DOT/FAA/AM-93-8

Variations in Time-to-Incapacitation and Blood Cynanide Values for Rats Exposed to Two Hydrogen Cyanide Gas Concentrations

Office of Aviation Medicine
Washington, D.C. 20591

Arvind K. Chaturvedi
Boyd R. Endecott
Roxane M. Ritter
Donald C. Sanders

Civil Aeromedical Institute
Federal Aviation Administration
Oklahoma City, Oklahoma 73125

May 1993

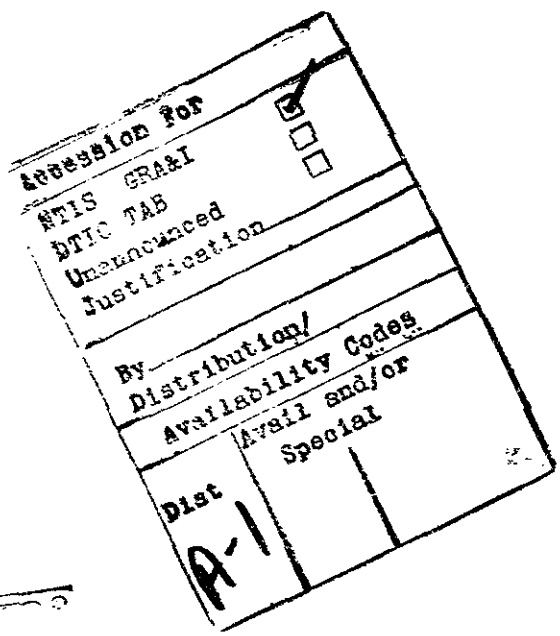
Final Report

U.S. Department
of Transportation
Federal Aviation
Administration

NOTICE

This document is disseminated under the sponsorship of the U. S. Department of Transportation in the interest of information exchange. The United States Government assumes no liability for the contents or use thereof.

The use of animals for this study was approved by the Institutional Laboratory Animal Use Review Committee under the U. S. Department of Agriculture Animal Welfare Act. The animals were cared for and humanely used in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, PHS Publication (NIH) No. 86-23, revised 1985.



Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-93/8		

4. Title and Subtitle	5. Report Date
VARIATIONS IN TIME-TO-INCAPACITATION AND BLOOD CYANIDE VALUES FOR RATS EXPOSED TO TWO HYDROGEN CYANIDE GAS CONCENTRATIONS	May 1993
	6. Performing Organization Code
7. Author(s) Arvind K. Chaturvedi, Boyd R. Endecott, Roxane M. Ritter, and Donald C. Sanders	8. Performing Organization Report No.
9. Performing Organization Name and Address FAA Civil Aeromedical Institute P. O. Box 25082 Oklahoma City, OK 73125-5066	10. Work Unit No. (TRAIS)
	11. Contract or Grant No.
	13. Type of Report and Period Covered
12. Sponsoring Agency Name and Address Office of Aviation Medicine Federal Aviation Administration 800 Independence Avenue, SW Washington, DC 20591	14. Sponsoring Agency Code

15. Supplementary Notes

This work was performed under task AM-B-92-TOX-60.

16. Abstract

It has been suggested that protective breathing devices protect aircraft passengers from combustion products for 5 min during evacuation and for 35 min during in-flight-plus-evacuation. Hydrogen cyanide (HCN), a combustion gas, produces incapacitation at relatively low concentrations, and time-to-incapacitation (t_i) is an applicable index for predicting escape from a fire. Variations in t_i and blood cyanide (CN^-) at specific HCN gas exposure concentrations have not been evaluated. Therefore, t_i and blood CN^- at t_i for two HCN concentrations that produce 5- and 35-min t_i were determined in male Sprague-Dawley rats. Blood CN^- levels as a function of HCN exposure time were measured. Animals were individually exposed to HCN gas in a chamber equipped with a rotating cage, and t_i was recorded as the time from insertion of the animal into the cage until it could no longer walk. At incapacitation and at selected intervals prior to t_i, rats were quickly removed from the cage and killed for blood collection and CN^- quantitation. Chamber HCN concentrations were monitored during the exposures. For the 5-min test (mean ± SD; n = 50), HCN gas = 184 ± 10.0 ppm, t_i = 5.1 ± 0.8 min, and blood CN^- = 2.3 ± 0.5 µg/mL; for the 35-min test, HCN gas = 64 ± 6.1 ppm, t_i = 31.1 ± 11.2 min, and blood CN^- = 4.2 ± 1.3 µg/mL. Blood CN^- levels increased as a function of HCN exposure time, but the blood CN^- level at the 5-min t_i was half of the 35-min blood CN^- level; the HCN gas uptake rate at 184 ppm was about 3 times that at 64 ppm. These findings suggest that the blood CN^- level at incapacitation may vary substantially, depending upon the HCN exposure concentration; an equation is proposed for predicting blood CN^- levels in rats.

17. Key Words	18. Distribution Statement
Inhalation toxicology; combustion gas/ hydrogen cyanide; time-to-incapacitation; blood cyanide level; hydrogen cyanide uptake; animal exposure system	Document is available to the public through the National Technical Information Service, Springfield, VA 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	20	

VARIATIONS IN TIME-TO-INCAPACITATION AND BLOOD CYANIDE VALUES FOR RATS EXPOSED TO TWO HYDROGEN CYANIDE GAS CONCENTRATIONS

INTRODUCTION

Inhalation of toxic combustion gases is a significant cause of deaths in aircraft fires. During in-flight fires, viable escape options do not exist for 20 to 30 min before the aircraft can land at a suitable location (Crane, 1984), and postcrash fire survival scenarios usually span a shorter period of time. An individual incapacitated from smoke gases has a negligible chance for escape from a fire environment. To enhance survivability, the development of passenger protective breathing equipment (PPBE) was initiated by both industry and government (Higgins, 1987). Concentrations of toxic gases normally encountered in aircraft fires and PPBE maximum pass limits that would provide the wearer a reasonable time to escape from a fire have been defined by the European Organization for Civil Aviation Equipment (EUROCAE, 1991). Accordingly, the prevailing view is that these devices should at least protect individuals for 5 min during an evacuation phase and for 25 min (20 min + 5 min) during an in-flight-plus-evacuation phase. However, an in-flight period of 30 min might be necessary in certain scenarios. Variations in the onset of incapacitation at specific fixed concentrations of toxic combustion gases for these evacuation periods have not been clearly established; levels of the toxic gases in blood should be quantitated to correlate with the incapacitation response.

One of the principal toxicants generated in potentially lethal amounts during fires is hydrogen cyanide (HCN) gas (Hartzell, 1989; Gad, 1990). This gas can produce incapacitation at relatively low concentrations (Crane, et al., 1989; Hartzell, 1989; Gad, 1990), and time-to-incapacitation (t_i) is an applicable toxicological index for predicting escape time from a fire (Crane, et al., 1977; Spurgeon, et al., 1979; Sanders, et al., 1991). In HCN poisonings, blood cyanide (CN^-) levels are frequently measured to explain the severity of HCN gas exposure; how blood CN^- levels are related to HCN exposure concentrations and exposure times is not adequately defined. Therefore, this study was conducted to evaluate variations in t_i and blood CN^- measurements at the 2 HCN concentrations that would incapacitate the laboratory rats at 5 and 35 min, representing durations of fire exposure in typical aircraft accidents. Relative HCN gas uptakes, measured as increases in blood CN^- levels as a function of exposure time at the 2 HCN concentrations, were included to establish a possible interrelationship between HCN exposure concentration, exposure time, and blood CN^-. Information from this study might be helpful in the interpretation of postmortem blood CN^- levels in fire victims.

MATERIALS AND METHODS

Materials

Chemicals used for analyses were of reagent grade and obtained from commercial sources. Compressed HCN gas (1% in N_2) and breathing air were purchased locally in cylinders. Chemical solutions were prepared in deionized water.

Animals

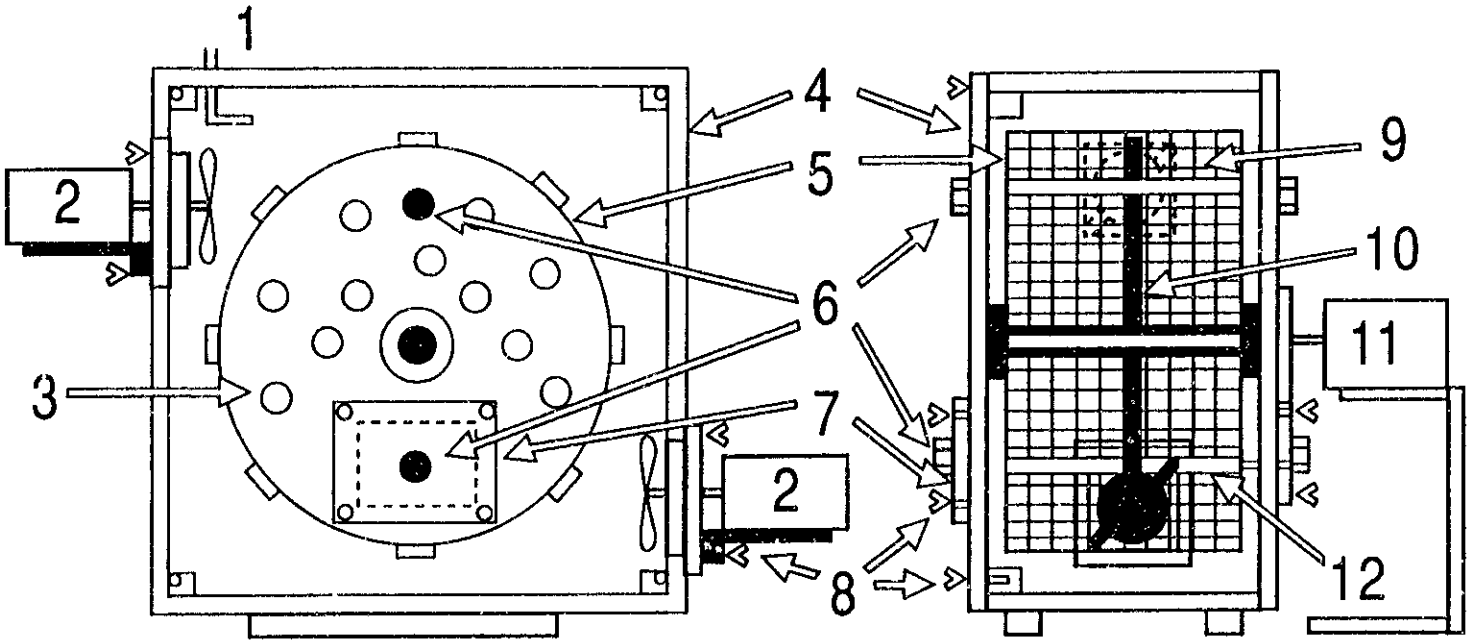
Male Sprague-Dawley rats (100 to 125 g) were supplied by Charles River Breeding Laboratories, Wilmington, MA. Animals were inspected by a veterinarian upon receipt and housed (4 to 6 per cage) in stainless steel cages (61 cm L X 45 cm W X 27 cm H) with mesh-wire floors in the centralized animal care facility of the Institute. The facility was maintained at 22 to 24°C with a relative humidity of 40 to 60% and a 12-hr on/off fluorescent light cycle (lights on 7 a.m. to 7 p.m.). Rats were held in isolation for 8 days prior to use and allowed food and water *ad libitum* during, and following, the isolation period. All animals were fasted overnight before the gas exposures.

Animal Exposure Chamber

The exposure chamber constructed from 1.3-cm polymethylmethacrylate sheets had internal dimensions of 50.8 cm L X 26.6 cm W X 50.6 cm H (Figure 1). In the chamber, there was a cylindrical cage (40.5 cm

Figure 1. Animal Exposure Chamber.

1. HCN gas-air inlet; 2. Fans (1/15 hp motor, 5,000 rpm, fitted 7-cm, 4-bladed Nylon fan); 3. Ventilation holes, 12-mm diameter, cut through center divider of rotating cage; 4. Exposure chamber walls constructed from 1.3-cm polymethylmethacrylate; 5. Rotating cage assembly (divider and outer rim are 6-mm polymethylmethacrylate; surface is polyethylene mesh); 6. Ports sealed with rubber septa; 7. Access-door for animal insertion and removal; 8. Thumbscrew fasteners; 9. Polyethylene mesh cover, mesh openings are approximately 7-mm square; 10. Center divider and support for rotating cage; 11. Cage drive motor (4-rpm); 12. Cross supports for chamber rims and plastic mesh cover.



diameter; 25.0 cm W) vertically divided into 2 equal compartments each 12.5-cm wide. The cage was rotated horizontally by a 4-rpm geared motor to provide a circumferential velocity of 8.5 cm/sec. The front compartment of the cage was used for the animal tests. A gasketed access-door (10.4 cm X 10.4 cm) on the front panel of the chamber at the cage floor level allowed rapid animal insertion into, and removal from, the cage. There were 2 fans, 1 on each side of the chamber; 1 fan was at the upper part and the other at the lower part of the chamber. These fans were for homogeneous mixing and circulation of the gas-air mixture in the flow-through chamber atmosphere. There were 2 ports sealed with rubber septa on the front panel of the chamber.

The HCN gas and air from cylinders were mixed by passing through a baffled cylindrical mixing tube before entering the chamber. Flow rates of the gas and air were regulated automatically using Scott model 5850E mass flow controllers attached to a Scott model 5878A power supply/control unit (Scott Environmental Technology, Inc., Plumsteadville, PA). The input of gas-air mixtures was through a port in the top of the chamber. The entire chamber was installed in a fume hood into which the chamber exhaust was vented.

Experimental Protocol

Preliminary HCN gas concentrations for producing incapacitation at 5- and 35-min exposure times were calculated from the concentration-t_i curve described in a previous study (Crane, et al., 1989). The airflow into the chamber was established at 4 L/min; the HCN gas flow was adjusted to produce the estimated gas concentrations required in the chamber. The HCN concentrations were refined by t_i measurements using 32 rats. Based on these experiments, gas concentrations produced by flow rates of (80 mL/min of 9239 ppm HCN + 4 L/min air) and (25 mL/min of 9239 ppm HCN + 4 L/min air) were adopted for the 5- and 35-min t_i study, respectively. The nominal dynamic flow of gas-air mixtures at 4 L/min prevented major HCN concentration changes during the rat insertion, exposure, or removal. Initial experiments suggested that ambient O_2 levels did not change for single rat exposures by the HCN-air mixture flows through the chamber; therefore, O_2 concentration was not monitored during the animal exposure experiments.

On every day of the experiments, the chamber flow-through atmosphere was equilibrated and stabilized and its HCN gas concentration was determined. When the HCN concentration stabilized at the desired exposure level, a chamber atmosphere sample for zero min was withdrawn from the access-door port for the HCN analysis. Following this, the chamber fans and cage motor were turned off, timer was set to zero, and retaining screws on the chamber access-door were removed. Then, in rapid succession, the door was opened, a rat was inserted into the cage, the door was closed, and the timer, cage motor, and fans were activated.

Fifty rats were individually exposed to the gas at each of the 2 concentrations to determine variations of t_i and of blood CN^- at incapacitation. For the relative rates of HCN uptake at the 2 exposure concentrations, additional rats were singly exposed for intervals less than t_i; the exposure intervals were 1, 2, 3, and 4 min for the 5-min experiments (4 rats/exposure interval), while they were 2.5, 5, 10, 15, and 25 min for the 35-min tests (3 animals/exposure interval). At incapacitation or at the end of each exposure interval, rats were quickly removed from the chamber and killed (by cervical dislocation) for blood collection and CN^- determination.

The criterion for incapacitation was the inability of the rat to walk, i.e., when tumbling or sliding began, as subjectively determined by 2 individuals. The t_i was recorded as the time from insertion of the rat until it could no longer walk in the rotating cage. Besides the zero-min samples, chamber HCN samples were collected at 1 and 4 min in the 5-min t_i study and at 1, 5, 15, and occasionally, at 30 min in the 35-min t_i study. HCN gas measurements at these intervals were conducted to describe the gas concentration with time during the animal exposure. Findings from these measurements indicated that the gas concentration did not significantly change during the exposure; typical concentration-time relationships for the 5- and 35-min study are illustrated in Figure 2. This allowed the assumption that the gas concentration at t_i (or the end of the applicable selected exposure intervals) was essentially identical to that in the chamber sample immediately preceding incapacitation (or the exposure interval). Since the rapid removal of the exposed animal at a particular time prohibited the simultaneous manual sampling of the chamber atmosphere, the HCN gas

concentration at incapacitation (or the exposure interval) was estimated by extrapolating the value of the preceding concentration to the t_i (or exposure time). HCN exposure concentration for each experiment was obtained by the integration of chamber HCN concentration as a function of exposure time from $t = 0$ to $t = t_i$ (or exposure time) and dividing the resulting product by t_i (or exposure time), i.e.,

$$\textit{Exposure Concentration} = \frac{\int_{t=0}^{t=t_i} C\,dt}{t_i}, \qquad (1)$$

where C = HCN concentration in ppm and t = exposure time in min.

Chamber HCN Gas and Blood CN^- Determinations

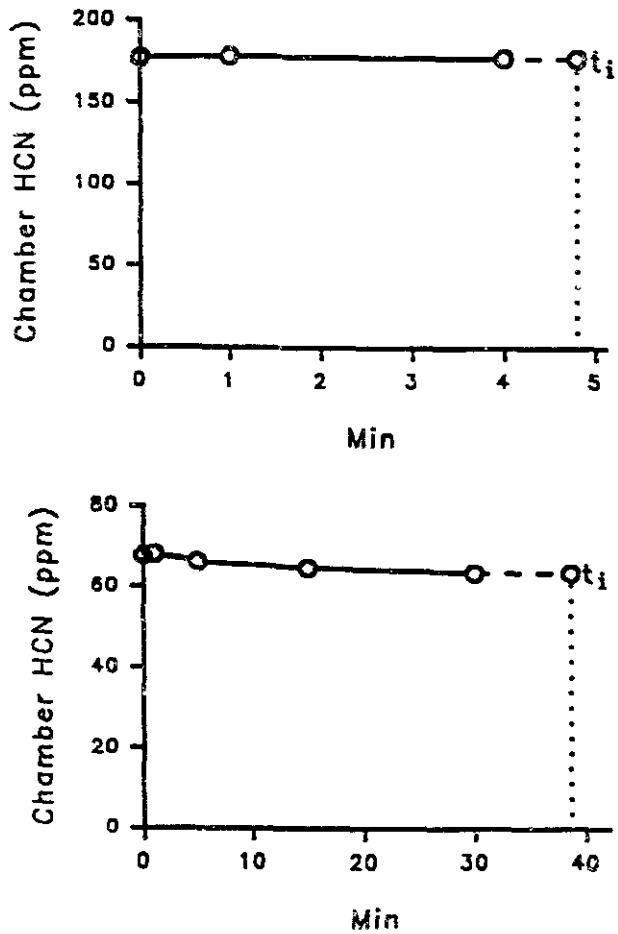
Chamber HCN gas concentrations and blood CN^- levels were determined using an automated Technicon AutoAnalyzer™ II System consisting of a sampler IV, a 2-speed proportioning pump III, a single-channel colorimeter, and a pen recorder II (Technicon Instruments Corporation, Tarrytown, NY). The manifold, a Technicon cyanide analytical cartridge, incorporated a distillation coil. The coil was attached by a short length of acid-flex tubing to a distillation head and condenser. The methodology employed was a modification of the Technicon Industrial Method No. 312-74W (Technicon, 1974).

Reagents used in the assay were: *o*-phosphoric acid (1.74 M); phosphate buffer (0.01 M; pH 5.2); chloramine-T (0.0142 M); pyridine (0.931 M)-barbituric acid (0.117 M) solution in 0.17 M HCl. Initially, sample (chamber sample or blood in 0.1 N NaOH; 1.0 mL/min), *o*-phosphoric acid (0.6 mL/min), and air (a bubble pattern of 2.5 mL/min) were mixed (in a double mixing coil) and passed thorough the distillation coil immersed in a 155° C oil bath; this digestive distillation stage converts complexed CN^- present in the sample to HCN. Vapors, including HCN gas, from the coil were advanced through the distillation head and, then, through a condenser having cold (13° C) water circulated in its outer jacket; the waste (*o*-phosphoric acid and/or blood residues) from the distillation head was removed at the rate of 0.4 mL/min. The condensate (resampled at 0.8 mL/min), phosphate buffer (0.42 mL/min), air (0.6 mL/min), chloramine-T (0.1 mL/min), and then, the pyridine-barbituric acid reagent (0.8 mL/min) were sequentially mixed and flowed through the colorimeter cell. The color intensity of the reaction mixture was measured using a 570 nm filter; the absorption for each sample was registered on the recorder. The flow rates of wastes from the condenser, and from the flow cell, were 1.0 mL/min. A water blank was inserted between each of the test samples. This system operated at a sampling rate of 20 samples/hour with a sample:wash ratio of 1:5. These precautions were taken to prevent sample carryover and achieve baseline separation on the recorder trace. Chamber atmosphere and blood samples were analyzed for CN^- immediately following the sample collection. A stock CN^- solution (100 mg/L), prepared from NaCN (purity: 96% by analysis), in 0.1 N NaOH was used for the preparation of working standards.

For chamber HCN gas concentrations, a 17-mL sample of chamber atmosphere was manually withdrawn into an acid-washed and oven-dried 30-mL glass syringe from the port on the access-door. The volume was immediately adjusted to exactly 15 mL, and then, 0.1 N NaOH was drawn until the plunger reached the 30-mL index. The syringe was closed with a plastic cap, and the gas-liquid mixture was allowed to equilibrate on a mechanical rocker for 5 min. Portions of the NaOH solution containing CN^- and 5 working standards were transferred into the AutoAnalyzer sample cups for the CN^- analysis. These standards were prepared from the stock CN^- solution; CN^- concentrations in the standards were 50, 100, 200, 300, and 400 µg/L. Standards were analyzed in triplicate and chamber samples in duplicate. Standard curves were constructed each day by plotting the absorbance peak heights *versus* CN^- concentrations. The analytical response was linear over the selected concentration range; average slope, *y*-intercept, and correlation coefficient values were 0.4731, -2.0306, and 0.9999, respectively. Concentrations of HCN gas in the chamber samples were expressed as ppm (v/v) at ambient temperature and pressure. HCN concentration in the gas cylinder was similarly determined.

Figure 2. Typical Chamber HCN Concentration-Exposure Time Relationships for the 5- and 35-Min Study.

During the exposure of animals to HCN gas, chamber atmosphere samples were manually collected at selected time intervals, and HCN concentrations in the samples were determined. The gas concentration at incapacitation was estimated by extrapolating the value of the preceding concentration to the t_i. The HCN exposure concentration was then calculated by Equation (1), as described in the text.



For blood CN^- levels, body cavities of the killed rats were quickly opened, and blood was drawn from the descending aorta using a 2.5-cc glass syringe and an 18-G needle. The collected blood samples were immediately injected into stoppered glass tubes containing solid sodium heparin (143 USP units) and mixed on a mechanical rocker for 5 min. The heparinized blood samples from the test animals were diluted 1:20 with 0.1 N NaOH in 10-mL stoppered volumetric flasks prior to the CN^- analysis. Also, 5 working standards were prepared by adding 0.5 mL aliquots of pooled blood from untreated rats to 10-mL volumetric flasks containing approximately 8.0 mL of 0.1 N NaOH. To this mixture, the requisite volumes of the stock CN^- solution were added, and the volume was adjusted to 10 mL with the NaOH solution. This yielded working standards for blood matrix containing 50, 100, 200, 300, and 400 µg of CN^-/L. Portions of these samples and standards were transferred into the AutoAnalyzer sample cups for the CN^- analysis in triplicate. Standard curves, prepared by plotting the absorbance peak heights *versus* CN^- concentrations, were linear for the concentration range. Average values of slope, *y*-intercept, and correlation coefficient were correspondingly 0.2190, 16.3554, and 0.9999.

Statistics

Values are presented as the mean ± SD, and a difference between means was considered significant at $p \leq 0.05$. Where possible, data were analyzed at $\alpha = 0.05$ using the analysis of variance and Tukey's HSD multiple comparison test for statistical pairwise differences between the groups (Wilkinson, 1989); otherwise, the significance of differences between means was checked by the Student's *t*-test (SigmaPlot, 1991). The normality of distribution of measurements was established by performing the Kolmogorov-Smirnov one-sample test at $\alpha = 0.05$ (Miller and Miller, 1988; Wilkinson, 1989). Slope, *y*-intercept, and correlation coefficient were calculated by linear regression analysis.

RESULTS

The HCN exposure concentration for producing the 5-min t_i was 184 ppm, while it was 64 ppm for the 35-min t_i (Table 1); coefficients of variation for these HCN concentrations were correspondingly 5.4 and 9.5%. The distribution of 5-min t_i values was moderate with a spread of 3.5-min from minimum to maximum, but the 35-min t_i measurements had a wide range of 56-min with the 31.1-min mean. Blood CN^- levels at incapacitation were 2.3 µg/mL for 184 ppm HCN and 4.2 µg/mL for 64 ppm HCN ($p \leq 0.05$); the variation in the blood CN^- values was more at the 35-min t_i than that at the 5-min t_i. In general, variations in the 35-min values of these parameters were about 2 times that for the 5-min values. In comparison to the HCN gas values, variations in the t_i and blood CN^- values were more; however, variation coefficients for the t_i and blood CN^- values were not much different from each other within each set of studies. Except for the 5-min t_i and 35-min HCN gas values, the 5-min HCN concentration, 35-min t_i, and 5- and 35-min blood CN^- measurements had normal distribution patterns (Figure 3), as they were not statistically different from their corresponding standard normal population forms ($p > 0.05$). The 35-min HCN gas measurements extended towards left from the mean. Although the 35-min t_i and CN^- measurements followed the normal distribution patterns at $p \leq 0.05$, these measurements were distributed more towards right from their means.

As is depicted in Figure 4, the blood CN^- level increased as a function of exposure time for both HCN exposure concentrations. Mean HCN exposure concentrations for the 5- and 35-min uptake study were 183 ± 4.4 (n = 16) and 71 ± 3.4 (n = 15) ppm, respectively. Within each set of studies, HCN concentrations for the exposure intervals were not different from each other ($p > 0.05$). The HCN gas uptakes, as represented by the blood CN^- levels *versus* exposure times, were linear; the CN^- in blood did not appear to attain a steady state prior to incapacitation. Mean values of slope, *y*-intercept, and correlation coefficient for the 5-min uptake study were 0.4007, 0.1666, and 0.9697, respectively; values of these regression functions were correspondingly 0.1518, 0.1145, and 0.9920 for the 35-min uptake study. Slopes of the regression indicated that blood CN^- increased at the rate of 0.401 µg/mL/min for 183 ppm HCN and at 0.152 µg/mL/min for 71 ppm HCN.

Table 1. Time-to-Incapacitation (t_i) and Blood Cyanide (CN^-) Values for Rats Exposed to Two Hydrogen Cyanide (HCN) Gas Concentrations.

Parameters	Values[a, b]		
	Mean (Range)	SD	CV%
	For 5-Min Study		
HCN (ppm)	184 (159 - 202)	10.0	5.4
t_i (min)	5.1 (4.0 - 7.5)	0.8	15.7
Blood CN^- (μg/mL) at t_i	2.3 (1.5 - 3.7)	0.5	21.7
	For 35-Min Study		
HCN (ppm)	64 (46 - 75)	6.1	9.5
t_i (min)	31.1 (14.0 - 70.0)	11.2	36.0
Blood CN^- (μg/mL) at t_i	4.2 (2.3 - 9.1)	1.3	31.0

[a]Mean values are derived from 50 rats individually exposed to HCN gas; SD = Standard Deviation; CV = Coefficient of Variation, (SD ÷ Mean) x 100.

[b]The data from which values were calculated are listed in Tables 1 and 2 of the Appendix.

Figure 3. Distribution of HCN Exposure Concentrations, t_i Values, and Blood CN^- Levels for the 5- and 35-Min Study.

The frequency distributions for these parameters were based on the measurements derived from 50 rat experiments (n = 50) for each of the 2 studies. The normality of distribution of measurements was established by the Kolmogorov-Smirnov one-sample test at α = 0.05. Details are given in the text.

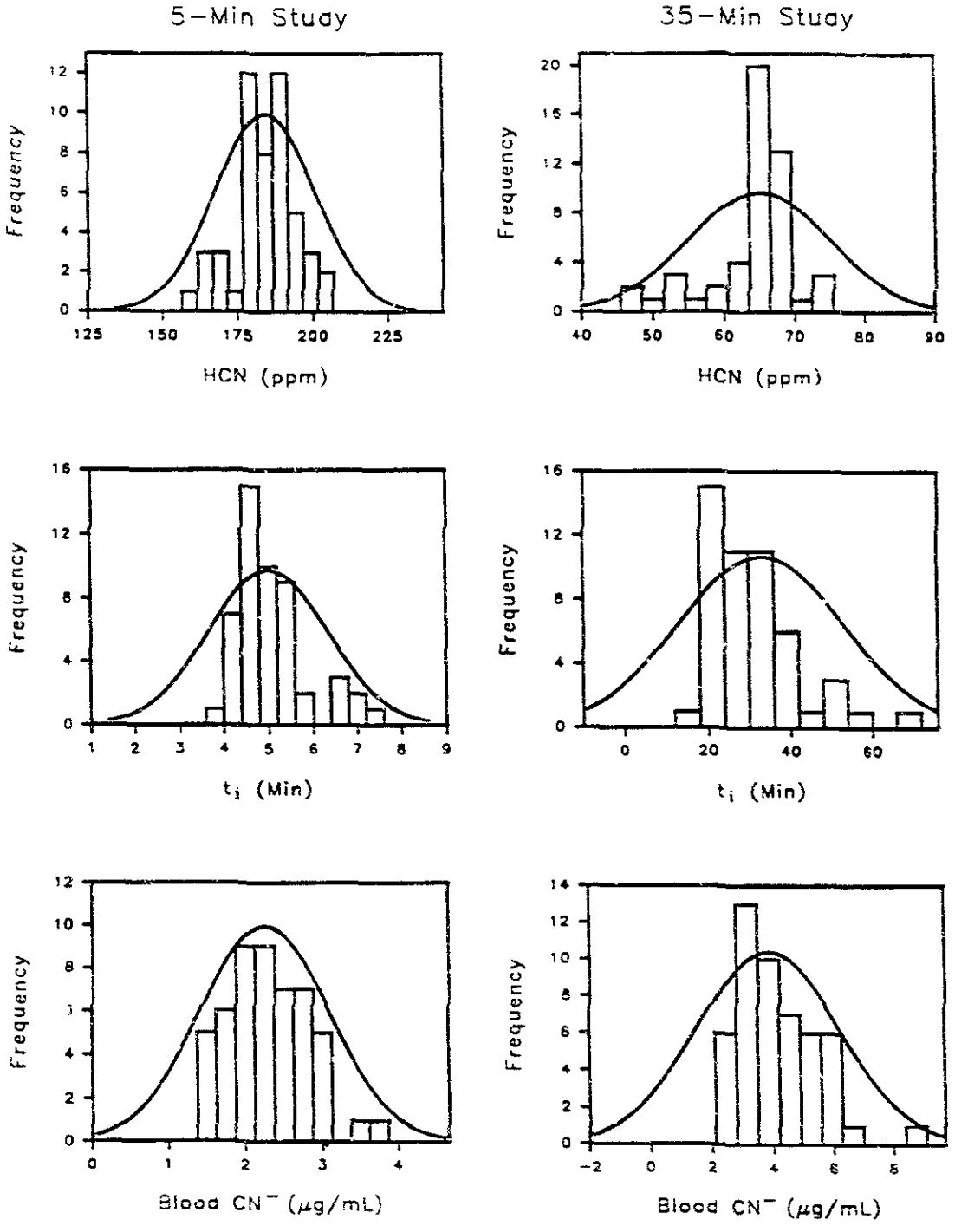
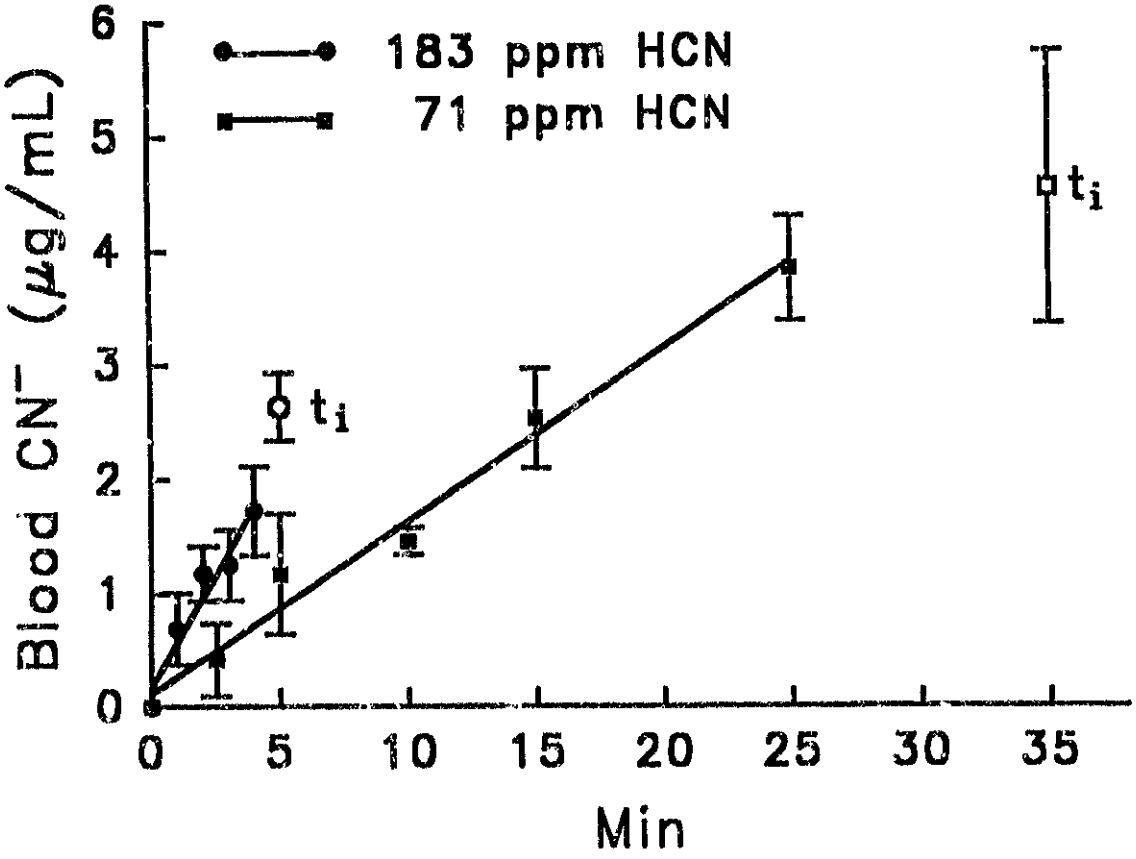


Figure 4. Blood CN^- Levels as a Function of Exposure Time at Two HCN Gas Concentrations.

Animals were individually exposed to 183 ± 4.4 ppm HCN (n = 16) for 1, 2, 3, or 4-min interval (4 rats/exposure interval) or to 71 ± 3.4 ppm HCN (n = 15) for 2.5, 5, 10, 15, or 25-min interval (3 rats/exposure interval). The HCN exposure concentrations were calculated from t = 0 to t = "exposure interval" using Equation (1) given in the text and represent the mean of all exposure intervals within each set of the uptake studies. Each point depicts the mean of blood CN^- values (n = 4 for 183 ppm HCN or 3 for 71 ppm HCN); bars represent SD. Blood CN^- levels as a function of gas exposure time are represented by a least-squares linear regression for both exposure conditions. The points marked "t_i" at 5 and 35 min represent the blood CN^- levels for animals incapacitated at exactly 5 and 35 min, respectively, during the t_i-variation study; these values were not included in the regression analysis, since the animals were incapacitated at the time of removal from the chamber. Although the 35-min blood CN^- level (n = 3) for the low HCN concentration was not different from the 25-min CN^- value, it was significantly different from the 15-min CN^- ($p \leq 0.05$). The 5-min blood CN^- value (n = 4) for the high HCN concentration was different from the corresponding 4-min value. The HCN concentrations for each exposure interval, including t_i, did not significantly change during either the 5- or 35-min study ($p > 0.05$). The data from which these values were calculated are listed in the Appendix (Tables 3 and 4).



DISCUSSION

The HCN exposure concentrations of 184 and 64 ppm were determined to produce the 5- and 35-min t_i in rats, respectively. The nominal variations in the HCN concentrations suggested that the fluctuations in t_i values would be primarily attributed to the changes in the individual animal response. The variations in the 35-min t_i and blood CN^- values were consistent with the 35-min gas value distribution and could have been partially attributed to the increasing difficulty in the t_i judgment, when the onset of incapacitation is less acute than at the 5-min HCN gas concentration; Haber's rule (Packham and Hartzell, 1981) becomes less applicable in the concentration-t_i curve at a lower gas concentration and longer t_i, where the "t_i" rational function, as a vertical asymptote, is less defined (Crane, et al., 1989). Therefore, a higher coefficient of variation would occur for a "longer t_i" in relation to a "shorter t_i." The HCN gas accumulated "dose" levels derived from the C•t product (C = HCN exposure concentration in ppm; t = t_i in min) were calculated to be 938 and 1990 ppm•min at 184 and 64 ppm, respectively. These C•t values fall within the reported ranges for incapacitation in humans (750-2500 ppm•min by 200-100 ppm HCN) (Hartzell, 1989), cynomolgus monkeys (1248-1900 ppm•min by 156-100 ppm HCN) (Purser, et al., 1984), and rats (1200-2700 ppm•min by 250-130 ppm HCN) (Kaplan and Hartzell, 1984). Thus, the C•t values for these species are similar. It appears that the rat could be a reasonable model for predicting escape time for humans exposed to HCN gas, a view also expressed by Kaplan and Hartzell (1984).

The increase in t_i by decreasing the HCN exposure concentration was not consistent with an expected decrease in the 35-min t_i blood CN^- level; instead, there was a substantial increase in blood CN^-. The C•t value appeared to be a better parameter than the HCN exposure concentration for correlating with the blood CN^- level, since the nearly 2-fold increase in the blood CN^- was compatible with the 2-fold increase in the C•t value. Even then, assuming that the measured blood CN^- level is directly related to the onset of incapacitation, the blood CN^- level should theoretically be the same at the pharmacological response, regardless of the HCN exposure concentration. The high CN^- level at the 35-min t_i could be explained by the (i) possible time dependent binding of CN^- to non-critical sites, sequestering of CN^- in erythrocytes (a CN^- detoxification mechanism) (Vesey and Wilson, 1978; McMillan and Svoboda IV, 1982), and/or enzymatic conversion of CN^- to a non-toxic form (Klaassen, 1990), and (ii) inability of our method to selectively quantitate critical CN^-. At the low HCN concentration, the slower gas uptake conceivably allowed a larger fraction of CN^- to be utilized in the detoxification processes, thereby retarding the critical CN^- reaching a threshold level necessary for the onset of incapacitation. Since the method employed quantitates total blood CN^-, the determined blood CN^- level may not necessarily represent a specific level for incapacitation; however, it is at least an indication of the severity of HCN exposure.

The blood CN^- range observed in our study (1.5-9.1 μg/mL) is in reasonable agreement with the reported ranges in humans (0.7-5.4 μg/mL) dying from smoke inhalation (Baud, et al., 1991) and in monkeys (1.2-3.0 μg/mL) at incapacitation (Purser, et al., 1984) and rats (3.1-3.7 μg/mL) at death (Yamamoto, 1977) caused by the HCN inhalation. Furthermore, the blood CN^- level of as high as 8.4 μg/mL has been quantitated in aircraft fire victims (Mayes, 1991; Veronneau, et al., 1992). While the influence of other combustion products, e.g., carbon monoxide, on the overall toxicity in fire victims cannot be ruled out, many of the blood CN^- values at t_i are equal to or higher than the levels generally considered to be lethal in humans (Baselt and Cravey, 1989; Hartzell, 1989).

There was a direct relationship between the HCN exposure concentration and uptake, as the decrease in the HCN concentration by a factor of 2.6 also decreased the blood CN^- uptake rate by 2.6. Calculated uptake rates in (μg CN^-/mL)/min/ppm HCN for both exposures were almost identical, i.e., 2.19×10^{-3} for 183 ppm HCN and 2.14×10^{-3} for 71 ppm HCN. Thus, this relationship can be described by

$$\frac{(CN^-)}{C \bullet t} = K \ , \qquad (2)$$

which can be rearranged to the form

$$(CN^-) = C \bullet t \bullet K \ , \qquad (3)$$

where CN^- = blood CN^- in μg/mL; C = HCN exposure concentration in ppm; t = exposure time in min; K (constant) = 2.2×10^{-3}. This equation may have some utility for predicting blood CN^-, HCN exposure concentration, or exposure time value by knowing the values of 2 of the 3 parameters (variables). From Equation (3), the calculated blood CN^- levels at incapacitation for the rats exposed to HCN in each of the 2 t_i studies were 2.1 μg/mL for the 5-min t_i and 4.4 μg/mL for the 35-min t_i, which corresponded very closely to the experimental blood CN^- mean values (Table 1). When K was derived from parameters obtained in the 2 t_i studies, a mean value of $(2.3 \pm 0.5) \times 10^{-3}$ was obtained (n = 100; t = t_i). This value is exceptionally close to the K value calculated from the uptake study, suggesting that Equation (2) holds true up to the "exposure time" equivalent to the onset of incapacitation, as well. The equation's effectiveness is further supported by the apparent nonexistence of steady-state blood CN^- levels in uptake studies prior to t_i. Therefore, blood CN^-, HCN exposure concentration, or t (up to t_i) can be predicted from the equation. Since the blood CN^- levels were considerably different at the 5- and 35-min t_i, no specific CN^- level could be linked to the onset of incapacitation.

SUMMARY AND CONCLUSIONS

The HCN exposure concentrations that produced incapacitation at 5 and 35 min were 184 and 64 ppm, respectively. Coefficients of variation corresponding to these HCN concentrations were 5.4 and 9.5%. Variations in the observed incapacitation response were 15.7% in the 5-min study and 36% in the 35-min study. No specific blood CN^- level could be linked to the onset of incapacitation. Uptake of HCN, measured as blood CN^-, was proportional to both HCN concentration and exposure time. An equation is proposed that may be of use in predicting total blood CN^- in the laboratory rat from HCN exposure concentration and exposure time parameters.

REFERENCES

Baselt RC, Cravey RH (1989). Disposition of Toxic Drugs and Chemicals in Man, 3rd edition, pp 224-9. Year Book Medical Publishers, Inc., Chicago, IL.

Baud FJ, Barriot P, Toffis V, Riou B, Vicaut E, Lecarpentier Y, Bourdon R, Astier A, Bismuth C (1991). Elevated blood cyanide concentrations in victims of smoke inhalation. N. Engl. J. Med., 325, 1761-6.

Crane CR, Sanders DC, Endecott BR, Abbott JK, Smith PW (1977). Inhalation toxicology: I. Design of a small-animal test system. II. Determination of the relative toxic hazards of 75 aircraft cabin materials. Department of Transportation/ Federal Aviation Administration, Washington, DC. Publication No. FAA-AM-77-9. Available from National Technical Information Service, Springfield, VA 22161. Order No. ADA043646/ 9GI.

Crane CR (1984). Inflight aircraft fires: Toxicological aspects. Panel presentation at the Aerospace Medical Association Scientific Meeting, May 7, 1984, San Diego, CA.

Crane CR, Sanders DC, Endecott BR (1989). Inhalation toxicology: IX. Times-to-incapacitation for rats exposed to carbon monoxide alone, to hydrogen cyanide alone, and to mixtures of carbon monoxide and hydrogen cyanide. Department of Transportation/Federal Aviation Administration, Washington, DC. Publication No. DOT/FAA/ AM-89/4. Available from National Technical Information Service, Springfield, VA 22161. Order No. ADA208195.

EUROCAE (1991). Minimum operational performance specification for passenger protective breathing equipment. EUROCAE Document No. ED-65. The European Organisation for Civil Aviation Equipment, Paris, France.

Gad SC (1990). The toxicity of smoke and combustion gases. In: Combustion Toxicology, SC Gad and RC Anderson, eds., pp 63-80. CRC Press, Boca Raton, FL.

Hartzell GE (1989). Understanding of hazards to humans. In: Advances in Combustion Toxicology, Vol. 1, GE Hartzell, ed., pp 19-37. Technomic Publishing Co., Inc., Lancaster, PA.

Higgins EA (1987). Summary report of the history and events pertinent to the Civil Aeromedical Institute's evaluation of providing smoke/fume protective breathing equipment for airline passenger use. *Department of Transportation/Federal Aviation* Administration, Washington, DC. Publication No. DOT/FAA/AM-87/5. Available from National Technical Information Service, Springfield, VA 22161. Order No. ADA184499.

Kaplan HL, Hartzell GE (1984). Modeling of toxicological effects of fire gases: I. Incapacitating effects of narcotic fire gases. J. Fire Sci., 2, 286-305.

Klaassen CD (1990). Nonmetallic environmental toxicants: Air pollutants, solvents and vapors, and pesticides. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, AG Gilman, TW Rall, AS Nies, P Taylor, eds., 8th edition, pp 1615-39. Pergamon Press, New York, NY.

Mayes RW (1991). The toxicological examination of the victims of British Air Tours Boeing 737 accident at Manchester in 1985. J. Forensic Sci., 36, 179-84.

McMillan DE, Svoboda AC IV (1982). The role of erythrocytes in cyanide detoxification. J. Pharmacol. Exp. Ther., 221, 37-42.

Miller JC, Miller JN (1988). Statistics for Analytical Chemistry, 2nd edition. Ellis Horwood Limited, Chichester, West Sussex, England.

Packham SC, Hartzell GE (1981). Fundamentals of combustion toxicology in fire hazard assessment. J. Testing and Evaluation (JTEVA), 9, 341-7.

Purser DA, Grimshaw P, Berrill KR (1984). Intoxication by cyanide in fires: A study in monkeys using polyacrylonitrile. Arch. Environ. Health, 39, 394-400.

Sanders DC, Endecott BR, Chaturvedi AK (1991). Inhalation toxicology: XII. Comparison of toxicity rankings of six polymers by lethality and by incapacitation in rats. Department of Transportation/Federal Aviation Administration, Washington, DC. Publication No. DOT/FAA/AM-91/17. Available from National Technical Information Service, Springfield, VA 22161.

SigmaPlot (1991). SigmaPlot®: Scientific Graphing Software. Jandel Scientific, Corte Madera, CA.

Spurgeon JC, Filipczak RA, Feher RE, Sternik SJ (1979). A procedure for electronically monitoring animal response parameters using the rotating wheel. J. Combustion Toxicol., 6, 198-207.

Technicon (1974). Cyanide in Water and Wastewater (Industrial Method No. 312-74W; March 18, 1974). Technicon Instruments Corporation, Tarrytown, NY.

Veronneau S, Ribe JK, Sathyavagiswaran L, Lewis I, Muto J (1992). Lessons learned from the 1991 USAir/SkyWest collision at LAX, presented at the 44th Annual Meeting of the American Academy of Forensic Sciences, New Orleans, LA, February 17-22, 1992.

Vesey CJ, Wilson J (1978). Red cell cyanide. J. Pharm. Pharmac., 30, 20-6.

Wilkinson L (1989). SYSTAT: The System for Statistics. SYSTAT, Inc., Evanston, IL.

Yamamoto K (1977). Acute combined effects of HCN and CO, with special reference to a theoretical consideration of acute combined effects on the basis of the blood cyanide and COHb analyses. J. Combustion Toxicol., 4, 69-78.

APPENDIX

TIME-TO-INCAPACITATION (t_i) VALUES AND BLOOD (CN^-) LEVELS AT INCAPACITATION FOR RATS EXPOSED TO HYDROGEN CYANIDE (HCN) GAS

Table 1.

Data for 5-Min Study.

Rat No.	t_i (min)	HCN* (ppm)	Blood CN^- (µg/mL)	Rat No.	t_i (min)	HCN* (ppm)	Blood CN^- (µg/mL)
1	4.7	190	1.68	26	5.6	193	2.88
2	5.7	171	2.05	27	4.7	198	2.74
3	5.0	161	1.79	28	4.5	202	2.66
4	5.3	181	1.55	29	7.0	182	3.41
5	4.9	181	2.11	30	5.5	183	2.72
6	4.2	177	2.16	31	4.7	186	2.66
7	7.5	181	1.89	32	4.8	188	2.48
8	4.9	190	1.52	33	4.8	178	2.42
9	4.3	178	1.87	34	5.0	179	2.42
10	5.4	184	1.71	35	5.9	173	2.19
11	5.3	184	2.08	36	5.0	190	3.01
12	4.5	187	2.53	37	5.6	179	2.77
13	4.5	193	1.95	38	4.0	191	1.84
14	5.3	193	2.19	39	4.7	192	2.24
15	4.4	181	1.52	40	4.6	197	2.61
16	4.9	171	2.29	41	4.4	202	2.42
17	5.6	159	2.11	42	4.8	190	2.80
18	6.6	163	2.98	43	4.5	188	1.87
19	6.8	165	1.95	44	5.6	188	2.13
20	6.6	168	2.19	45	4.1	193	2.56
21	4.6	188	2.88	46	4.5	180	1.89
22	4.2	185	2.64	47	5.0	184	2.37
23	7.1	181	3.01	48	4.9	200	1.52
24	5.1	188	2.24	49	4.4	183	2.11
25	5.0	190	3.70	50	4.7	180	1.52

*HCN exposure concentration, see text for definition.

Table 2.

Data for 35-Min Study.

Rat No.	t_i (min)	HCN* (ppm)	Blood CN^- (µg/mL)	Rat No.	t_i (min)	HCN* (ppm)	Blood CN^- (µg/mL)
1	40.9	54	4.50	26	43.1	64	6.29
2	22.0	52	3.11	27	41.2	66	3.26
3	28.0	51	2.81	28	53.1	60	6.59
4	35.7	46	2.83	29	22.1	66	3.21
5	34.6	53	3.16	30	30.5	67	5.76
6	34.1	48	3.13	31	38.3	64	5.46
7	26.0	60	3.24	32	26.2	66	4.90
8	56.0	57	5.63	33	24.9	65	4.17
9	23.0	65	4.53	34	38.7	65	4.40
10	21.1	65	3.79	35	21.0	66	4.45
11	33.5	61	5.00	36	14.0	69	2.38
12	53.5	69	6.08	37	18.2	66	3.29
13	29.6	65	6.06	38	26.5	69	5.00
14	35.0	66	5.23	39	19.4	67	2.51
15	70.0	63	9.06	40	20.2	64	2.28
16	33.5	61	4.62	41	39.1	66	4.68
17	20.5	67	3.13	42	37.5	67	5.71
18	19.0	66	2.51	43	34.8	71	5.25
19	22.5	67	3.57	44	22.0	74	4.17
20	29.0	66	3.81	45	22.8	67	3.47
21	31.7	65	4.15	46	25.9	74	2.61
22	48.4	68	3.89	47	20.8	75	3.34
23	27.5	65	3.72	48	25.4	68	3.66
24	28.3	64	3.94	49	31.5	67	3.26
25	33.2	62	4.53	50	20.0	68	2.33

*HCN exposure concentration, see text for definition.

Table 3.

Data for Exposure Periods Less Than t_i at Nominal 183 ppm HCN.

Rat No.	Exposure Time (min)	HCN (ppm)	Blood CN^- (μg/mL)
1	1.0	182	1.07
2	1.0	183	0.49
3	1.0	180	0.38
4	1.0	182	0.83
5	2.0	186	1.49
6	2.0	187	1.17
7	2.0	189	1.12
8	2.0	180	0.91
9	3.0	182	1.28
10	3.0	190	1.31
11	3.0	176	1.58
12	3.0	178	0.83
13	4.0	183	1.87
14	4.0	181	1.25
15	4.0	174	2.17
16	4.0	187	1.60
17	5.0*	176	2.74
18	5.0*	179	2.42
19	5.0*	190	3.01
20	5.0*	184	2.37

*Data for rats listed at the 5-min exposure time were selected from the animals in the Appendix Table 1 for the purpose of comparison only; these animals (No. 17, 18, 19, and 20) were incapacitated at the time of blood removal for CN^- analyses.

Table 4.

Data for Exposure Periods Less Than t_i at Nominal 71 ppm HCN.

Rat No.	Exposure Time (min)	HCN (ppm)	Blood CN^- (µg/mL)
1	2.5	70	0.14
2	2.5	67	0.77
3	2.5	77	0.34
4	5.0	75	1.70
5	5.0	69	0.64
6	5.0	74	1.17
7	10.0	72	1.52
8	10.0	68	1.32
9	10.0	74	1.52
10	15.0	66	2.03
11	15.0	67	2.81
12	15.0	72	2.76
13	25.0	69	3.54
14	25.0	71	3.62
15	25.0	67	4.37
16	35.0*	53	3.17
17	35.0*	66	5.23
18	35.0*	71	5.25

*Data for rats listed at the 35-min exposure time were selected from the animals in the Appendix Table 2 for the purpose of comparison only; these animals (No. 16, 17, and 18) were incapacitated at the time of blood removal for CN^- analyses.

*U.S. GOVERNMENT PRINTING OFFICE: 1993-761-068-60066