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Toxicity of Carbon Monoxide-Hydrogen Cyanide Gas Mixtures: Exposure Concentration, Time-to-Incapacitation, Carboxyhemoglobin, and Blood Cyanide Parameters

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16. Abstract

During aircraft interior fires, carbon monoxide (CO) and hydrogen cyanide (HCN) are produced in sufficient amounts to cause incapacitation and death. Time-to-incapacitation (t_i) is a practical parameter for estimating escape time in fire environments. Exposures to CO-HCN mixtures have demonstrated that these gases have additive effects (producing shorter times to incapacitation), but the resulting concentrations of carboxyhemoglobin (COHb) and blood cyanide (CN⁻) at incapacitation are not well defined. These undefined relationships between COHb and blood CN⁻ levels and the onset of incapacitation make the interpretation of postmortem levels difficult for medical accident investigators. To explore these relationships, t_i was determined in laboratory rats exposed to 2 CO-HCN mixtures consisting of CO and HCN concentrations that produce 5- and 35-min t_i in individual gas exposures; COHb and blood CN⁻ concentrations were determined at incapacitation. In the high concentration CO-HCN mixture, the resultant t_i was shortened from 5 min to 2.6 min; COHb dropped from 81% to 55% and CN⁻ from 2.3 µg/mL to 1.1 µg/mL. At the lower concentration CO-HCN mixture, where the resultant t_i was reduced from 35 min to 11.1 min, COHb dropped from 71% to 61% and blood CN⁻ decreased from 4.2 µg/mL to 1.1 µg/mL. Comparison of the COHb and blood CN⁻ values with the values from our single gas exposure studies indicated that any alteration of the uptake of either gas in blood by the presence of the other was minimal. These findings suggest that changes in COHb and blood CN⁻ may not be directly correlated with the onset of incapacitation and that postmortem blood levels should be carefully evaluated, particularly when both gases are present in fire victims.

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TOXICITY OF CARBON MONOXIDE-HYDROGEN CYANIDE GAS MIXTURES: EXPOSURE CONCENTRATION, TIME-TO-INCAPACITATION, CARBOXYHEMOGLOBIN, AND BLOOD CYANIDE PARAMETERS

INTRODUCTION

During aircraft interior fires, carbon monoxide (CO) and hydrogen cyanide (HCN) gases are likely to be generated in sufficient amounts to cause lethal effects (Hartzell, 1989; Gad, 1990; Mayes, 1991; Veronneau, et al., 1992). Inhalation of less-than-lethal amounts of these gases can produce physical incapacitation, resulting in the inability to escape from a fire environment, and time-to-incapacitation (t_i) has been considered as an applicable toxicological index for escape time (Crane, et al., 1989; Sanders, et al., 1992). Since both gases are generally present in substantial amounts in smoke, there is a considerable potential for simultaneous exposures to these gases, thereby resulting in their combined effects on biological systems. Although the inhalation toxicology of each of these gases has been widely studied, toxicity of their mixtures has been defined to a lesser extent (Kaplan, 1988; Gad, 1990). Simultaneous exposures to CO and HCN have demonstrated that these gases might produce additive effects on t_i (Kaplan, 1988; Crane, et al., 1989; Gad, 1990); however, carboxyhemoglobin (COHb) and blood cyanide (CN⁻) parameters at incapacitation have not been well explored for CO-HCN mixtures.

Recently, variations in 5- and 35-min t_i from exposures to fixed concentrations of CO (Sanders, et al., 1993) and of HCN (Chaturvedi, et al., 1993) were investigated in rats; concentrations of CO and of HCN in these studies were selected to induce 5- and 35-min t_i, representing the probable durations of fire exposure times in typical post-crash and in-flight aircraft fire accidents, respectively (Crane, 1984; EUROCAE, 1991). In these earlier studies, the patterns of CO uptake as %COHb were proportional to the CO concentration and exposure time and were consistent with those predicted by Hartzell, et al. (1989a); COHb levels at incapacitation for the 2 CO concentrations were different. The uptake of CN⁻ by blood was also concentration and time dependent; blood CN⁻ at the 5-min t_i was half that at the 35-min

t_i and the HCN uptake at the high gas concentration was about 3 times that at the low concentration. The interrelationship between blood CN⁻ (CN⁻; μg/mL of blood), HCN exposure concentration (C; ppm), and exposure time (t; min) was represented by

$$(CN^-) = C \bullet t \bullet K \qquad (1)$$

where K (constant) = 2.2 x 10⁻³ (Chaturvedi, et al., 1993). This equation was determined to be effective up to the "exposure time" equivalent to the onset of incapacitation, i.e., $t = t_i$.

To evaluate the influence of simultaneous exposures to both gases, effects of 2 CO-HCN mixtures on blood COHb saturation and CN⁻ level at incapacitation were examined in rats. These values were compared with those from earlier single gas studies; concentrations of CO or HCN used in the mixtures produce nominal 5- and 35-min t_i in single gas exposures. Information from this CO-HCN interaction study would have application in the interpretation of COHb and blood CN⁻ levels in fire victims.

MATERIALS AND METHODS

Materials

Compressed CO (99.5%), HCN gas (1% in N_2), and breathing air were purchased locally in cylinders. Reagent grade chemicals for analyses were obtained from commercial sources. Specific reagents and chemicals for COHb determinations were supplied by the CO-Oximeter manufacturer (Instrumentation Laboratories, Inc., Lexington, MA). Solutions of chemicals were prepared in deionized water.

Animals

Male albino rats of Sprague-Dawley origin were obtained from the Charles River Breeding Laboratories, Wilmington, MA, in a 100 to 125 g weight range. Rats (4 to 6 per cage) were housed 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 Civil

Aeromedical Institute. The facility was maintained at 22 to 24°C with a 12-hr (7 a.m. to 7 p.m.) light cycle from fluorescent lights and a relative humidity of 40 to 60%.

Upon arrival, animals were inspected by a veterinarian and held in isolation for 8 days prior to use. Animals were provided with food and water *ad libitum* during, and following, the isolation period. All animals were fasted overnight before the CO-HCN exposures.

Animal Exposure Chamber

The exposure chamber was constructed from 1.3-cm polymethylmethacrylate sheets with 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 diameter; 25.0 cm W), which was vertically divided into 2 equal compartments, each 12.5-cm wide, and was horizontally rotated by a 4-rpm geared motor to provide an 8.5 cm/sec circumferential velocity; the front compartment 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, to provide mixing and circulation of the CO-HCN-air mixture in the flow-through chamber atmospheres and 2 ports sealed with rubber septa on the front panel of the chamber for the atmosphere sampling.

CO, HCN gas, and breathing air were mixed by passing through a baffled cylindrical mixing tube before entering the chamber; flow rates of CO, HCN, 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 CO, HCN, and air mixtures was through a port in the top of the chamber, and an exhaust port was located in the rear panel. The entire chamber was installed in a fume hood, into which the chamber exhaust was vented.

Experimental Design

Gas concentrations for the CO-HCN mixture exposure experiments were determined from previous studies (Crane, et al., 1989; Chaturvedi, et al., 1993; Sanders,

et al., 1993). The initial airflow into the chamber was established at 4 L/min; CO and HCN gas flows were adjusted to produce the estimated chamber concentrations required. CO-HCN mixtures used in this study were (25 mL/min CO + 80 mL/min of 9239 ppm HCN + 4 L/min air), giving mean exposure concentrations of 5379 ppm CO and 183 ppm HCN, and (8.5 mL/min CO + 25 mL/min of 9239 ppm HCN + 4 L/min air), resulting in mean exposure concentrations of 1831 ppm CO and 64 ppm HCN. The high concentration of CO or HCN causes incapacitation at 5 min in rats, while the low concentration of each of these gases produces the 35-min t_i. Preliminary experiments suggested that ambient O_2 levels did not change for single rat exposures by the CO-HCN-air mixture flow rates through the chamber; therefore, concentration of O_2 was not monitored during the animal exposure experiments. The dynamic flow of CO-HCN-air mixtures through the chamber also prevented major changes in the CO and HCN concentration during rat insertion, exposure, or removal.

Ten rats were individually exposed to each of the 2 CO-HCN mixtures to measure t_i, COHb, and blood CN⁻. When chamber gas concentrations reached the desired levels, zero-min chamber atmosphere samples were withdrawn for the quantitation of both gases. The chamber fans and cage motor were turned off, the timer was set to zero, and the retaining screws on the chamber access door were removed. Then, in rapid succession, the door was opened, a rat was inserted, the door was closed, and the timer, cage motor, and fans were activated. The t_i was recorded as the time from insertion until the rat could no longer walk, i.e., when tumbling or sliding began, in the rotating cage—as subjectively determined by 2 individuals. At incapacitation, the rat was quickly removed and killed by cervical dislocation; the body cavity was immediately opened, and blood was drawn into a 2.5-cc glass syringe from the descending aorta. The blood was immediately injected into a stoppered glass tube containing solid sodium heparin, then mixed on a mechanical rocker for 5 min. The heparinized blood was then divided into 2 parts; 1 aliquot was used for COHb determination and the other aliquot was analyzed for blood CN⁻, utilizing methods to be described later.
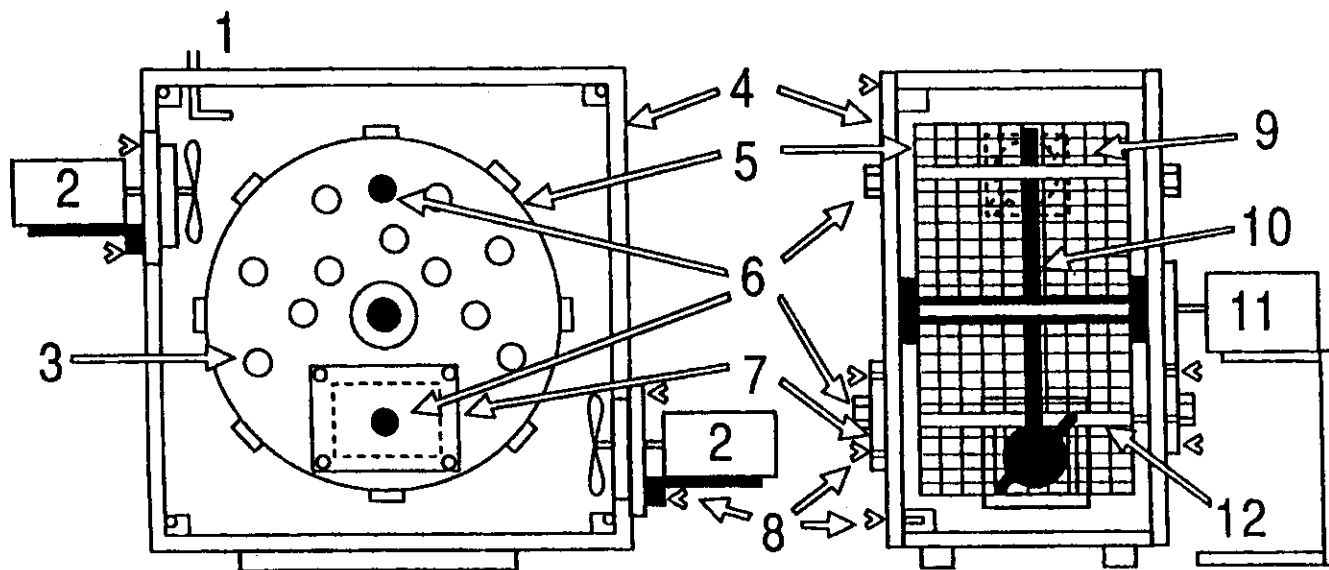
Figure 1. Animal Exposure Chamber.

1. CO-HCN-air mixture inlet; 2. Mixing fan assembly (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 13-mm 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.

To determine gas concentrations during the animal exposures, chamber samples were collected at defined intervals for the determination of CO and HCN concentrations. Besides the zero-min samples, chamber samples were removed at 1 min and then at 1.5-min intervals for CO determinations; samples for HCN quantitation were collected at 0 and 1 min and at 0, 1, and 5 min for the high and low mixture concentrations, respectively. Findings from these measurements suggested that any changes in the CO and HCN levels during the exposure were minimal, allowing the assumption that the concentration of each gas at t_i was identical to their concentration in the chamber sample immediately preceding incapacitation. Therefore, gas concentrations at incapacitation were estimated by extrapolating the values of the preceding concentrations to the t_i. The exposure concentrations of CO and HCN for each experiment were obtained by integrating the chamber concentration of the respective gas as a function of exposure time from $t = 0$ to $t = t_i$ and dividing the products by t_i, i.e.,

$$\text{Exposure Concentration} = \frac{\int_{t=0}^{t=t_i} c\,dt}{t_i} \qquad (2)$$

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

Chamber CO Analysis

A Carle series 100 gas chromatograph (GC) equipped with 3.2-mm (i.d.) packed columns and a thermistor detector was used to quantitate CO in the chamber samples. Sampling was accomplished automatically by a continuous flow of chamber atmosphere (55 mL/min) pumped from the port on the access-door by a ceramic-piston pump (FMI model RRP, Fluid Metering, Inc., Oyster Bay, NY), through the GC sample loop, then back to the chamber. Injections were controlled by activation of an injector valve with an interval timer at 1 min after rat insertion and then at 1.5-min intervals. CO concentration values in samples were obtained from standard curves prepared daily from syringe dilutions of tank CO in air.

COHb Determination

COHb was determined by a model IL 282 CO-Oximeter modified for use with rat blood. This instrument measures the absorbance of diluted blood samples at 4 wavelengths in the visible spectrum (Instrumentation Laboratories, Inc., Lexington, MA). Each sample was analyzed in duplicate.

Chamber HCN Gas and Blood CN⁻ Analyses

Chamber HCN gas concentrations and blood CN⁻ levels were determined colorimetrically 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 procedure employed was a modification of Technicon Industrial Method No. 312-74W (Technicon, 1974); it includes conversion of complexed CN⁻ to HCN by digestive distillation of samples at 155°C and resampling of the condensate. Concentration of CN⁻ in the condensate was determined by measuring the absorbance of chromophores formed by interaction of CN⁻ with chloramine-T and pyridine-barbituric acid reagent in the presence of phosphate buffer. The CN⁻ quantitation in chamber and blood samples has been previously described by Chaturvedi, et al. (1993). The chamber HCN concentrations were expressed as ppm (v/v) at ambient temperature and pressure. This method was also used to determine the concentration of gas cylinder HCN employed for the preparation of exposure atmospheres.

Data Analysis

Values are presented as the mean ± SD (n = 10). 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). A difference between means was checked by the Student's t-test (SigmaPlot, 1991) and considered significant at $p \leq 0.05$.

RESULTS

Exposures to the mixtures of CO and HCN, at the equipotent concentrations of each gas that produce 5- and 35-min t_i, reduced the onset of incapacitation; mean t_i values for the mixtures were correspondingly 2.6 and 11.1 min (Table 1). Distribution of the 2.6-

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Table 1

Time-to-Incapacitation (t_i), Carboxyhemoglobin (COHb), and Blood Cyanide (CN⁻) Values for Rats Exposed to Two Carbon Monoxide (CO)-Hydrogen Cyanide (HCN) Gas Mixtures

Parameters	Values[a, b]		
	Mean (Range)	SD	CV%
High CO-HCN Mixture Concentration			
CO (ppm)	5379 (5297 - 5482)	70	1.3
HCN (ppm)	183 (173 - 200)	8	4.4
t_i (min)	2.6 (2.2 - 3.0)	0.26	10.0
COHb (%) at t_i	54.7 (31.2 - 62.0)	8.7	15.9
Blood CN⁻ (μg/mL) at t_i	1.1 (0.62 - 1.37)	0.29	26.4
Low CO-HCN Mixture Concentration			
CO (ppm)	1831 (1753 - 1899)	58	3.2
HCN (ppm)	64 (60 - 70)	3	4.7
t_i (min)	11.1 (9.0 - 14.7)	1.8	16.2
COHb (%) at t_i	60.9 (58.0 - 66.1)	2.5	4.1
Blood CN⁻ (μg/mL) at t_i	1.1 (0.24 - 1.80)	0.49	44.5

[a]Mean values are derived from rats individually exposed to CO-HCN mixtures (n = 10). SD = Standard Deviation; CV = Coefficient of Variation, (SD/Mean) x 100.

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

min t_i measurements was uniform, with most of the values grouped around 2.7 min; variation in t_i measurements was greater at the lower CO-HCN concentration, as the value range was from 9.0 to 14.7 min. Mean COHb values at incapacitation for the 2 CO-HCN mixtures were statistically different at the 0.05 p level, but the difference was only 10%. Blood CN^- levels at the 2.6- and 11.1-min t_i were not significantly different from each other ($p > 0.05$). The coefficient of variation in the COHb values was higher for the high CO-HCN concentration than for the low CO-HCN concentration. However, the variation coefficient in blood CN^- measurements with the high mixture concentration was about half of the coefficient of variation observed at the low CO-HCN concentration. Values of all measured parameters were normally distributed, as they were not statistically different from their corresponding standard normal population forms ($p > 0.05$).

DISCUSSION

Equipotent concentrations of CO and of HCN, used in the 2 CO-HCN mixtures, were comparable to the concentrations that produce nominal 5- and 35-min t_i by the individual gas exposures (Chaturvedi, et al., 1993; Sanders, et al., 1993). With respect to the 5- and 35-min t_i, decreases of 48 and 68% in the onset of incapacitation by the CO-HCN mixtures suggested that CO and HCN had some type of interactive effect(s) on t_i. This aspect was supported by respective decreases of 32 and 14% in COHb and of 52 and 74% in blood CN^- at the 2.6- and 11.1-min t_i with the CO-HCN mixtures in relation to the values at 5- and 35-min t_i from the single gas exposures.

To be an additive effect, the reciprocal of t_i value for the combined gases should be equal to the sum of the reciprocal t_i values observed with the individual gases, as

$$\frac{1}{t_{i(CO+HCN)}} = \frac{1}{t_{i(CO)}} + \frac{1}{t_{i(HCN)}} \quad (3)$$

The calculated $t_{i(CO+HCN)}$ of 2.5 min from Equation (3) for the 5-min t_i by single gases is very close to the experimental $t_{i(CO+HCN)}$ of 2.6 min. For the 35-min t_i by individual gases, the observed $t_{i(CO+HCN)}$ was 11.1 min

and the calculated $t_{i(CO+HCN)}$ was 16.4 min. Both calculated values were in reasonable agreement with the respective $t_{i(CO+HCN)}$ values of 2.9 and 15.6 min from another equation developed for predicting t_i produced by CO-HCN mixtures (Crane, et al., 1989). Applying the "Fractional Effective Dose" (FED) concept (Hartzell, et al., 1989b), $FED_{(gas\ 1)} + FED_{(gas\ 2)}$ should equal unity for an additive effect. FED of each gas in the mixture is calculated by dividing the C • t product (where C = gas exposure concentration in ppm and t = exposure time in min, i.e., t_i) of the gas in the mixture by the corresponding C • t value at incapacitation for exposure to the same gas alone, i.e.,

$$FED_{CO} = \frac{C_{COin(CO+HCN)} \bullet t_{i(CO+HCN)}}{C_{CO} \bullet t_{i(CO)}} \quad (4)$$

and

$$FED_{HCN} = \frac{C_{HCNin(CO+HCN)} \bullet t_{i(CO+HCN)}}{C_{HCN} \bullet t_{i(HCN)}} \quad (5)$$

Employing the mean C • t products for 50 rats exposed to the individual gas concentrations (Sanders, et al., 1993; Chaturvedi, et al., 1993), FED values from Equations (4) and (5) for the 2.6- and 11.1-min t_i based on the corresponding 5- and 35-min t_i were:

$$FED_{CO} + FED_{HCN} = \frac{(5379) \bullet (2.6)}{(5706) \bullet (5.0)} + \frac{(183) \bullet (2.6)}{(184) \bullet (5.1)} = 0.49 + 0.51 = 1.0$$

and $\quad (6)$

$$FED_{CO} + FED_{HCN} = \frac{(1831) \bullet (11.1)}{(1902) \bullet (34.8)} + \frac{(64) \bullet (11.1)}{(64) \bullet (31.1)} = 0.31 + 0.36 = 0.67$$

$\quad (7)$

respectively. Thus, the fractional contributions of CO and HCN in the high mixture concentration are essentially equal and the sum of the fractions is equal to unity, indicative of additive effect. At the low concentration, FED values were nearly the same, but their sum was much less than unity, implying other than additive type of effect.

These t_i and FED calculations suggest that CO and HCN have additive effects on the incapacitation onset, at least, at the shorter t_i. The value of less than 1 in Equation (7) could be due to the lessened applicability of Haber's rule, and its dependent FED concept, as "C" and "t" values may simply be too close to the

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asymptotic portion of the "C-t_i" curve to be valid (Packham and Hartzell, 1981; Kaplan and Hartzell, 1984).

Theoretical COHb levels for 5379 ppm CO at 2.6 min and for 1831 ppm CO at 11.1 min from the equation of Hartzell, et al. (1989a) were calculated to be 60 and 59%, respectively; these COHb levels were in reasonable agreement with the experimental COHb values in the present study and with the COHb levels previously observed in the CO uptake study by Sanders, et al. (1993). From Equation (1), "K" values at the 2.6-min t_i for 183 ppm HCN and at the 11.1-min t_i for 64 ppm HCN were calculated to be 2.3 x 10^{-3} and 1.7 x 10^{-3}, respectively. The respective blood CN$^-$ values of 1.05 and 1.56 µg/mL, predicted by Equation (1) using K = 2.2 x 10^{-3}, were in reasonable agreement with the corresponding experimental blood CN$^-$ values of 1.06 ± 0.29 and 1.15 ± 0.49 µg/mL. These comparisons suggest that any alteration in the uptake of either gas in blood by the presence of the other gas was minimal.

When the FED model was employed using blood COHb saturation and CN$^-$ levels at incapacitation, FED for each gas in blood was obtained by dividing values of these parameters in the CO-HCN mixture exposures by the corresponding values at t_i in the single gas exposures, i.e.,

$$FED_{COHb} = \frac{COHb_{(CO+HCN)}}{COHb_{CO}} \qquad (8)$$

and

$$FED_{CN^-} = \frac{(CN^-)_{(CO+HCN)}}{(CN^-)_{HCN}} \qquad (9)$$

where COHb = % blood COHb saturation and (CN$^-$) = CN$^-$ in µg/mL of blood.

For the high CO-HCN mixture concentration at t_i = 2.6 min:

$$FED_{COHb} + FED_{CN^-} = \frac{55}{81} + \frac{1.1}{2.3} = 0.68 + 0.48 = 1.16 \qquad (10)$$

For the low CO-HCN mixture concentration at t_i = 11.1 min:

$$FED_{COHb} + FED_{CN^-} = \frac{61}{71} + \frac{1.1}{4.2} = 0.86 + 0.26 = 1.12 \qquad (11)$$

This would indicate that the combined effect of CO and HCN is slightly less than additive (i.e., the sum is > 1). It should be noted that the COHb level was nearly linear up to 5 min at both CO concentrations and began reaching a steady state before 11.1 min at the CO exposure concentration that caused the 35-min t_i (Sanders, et al., 1993). This observation is in agreement that FED$_{COHb}$ is not linearly proportional to the length of exposure (Hartzell, et al., 1989a). Blood COHb saturation is regarded as a measure of the severity of CO exposure, but dissolved CO in plasma has been proposed for causation of toxicity by competing at the cellular level with O$_2$ for cytochrome a$_3$ (Goldbaum, 1976). The plasma CO level, in the above equations, might be a more suitable parameter for comparison than %COHb in explaining the CO-HCN interaction. Thus, it appears that the FED model, when applied to actual COHb and blood CN$^-$ levels, may not be applicable except in the extremely limited exposure period when both CO and HCN uptake are relatively linear with time.

Overall, this study suggests that changes in COHb and blood CN$^-$ may not always be correlated with the t_i response. Therefore, postmortem COHb and blood CN$^-$ levels in fire victims, particularly where there is a potential for exposure to both gases, should be carefully evaluated in assessing the probable antemortem states of the subjects.

Additional studies involving uptake and distribution processes, detoxification mechanisms, and sites of action specifically at low concentrations of these gases are clearly needed to further define predicted response times and blood concentrations for exposures to CO-HCN mixtures.

SUMMARY AND CONCLUSIONS

Ten laboratory rats, exposed to a mixture of CO and HCN at concentrations that cause nominal 5-min t_i in animals exposed to each gas individually, showed an average t_i of 2.6 min with a coefficient of variation of 10%. An additional 10 rats exposed to a second CO-HCN mixture, with the individual gases at concentrations that produce nominal 35-min t_i, exhibited an average t_i of 11.1 min with a variation coefficient of 16%. The shortening in the incapacitation onset by

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the CO-HCN mixtures was accompanied by decreases of 32 and 14% in COHb and of 52 and 74% in blood CN^- at t_i with respect to the values reported at 5- and 35-min t_i for the single gas exposures. Any changes in the uptake of either gas caused by the presence of the other appeared to be minimal; predicted values of both COHb and blood CN^- at the 2.6 and 11.1-min t_i were within the reasonable ranges of the observed values. The mean blood CN^- levels at t_i for the 2 CO-HCN mixture concentrations were not statistically different ($p > 0.05$); this observation was unlike the results of the previous 5- and 35-min t_i studies, where both COHb and blood CN^- proved to be gas concentration dependent and neither had a specific value that could be linked to the incapacitation onset. The limited scope of this study prohibited further definition of predicted response times to CO-HCN mixtures, particularly at low concentrations. More definitive studies pertaining to uptake and distribution processes, detoxification mechanisms, and sites of action will be needed before response times to very low (and possibly very high) concentrations of toxic gases can be predicted in CO-HCN mixtures.

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APPENDIX

TIME-TO-INCAPACITATION (t_i) VALUES AND

CARBOXYHEMOGLOBIN (COHb) AND BLOOD (CN^-) LEVELS

AT INCAPACITATION FOR RATS EXPOSED TO 2

CARBON MONOXIDE (CO)-HYDROGEN CYANIDE (HCN) GAS MIXTURES

TABLE 1

**Data for CO-HCN Mixture Containing Both Gases at the Concentrations
Capable of Producing 5-Min Time-to-Incapacitation (t_i) Individually**

Rat No.	t_i (min)	CO* (ppm)	COHb (%)	HCN* (ppm)	Blood CN⁻ (µg/mL)
1	2.5	5344	57.3	186	1.15
2	2.9	5303	59.8	174	1.35
3	2.2	5323	53.6	182	1.30
4	2.7	5315	57.2	200	0.62
5	2.7	5297	55.5	187	1.07
6	2.7	5482	58.6	186	1.27
7	3.0	5430	58.0	183	0.67
8	2.3	5407	53.2	173	1.05
9	2.9	5447	62.0	178	0.71
10	2.5	5444	31.2	178	1.37

*CO and HCN exposure concentrations, see text for definition.

TABLE 2

**Data for CO-HCN Mixture Consisting of Both Gases at the Concentrations
Capable of Producing 35-Min Time-to-Incapacitation (t_i) Individually**

Rat No.	t_i (min)	CO* (ppm)	COHb (%)	HCN* (ppm)	Blood CN⁻ (µg/mL)
1	11.4	1818	60.9	66	1.60
2	12.3	1801	62.2	69	1.32
3	9.0	1899	59.2	62	1.80
4	9.6	1860	59.9	61	1.55
5	10.0	1770	58.0	67	1.45
6	12.0	1755	61.1	65	1.17
7	9.8	1753	58.6	64	0.79
8	10.0	1883	59.1	70	0.24
9	14.7	1885	63.7	60	0.71
10	12.6	1885	66.1	61	0.82

*CO and HCN exposure concentrations, see text for definition.