation Matrix of Parameter Estimates
        ( *** The model parameter(s)  -background  have been estimated at a boundary point, or
        have been specified by the user, and do not appear in the correlation matrix )

                             intercept       slope
         intercept               1             -1
        slope                   -1              1


                  Parameter Estimates

            Variable         Estimate          Std. Err.
        background              0                 NA
        intercept           -64.9721           4558.92
        slope                 9.92993           713.606

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
    has no standard error.
                  Analysis of Deviance Table
```

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-3.74067			
Fitted model	-3.74067	5.37593e-008	1	0.9998
Reduced model	-31.199	54.9175	2	<.0001
AIC:	11.4813			

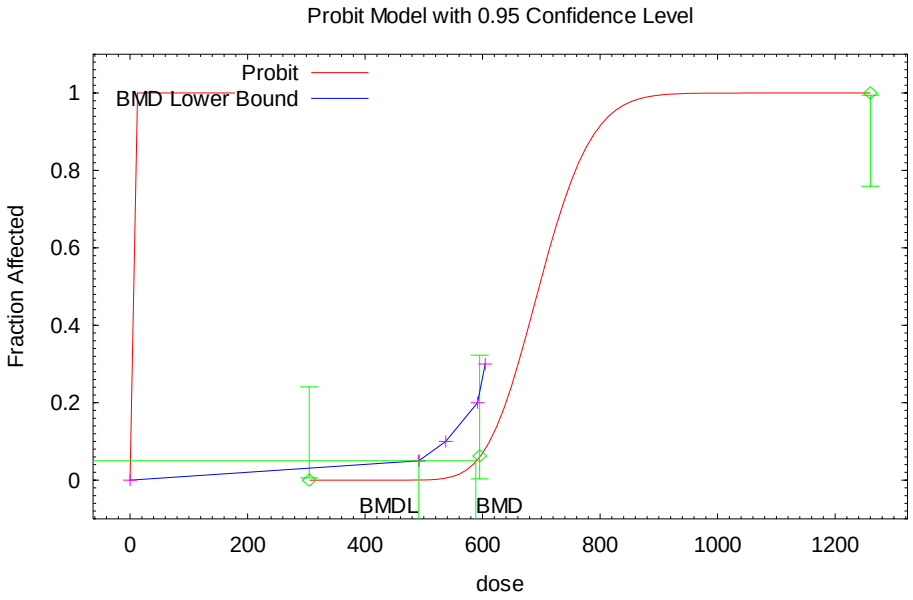
Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
305.0000	0.0000	0.000	0	16	-4.972e-008
595.0000	0.0625	1.000	1	16	-3.32e-005
1260.0000	1.0000	16.000	16	16	0.0001623

Chi-square = 0.00 DF = 1 P-value = 0.9999

Benchmark Dose Computation

Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMC = 588.401
BMCL = 491.304



$BMCL_{05}$ 4-hr exposure of rats (Dudley and Neal, 1942)

```
 ================================================================================
          Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
          Input Data File: C:\BMDS\UNSAVED1.(d)
          Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                  Thu Mar 01 08:43:13 2007
 ================================================================================

 BMDS MODEL RUN
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:
                 P[response] = Background + (1-Background) *
                 CumNorm(Intercept+Slope*Log(Dose)),  where CumNorm(.) is the cumulative
                 normal distribution function

  Dependent variable = COLUMN3
   Independent variable = COLUMN1
  Slope parameter is not restricted

  Total number of observations = 3
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008

  User has chosen the log transformed model

  Default Initial (and Specified) Parameter Values
        background    =          0
        intercept     =     -13.5273
        slope         =       2.34824

  Asymptotic Correlation Matrix of Parameter Estimates
        ( *** The model parameter(s)  -background  have been estimated at a boundary point, or
        have been specified by the user,  and do not appear in the correlation matrix )

                          intercept       slope
         intercept            1            -1
        slope                -1             1

                      Parameter Estimates
     Variable         Estimate          Std. Err.
   background            0                NA
    intercept         -50.8405          3148.13
      slope            8.75291          547.256

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
has no standard error.
```

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-9.93738			
Fitted model	-9.93738	2.60525e-007	1	0.9996
Reduced model	-32.8951	45.9154	2	<.0001
AIC:	23.8748			

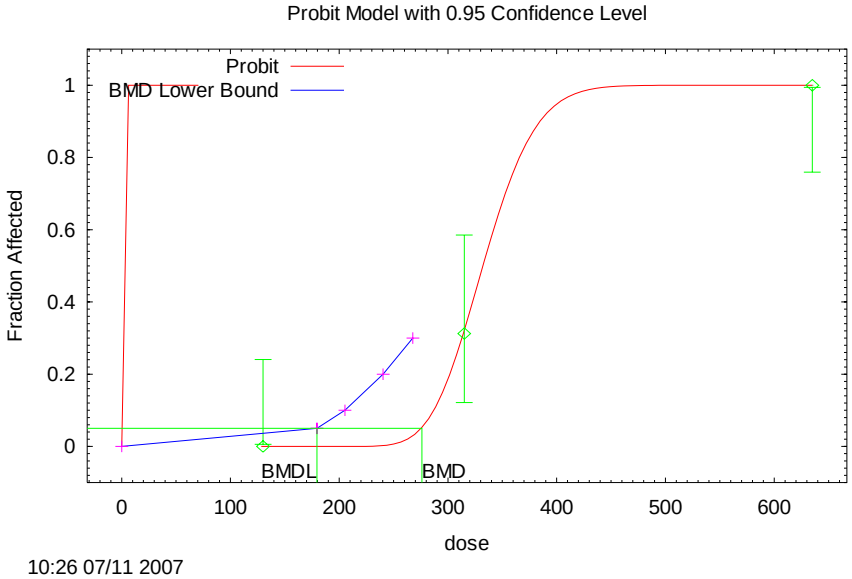
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
130.0000	0.0000	0.000	0	16	-3.783e-008
315.0000	0.3125	5.000	5	16	-3.304e-006
635.0000	1.0000	16.000	16	16	0.0003609

Chi-square = 0.00 DF = 1 P-value = 0.9997

Benchmark Dose Computation

Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMC = 276.026
BMCL = 179.532



$BMCL_{05}$ 8-hr exposure of rats (Dudley and Neal, 1942)

```
==========================================================================
        Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                Thu Mar 01 08:46:12 2007
==========================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
        P[response] = Background  + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
         where CumNorm(.) is the cumulative normal distribution function

 Dependent variable = COLUMN3
  Independent variable = COLUMN1
  Slope parameter is not restricted

 Total number of observations = 5
 Total number of records with missing values = 0
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

 User has chosen the log transformed model
      Default Initial (and Specified) Parameter Values
        background    =       0
        intercept     =    -13.
        slope         =     2.37276

 Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -background  have been estimated at a boundary point, or have
        been specified by the user,  and do not appear in the correlation matrix )

                     intercept      slope
        intercept          1          -1
        slope             -1           1


                Parameter Estimates
        Variable          Estimate        Std. Err.
        background           0               NA
        intercept          -40.1969        9.34116
        slope                7.18845       1.66722

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
    has no standard error.
```

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-18.4464			
Fitted model	-18.9141	0.935409	3	0.8169
Reduced model	-47.991	59.091	4	<.0001

AIC: 41.8281

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
90.0000	0.0000	0.000	0	16	-1.822e-007
135.0000	0.0000	0.000	0	16	-0.002528
210.0000	0.0392	0.628	1	16	0.479
270.0000	0.5188	8.300	7	16	-0.6506
320.0000	0.8977	14.363	15	16	0.5257

Chi-square = 0.93 DF = 3 P-value = 0.8184

Benchmark Dose Computation

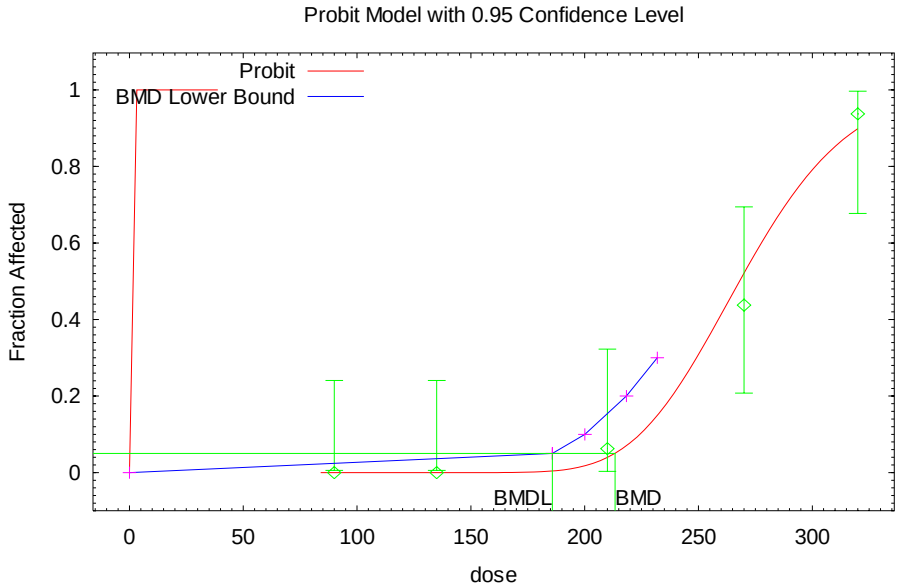
Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMC = 213.376

BMCL = 185.797



APPENDIX E: Litchfield and Wilcoxon LC_{50} Calculation

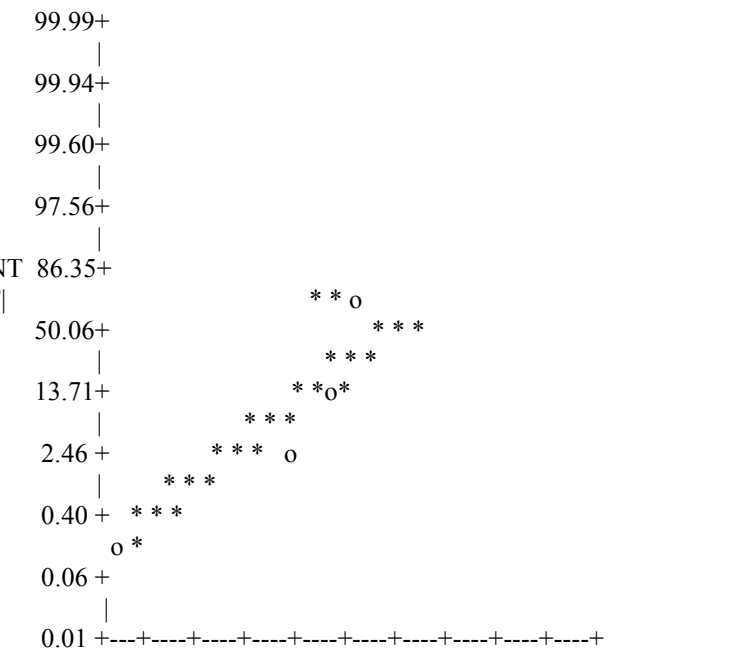
Dudley and Neal (1942): rat lethality 1-hr exposure to AN

Dose	Mortality	Observed%	Expected%	Observed-Expected	Chi-Square
665.000	0/ 16	0(0.30)	0.28	0.02	0.0000
1270.000	0/ 16	0(3.80)	9.95	-6.15	0.0422
1490.000	4/ 16	25.00	21.53	3.47	0.0071
2445.000	13/ 16	81.25	82.13	-0.88	0.0005

Values in parentheses are corrected for 0 or 100 percent Total = 0.0499

LC_{50} = 1870.153(1621.558 - 2156.859)*
Slope = 1.34(1.22 - 1.47)*
* These values are 95 percent confidence limits
Total animals = 64 Total doses = 4 Animals/dose = 16.00
Chi-square = total chi-square X animals/dose = 0.7986
Table value for Chi-square with 2 Degrees of Freedom = 5.9900

LC_{84} = 2502.530 LC_{16} = 1397.574 FED = 1.15 FS = 1.10 A = 1.07



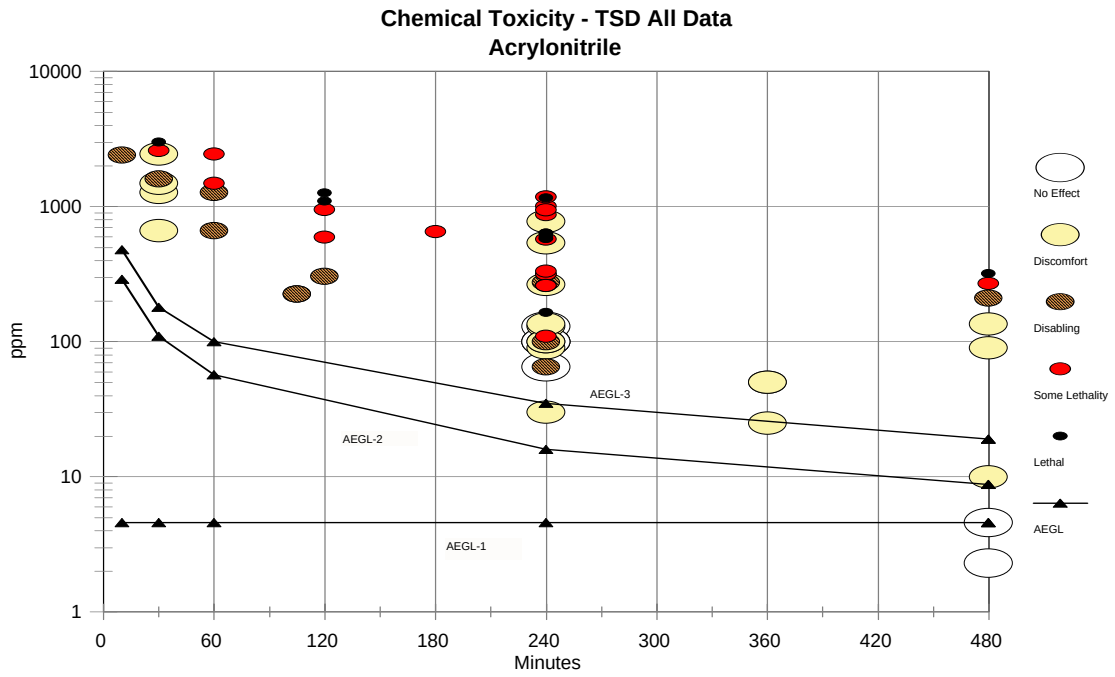
Expected Lethal Dose Values

$LC_{0.1}$	555.726
$LC_{1.0}$	834.159
$LC_{5.0}$	1114.816
LC_{10}	1271.215
LC_{25}	1541.871
LC_{50}	1870.153
LC_{75}	2268.330
LC_{90}	2751.283
LC_{99}	4192.812

APPENDIX F: CARCINOGENICITY ASSESSMENT FOR ACRYLONITRILE

Various inhalation unit risk values have been developed for acrylonitrile (see Section 2.5). IARC (1999) downgraded AN from category 2a to category 2b noting that data relative to human carcinogenicity are inadequate and that no causal association exists. Current data are sufficient for considering AN to be carcinogenic in animals (NTP, 2002). That AN would induce a carcinogenic response in humans following a single, once-in-a-lifetime acute exposure is remote.

APPENDIX G: CATEGORY PLOT FOR ACRYLONITRILE


Chemical Toxicity - TSD All Data
Acrylonitrile
10000
1000
100
10
1
ppm
0
60
120
180
240
300
360
420
480
Minutes
AEGL-3
AEGL-2
AEGL-1
No Effect
Discomfort
Disabling
Some Lethality
Lethal
AEGL