MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

August 14, 2014

To: File for 3-cyanopyridine (CAS No. 100-54-9)

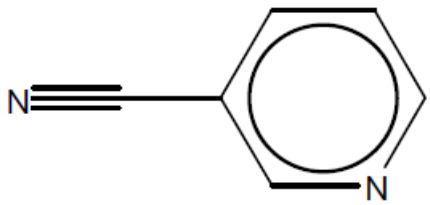
From: Michael Depa, Air Quality Division, Toxics Unit

Subject: Screening Level

The Initial Threshold Screening Level (ITSL) for 3-cyanopyridine (also known as nicotinonitrile) is 1.5 µg/m³ with annual averaging time.

The following information sources were searched in order to support the development of a screening level for 3-cyanopyridine: Chemical Abstract Service (CAS)- Online (through July 2014), United States Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS), the Registry of Toxic Effects of Chemical Substances (RTECS, 2014), the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLV), National Institute of Occupational Safety and Health (NIOSH) Pocket Guide to Hazardous Chemicals, Environmental Protection Bureau Library, International Agency for Research on Cancer Monographs, National Library of Medicine, and National Toxicology Program Status Report. The EPA has not established a reference concentration (RfC) for 3-cyanopyridine. California Office of Environmental Health Hazard Assessment (Cal-OEHHA) has not established reference exposure levels for 3-cyanopyridine. The U.S. Agency for Toxic Substances and Disease Registry (ATSDR) has not established minimal risk levels for 3-cyanopyridine. The ACGIH and NIOSH have not established occupational exposure levels. See Figure 1 for chemical and physical information for 3-cyanopyridine. Additional data is presented in the Appendix.

Figure 1. Chemical and Physical Information for 3-Cyanopyridine



Molecular Formula = $C_6H_4N_2$
Molecular Weight = 104.1g
Melting Point = 27℃
Boiling Point = 225.5℃ at 760 mmHg
Vapor Pressure = 0.296 mm Hg at 25℃ (measured)

The literature review revealed only one good quality repeated dose toxicity study suitable for ITSL derivation for this substance. Male and female rats (Crl:CD Sprague-Dawley) were gavaged once per day for 28 days at 0, 5, 30 and 180 mg/kg/day with 3-Cyanopyridine (CAS RN 100-54-9) (Purity: 99.9%) (Ichiki et al., 2003). Six animals per sex per group were assigned in the main study. Six additional animals per sex were assigned to the control and 180 mg/kg/day groups to determine the recovery of the animals 14 days after treatment was terminated.

Statistical Methods:
The Bartlett method was used to determine the homogeneity of variances. When homogeneous, the Dunnett multiple comparison calibration was employed, and when not homogeneous the Steel multiple comparison calibration was employed for comparison with the control group. Mann-Whitney U calibration was employed for evaluation of the histopathological examinations. A level of significance of 5% was employed in all cases.

Key Findings:
Body weights and food consumption were measured twice weekly during the dosing and recovery periods. Urinalysis was performed during the fourth day of administration and the second week of recovery. Hematology and blood chemistry parameters were evaluated at the end of the administration period and at the end of the recovery period after fasting the animals for 18 hours. Animals in the main study were sacrificed on day 29 and recovery animals were sacrificed on day 43. Organ weights were obtained and organ weights relative to body weight were evaluated. The following tissues from the control and high dose groups were examined histologically at the end of the administration period: salivary glands, heart, liver, kidneys, spleen, adrenals, testes, ovaries, bladder and any gross lesions. Further, since changes were observed in the high dose group in the liver, spleen, kidneys, bladder, testes and adrenals, these tissues in the middle dose group were also examined at the end of the dosing period. In addition, the liver and kidneys from the low dose group were examined. These tissues were also examined in the control and high dose group at the end of the recovery period. No ophthalmological examination was performed. A no-observed-adverse-effect-level (NOAEL) of 5 mg/kg/day was identified. A lowest-observed-adverse-effect-level (LOAEL) of 30 mg/kg/day based on centrilobular hypertrophy of hepatocytes observed in males and females of the 30 mg/kg/day and higher dosage groups. The incidence of hyaline droplets in proximal tubules of kidney was increased in males of the 30 mg/kg/day and high dose groups. Additionally, an increase in the absolute and relative weights of the liver and an increase in the relative weight of the kidneys were observed in females of the 30 mg/kg/day group.

Additional Toxicological Findings in the 28-day Oral Toxicity Study in Rats:
Salivation was observed in males and females in the 180 mg/kg/day group just after administration beginning on day 11 of the dosing period. Lacrimation was observed in one female of the 180 mg/kg/day group about 6 hours after administration on day 22 of dosing. Body weight gain of males in the 180 mg/kg/day group was suppressed throughout the dosing period. During the recovery period, the body weight of the males in the 180 mg/kg/day group remained significantly lower than the control group; however, the body weight gain over the recovery period was almost the same as that of the control group. A reduction or a tendency toward a reduction in food consumption was observed in males and females of the 180 mg/kg/day dose group during the beginning of the administration period, but a recovery exhibiting roughly the same transition as the control

group was exhibited over the remainder of the administration period and throughout the recovery period. Urinalysis showed increase in urine volume, decreases in osmotic pressure, specific gravity and pH, and pale coloration in males and females of the 180 mg/kg/day group. During the recovery period, recovery from these changes had occurred. Hematological examination showed increases in leukocyte counts, the reticulocyte ratio, MCV, MCH and decreased erythrocyte counts in males and females in the 180 mg/kg/day group; increased segmented neutrophil ratio and decreased lymphocyte ratio in males of the 180 mg/kg/day group; and increased prothrombin time in females of the 180 mg/kg/day group. Histologically, hemosiderin deposits in the red pulp of the spleen were seen as a sign of the destruction of erythrocytes and extramedullary hematopoiesis in the spleen was seen as a change corresponding to the decrease in erythrocytes in males and females of the 180 mg/kg/day group. Thus, the reduction in erythrocyte count was thought to be due to advanced destruction of erythrocytes. Further, in a two-week repeated dose toxicity preliminary test, an increase in leukocyte count was seen in both males and females of the 200 mg/kg/day group and above and was thought to be due to administration of the test substance. However, all of these changes were within the range of physiologically fluctuating values and none of the changes were toxicologically significant. Increases in MCV and MCH were seen in males and females of the 180 mg/kg/day group and a reduction in the erythrocyte count was seen in males of the same group at the end of the recovery period; however, the degree was slight as compared to the end of the administration period and a tendency to recover was thought to exist. Blood chemical examination showed increases in total protein, albumin, A/G ratio, GPT, total cholesterol and phospholipids in males and females of the 180 mg/kg/day group, and decreased triglyceride in males and decreased cholinesterase and acetylcholinesterase in females of the 180 mg/kg/day group. These blood chemistry values were not similarly affected during the recovery period. An increase, or a tendency to increase in the absolute and relative (to body weight) liver and kidney weights were observed in the males and females in the 180 mg/kg/day group, and the absolute and relative adrenal weights were increased in the males of this same group. Additionally, an increase in the absolute and relative weights of the liver and an increase in the relative weight of the kidneys were observed in females of the 30 mg/kg/day group. At the end of the recovery period, an increase in the relative weight of the kidneys and adrenals was observed in males of the 180 mg/kg/day group. Histopathologically, centrilobular hypertrophy of hepatocytes was observed in males and females of the 30 mg/kg/day and higher dosage groups. The incidence of hyaline droplets in proximal tubules was increased in males of the 30 mg/kg/day and high dose groups. Furthermore, hypertrophy of zona fasciculate of the adrenal, and extramedullary hematopoiesis and hemosiderin deposits in spleen were observed in males and females of the 180 mg/kg/day group. Necrosis of spermatocytes and spermatids, and vacuolation of Sertoli cells were noted in males of the 180 mg/kg/day group. In addition, cystitis and neutrophil infiltration in renal pelvis were observed in one female of the 180 mg/kg/day group. In animals dissected at the end of the recovery period, centrilobular hepatocytic hypertrophy was seen in the livers of three males and four females in the 180 mg/kg/day group. However, the rate of incidence and the degree of change were slight compared to what was observed at the end of the administration period. Although an increase in the relative weights of the kidneys and adrenals was seen in males of the 180 mg/kg/day group at the end of the recovery period, no histological changes were seen. Further, in the liver, spleen and bladder in which changes were observed at the end of the dosing period, the changes were eliminated or attenuated. Nor was necrosis of spermatocytes and spermatids seen in the testes, confirming the capacity for recovery. The toxicity of the test substance disappeared or

abated 14 days after cessation of administration and was a reversible change (as stated by authors of the report).

Derivation of the ITSL:
Since the key study available for 3-cyanopyridine is a 28-day oral toxicity study, Rule 232(1)(e) was used to derive the screening level. Rule 232(1)(e) is as follows:

$$ITSL = \frac{NOAEL}{35 \times 100} \times \frac{W_a}{I_a} \times \frac{a}{b}$$

Where NOAEL = the no-observed-adverse-effect-level
W_a = the weight of the animal
I_a = the inhalation rate (or IR) of the animal
a = the absorption efficiency in animals
b = the absorption efficiency in humans

Both male and female rats were observed to have centrilobular hypertrophy of hepatocytes at 30 mg/kg/day and higher dosage groups. Based on this critical effect, the NOAEL was identified as 5 mg/kg.

Since absorption efficiencies of 3-cyanopyridine in animals and humans are unknown, the default ratio of 1 was used for $\frac{a}{b}$.

The initial ranges of weights for male and female rats were given as 0.213-0.245 kg, and 0.158-0.18 kg, respectively. The weights at the end of the study were not given, nor were the inhalation rates. EPA (1988) estimated that the weights of male and female Sprague-Dawley rats after a subchronic study are 0.267 kg and 0.204 kg, respectively. These weights were used in the Rule 232(1)(e) equation as well as inputs into algorithms to calculate the inhalation rate.

The inhalation rate (IR) for male and female rats can be calculated based on their body weights using EPA (1988):

$IR_{MaleRat} = 0.883\ m^3/kg/day \times BW_{MaleRate}$
$IR_{MaleRat} = 0.883\ m^3/kg/day \times 0.267\ kg = 0.236\ m^3/day$
$IR_{FemaleRat} = 0.971\ m^3/kg/day \times BW_{FemaleRat}$
$IR_{FemaleRat} = 0.971\ m^3/kg/day \times 0.204\ kg = 0.198\ m^3/day$
Where BW = body weight

Using the male rats the ITSL can be calculated as follows:

$$ITSL = \frac{5\ mg/kg}{35 \times 100} \times \frac{0.267\ kg}{0.236\ m^3/day} \times 1$$
$ITSL = 0.00143\ mg/kg \times 1.13\ kg/m^3$
$ITSL = 0.00161\ mg/m^3 \times 1000\ \mu g/mg$
$ITSL = 1.6\ \mu g/m^3$

Using the female rats the ITSL can be calculated as follows:

$$\text{ITSL} = \frac{5\ \text{mg/kg}}{35 \times 100} \times \frac{0.204\ \text{kg}}{0.198\ \text{m}^3\text{/day}} \times 1$$

$$\text{ITSL} = 0.00143\ \text{mg/kg} \times 1.03\ \text{kg/m}^3$$

$$\text{ITSL} = 0.00147\ \text{mg/m}^3 \times 1000\ \mu\text{g/mg}$$

$$\text{ITSL} = 1.5\ \mu\text{g/m}^3$$

The ITSLs derived from the Ichiki et al. (2003) study are 1.6 µg/m³ and 1.5 µg/m³, for male and female rats, respectively. The female rat ITSL was slightly more restrictive than the male rat ITSL, and was used as the final ITSL in order to account for toxicodynamic differences that may be present between male and female humans.

It may be noted that the Rule 232(1)(e) equation includes in the denominator a factor of 35 for the extrapolation of a 7-day duration dose-response to an estimated chronic duration dose-response. The exposure duration in this key study (28 days) was substantially longer than 7 days, yet it was substantially less than a typical "subchronic" 90 day duration in rodents. There is a lack of guidance on any more appropriate extrapolation factor than 35 in such cases. Therefore, the factor of 35 is utilized here, with a note that this adds conservatism to the resulting ITSL.

References

EPA 1988. Recommendation for and documentation of biological values for use in risk assessment. PB 88-179874.

Ichiki, T., H. Ogata, H. Furukawa, K. Yuki, T. Saito, K. Kamiya, M. Hamamura. Twentyeight-day Repeat Dose Oral Toxicity Test of 3-Cyanopyridine in Rats. Panapharm Laboratories Co., Ltd., Kumamaoto, Japan. December 17, 2003. Remarks: Reliable without restriction; guideline study – report written in Japanese, fully translated. American Chemistry Council, Pyridine and Pyridine Derivatives HPV Work Group. http://www.epa.gov/hpv/pubs/summaries/pyriderv/c14925.pdf

Appendix

CASRNs 100-54-9 and 100-70-9 are solids at ambient temperature with high water solubility and moderate vapor pressure. They are expected to have high mobility in soil. Volatilization is considered moderate based on their Henry's Law constants. The rates of hydrolysis and atmospheric photooxidation are considered negligible. The rate of biodegradation is considered rapid to moderate and they are expected to have low persistence (P1) and low bioaccumulation potential (B1).

The acute toxicity of sub-category II is low via the oral (rats) and dermal (rabbits) routes of exposure. Repeated oral exposures with CASRN 100-54-9 in rats showed histopathological changes in the liver at 30 mg/kg-bw/day; the NOAEL for systemic toxicity was 5 mg/kg-bw/day. At higher doses, there was necrosis of spermatocytes and spermatids. No specific reproductive toxicity studies were available but effects on the male reproductive system were observed after repeated oral exposures to CASRN 100-54-9 in rats. No developmental toxicity studies were available. From: Hazard Characterization Document, SCREENING-LEVEL HAZARD CHARACTERIZATION, Pyridine and Pyridine Derivatives Category. U.S. Environmental Protection Agency September, 2009
http://www.epa.gov/hpvis/hazchar/Category%20Pyr%20and%20Pyr%20Derivs_Sept2009.pdf