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

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16. Abstract

Laboratory rats were exposed to experimental atmospheres that contained (a) carbon monoxide in air, (b) hydrogen cyanide in air, and (c) mixtures of CO and HCN in air. The toxic potency of each of the three types of environments was evaluated toxicokinetically by measurement of time-to-incapacitation as a function of the toxic gas concentrations. Regression equations were derived that describe those relationships for exposure to CO or HCN alone. Analysis of the data from the combined-gas exposures, and comparison of that data with the results obtained from the exposures to each gas alone, lead to the conclusion that the toxicity of the combination is definitely greater than would be produced by either gas alone. There was, however, no evidence for a synergistic action--in which the effect of the combination would have been greater than that predicted from the sum of the two individual effects. An empirical equation was derived that allows calculation of a predicted time-to-incapacitation for any combination of CO and HCN concentrations that are within the ranges utilized in the experimental exposures.

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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

INTRODUCTION

The inhalation of toxic thermal decomposition products is now recognized as the primary cause of death in most fire related accidents. It is further recognized that even sublethal concentrations of these fire-generated gases may lead to psychophysical incapacitation and thus prevent a victim's escape from the fire environment. Of these gases, two that are likely to be produced in quantities sufficient to pose a threat to life are carbon monoxide (CO) and hydrogen cyanide (HCN). CO is produced from all carbon-containing materials--and is produced in particularly high concentrations when thermal decomposition occurs under smoldering, low-oxygen conditions. HCN is a common thermal decomposition product of any material that contains nitrogen (wool, polyurethane, polyamides, acrylonitriles, etc.).

The Aviation Toxicology Laboratory of the Federal Aviation Administration has been concerned since the mid-1960's that, in the event of an inflight or postcrash fire, the diverse materials present in aircraft cabin interiors could generate thermal decomposition products of significant toxicity. Inhalation of these toxic gases by passengers and crew who had survived any attendant impact trauma could lead to their incapacitation and subsequent failure to evacuate successfully.

In 1967 a research effort was initiated that was designed to assist in the post-mortem identification of those fatalities, from aircraft accidents involving fire, that were attributable solely to smoke inhalation. This activity was placed in the Forensic Toxicology Research Unit, under the direction of Delbert J. Lacefield, and consisted of the post-mortem measurement of the blood carboxyhemoglobin (COHb) concentration in autopsy specimens from all victims of such accidents--for at this time it was, and to a large degree still is, common practice in the forensic medicine arena to describe death from smoke inhalation as "CO poisoning." By 1970 it had become obvious that many such victims, with no evidence of physical trauma, were reported to have blood COHb levels that were consistent with the diagnosis of death due to CO poisoning--and, thus, to "smoke inhalation." In a significant number of cases, however, the COHb saturation level was reported by Lacefield as considerably less than 50 percent and it was, therefore, questionable to assign the cause of death as CO poisoning alone (or even as smoke inhalation) on the basis of evidence at hand.

The authors were aware that smoke from burning materials contains a multitude of individual thermal decomposition products

and, although the contribution to lethality from CO alone might predominate in some instances, in others it would be a case of combined toxicity contributed from two or more individual components. We felt that a candidate likely to make a significant contribution to the overall toxicity, in addition to that due to CO, would be HCN. The measurement of blood cyanide concentration was therefore added to that for COHb in the standard protocol for forensic toxicological analysis of autopsy specimens from victims of aircraft accidents involving fire.

Our prediction concerning cyanide involvement was verified when levels of HCN higher than normal were found in the blood of victims of a DC-8 crash at Anchorage, Alaska, in which a post-crash fire occurred (1). Several practical questions, however, remained unanswered: What is the effective (incapacitating or lethal) dose for each of these gases acting alone? How does one add up their combined effects? Are their effects additive? Synergistic? Antagonistic?

Subsequently, we established incapacitating and lethal inhalation dose levels for CO and HCN and developed equations for predicting time-to-incapacitation (t_i) and time-to-death (t_d) for rats exposed to the individual gases. We also established a rationale for using experimental animal data to predict human inhalation toxicity for CO and HCN (2). In some preliminary experiments, we exposed rats to CO-HCN mixtures and found that the sum of the two fractional effective doses (i.e., the quantity of each gas that was inhaled divided by the quantity of that gas required to produce incapacitation) was approximately 1.2, which we felt probably represented experimental and biological variation about unity, or a simple additive effect (3).

Whether the effects of CO and HCN, when inhaled as a mixture, are additive or synergistic has been the subject of some research and considerable speculation over at least the past 60 years, since Hofer (4), in 1926, reported exposing cats to such mixtures and measuring the exposure time required to produce paralysis. A recent critique of 11 such studies was published by Tsuchiya (5) and, although it is not a complete review of the literature, it is interesting that the conclusions reached by the individual authors were almost equally divided for and against a synergistic combined effect. Tsuchiya attributed these differing conclusions to one or more of the following deficiencies: (a) improper use of the term synergism; (b) conclusions by the authors that were inconsistent with the experimental data; or (c) valid statistical treatments were not applied to the experimental data.

Our continuing interest in this long-standing problem prompted us, in 1985, to design a series of experiments that would re-examine the question in sufficient detail to allow a meaningful and useful interpretation of the results. This report describes the design, execution, results, and the authors' interpretation of the results of those experiments.

MATERIALS AND METHODS

Animals. Male albino rats of Sprague-Dawley origin were obtained from Charles River Breeding Laboratories, Wilmington, MA. They were ordered in a weight range of 100 to 120 g, were inspected by a veterinarian on receipt, and were held in isolation for 10 days prior to use. All were maintained for the first 5 days on drinking water containing 1.5 g/L of sulfathiazole, then on normal tap water for the remaining 5 days' isolation.

Rats were fasted overnight before testing in order to establish equivalent metabolic states; individual animal weights were determined post-exposure.

Exposure chamber. The animal exposure chamber used for this study was procured from Columbus Instruments (930 N. Hague Ave., Columbus, OH) and is illustrated in Figure 1. The assembly consisted of a motor-driven drum and a shock grid mounted inside a clear polymethylmethacrylate chamber (43.4 x 11.4 x 44.6 cm), along with the associated electronic control devices. The peripheral linear velocity of the drum was controlled at 6 cm/sec (0.134 mph) and the shock intensity was set at controller position #5 (grid potential of 61 volts and maximum current of 0.61 milliamperes). As can be seen from Figure 1, if the subject animal fails to maintain the selected walking speed he will be carried back onto the shock platform.

Gas handling equipment. Carbon monoxide (research grade) and breathing air were supplied as compressed gases by Big Three Industries, Inc. (11426 Fairmont Parkway, La Porte, TX 77571). Hydrogen cyanide was supplied by Matheson Gas Products (1920 West Fairmont Parkway, La Porte, TX 77471) as a nominal 1500 parts per million (ppm) mixture (volume/volume) in nitrogen.

The metered gases were mixed, as they were used, by passage through a baffled cylindrical mixing chamber (3-cm dia x 23 cm) before entering the animal exposure chamber. A gas sampling bulb and a flowmeter connected in line between the mixing chamber and the animal chamber allowed sampling (from the bulb sidearm) and adjustment of the flow rate of the final gas mixture without having to wait for equilibrium to be established in the large chamber.

HCN flow rates were regulated manually with a double needle valve assembly and monitored with a Matheson model 8116-0252 mass flowmeter (Matheson Gas Products, Lyndhurst, NJ 07071). The pure CO flow rate was regulated automatically with a Matheson model 8240 mass flow controller; air flow rates were controlled manually by use of a simple, tank-mounted, two-stage pressure regulator and a needle valve assembly.

The design of this gas delivery system is depicted schematically in Figure 2.

Gas input to the chamber was through a port in the lower front panel and exhaust was through a port in the rear panel.

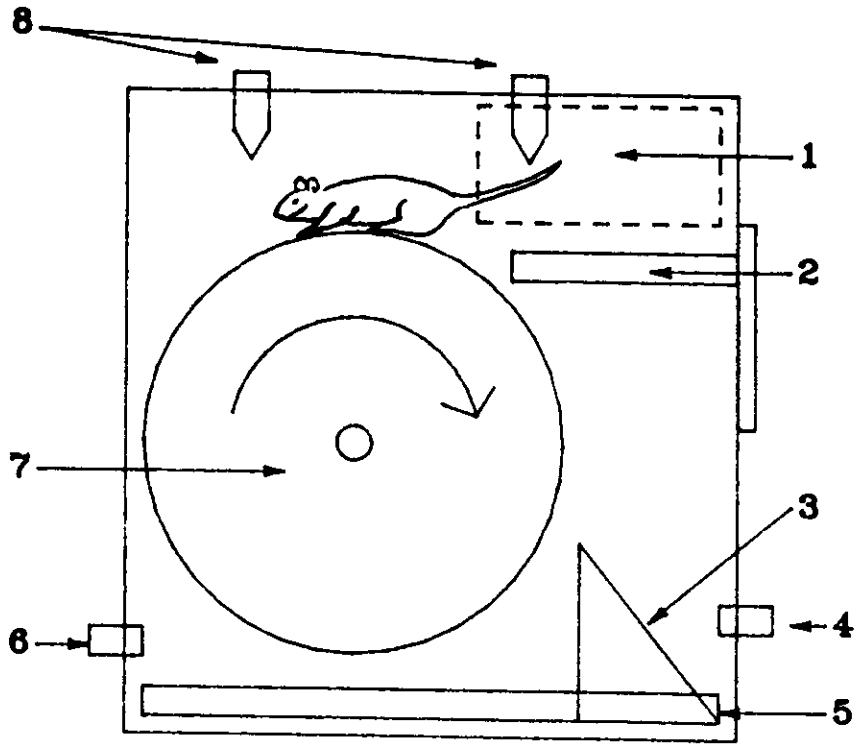

Figure 1. Animal exposure chamber

1. Sensor, activates shock assembly when rat is on grid
2. Shock grid
3. Gas deflector
4. Gas input port
5. Waste tray
6. Gas exhaust port
7. Rotating drum assembly
8. Latches for chamber cover

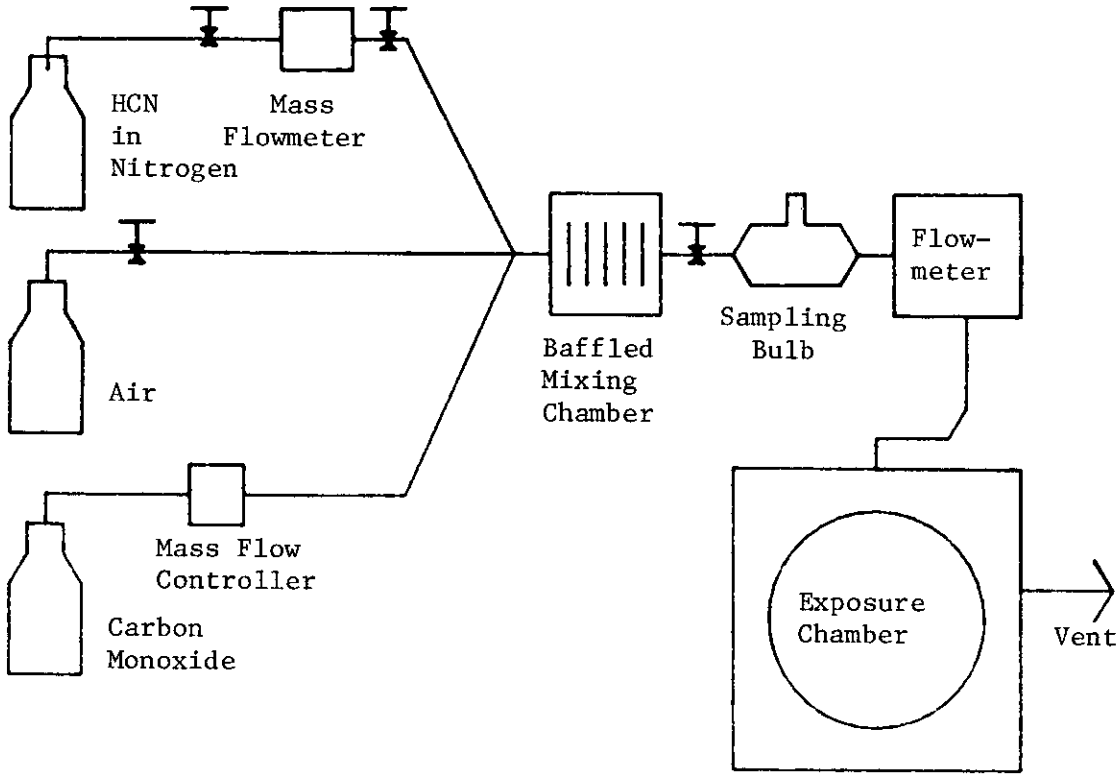

Figure 2. Diagram of gas delivery system

The incoming gas flow was deflected upward by an internal 45° deflector that was added to the system by the authors. Uniform mixing inside the chamber was accomplished by running the motor-driven drum while equilibrium was being established. The entire chamber was located inside a fume hood through which the exhausted gas mixture was vented.

Gas analysis. The chamber atmosphere was analyzed gas chromatographically for CO and oxygen concentrations. The gas chromatograph (GC) was equipped with 1/8-inch packed columns and a thermistor detector. A gas sampling port was located in the top of the exposure chamber, approximately 2 cm above the head of the walking rat.

For the experiments with CO alone, 30-mL samples of the chamber atmosphere were withdrawn manually into dry, plastic syringes. These aliquots were then manually flushed through the GC sample loop at a flow rate of 55 mL/min and injected from the loop while maintaining this flow, so that the gas pressure--and thus the concentration--in the loop would match that produced by the automatic pumping used later in the combined gas experiments.

For the combined gas studies, a continuous stream of the chamber atmosphere was pumped (at 55 mL/min) from the port and through the sample loop of the GC using a ceramic piston pump (FMI model RRP; Fluid Metering, Inc., 48 Summit St., Oyster Bay, NY 11771) with Saran tubing connections. Injections into the GC were accomplished automatically by driving the sample loop injector valve with an interval timer. Sampling interval was limited to 1.8 min by the column retention time for CO.

Samples for HCN analysis were withdrawn manually from the sampling port into acid-washed, all-glass hypodermic syringes--syringes with metal ferrules on the tip must not be used. The HCN was reacted, in the syringe, with the ammoniacal nickel reagent described by Scoggins (6) and by Pranitis and Stolman (7); the concentration of the resulting tetracyanonickelate anion complex was determined spectrophotometrically at 267 nm. Aqueous standards for the spectrophotometric method were prepared daily from reagent grade sodium cyanide (NaCN). The purity of the NaCN was determined by titration with silver nitrate solution that had been standardized previously against potassium chloride (primary standard grade).

Test procedure. Before each test, flow rate from the compressed air tank was adjusted to 1 L/min using the in line flowmeter. The HCN and/or CO flow rates were then adjusted to produce the concentration(s) desired at equilibrium, as determined by the analysis of samples from the gas sampling bulb. When the desired equilibrium concentration was achieved in the chamber, the drum rotation was stopped and the chamber cover latches were unfastened, but the cover was left on. The shock intensity switch was set at position #5. At time zero, the chamber cover was opened just enough to admit the rat, the rat was inserted, the cover was closed and secured, and the drum

rotation, shock grid, and sampling timer for the GC were all activated simultaneously. The rat was observed for any changes in behavioral pattern, such as slowed response to shock, gasping, collapse, convulsions, etc., and the time of onset and duration of such occurrences were noted. The rat was considered to be incapacitated when he no longer walked on the drum surface and was carried onto, and remained on, the shock grid. The elapsed time between insertion of the animal and this loss of the ability to walk was recorded as t_i. After incapacitation, the unconscious animal was removed from the chamber, weighed, and sacrificed by immersion in a closed container filled with CO.

Syringe samples for spectrophotometric analysis of HCN were removed at approximately 1, 2, 3, 5, 7, and 10 minutes, then, if necessary, at 3- to 4-min intervals until incapacitation was observed. Chamber atmosphere samples were analyzed gas chromatographically for CO and oxygen concentrations, with the sample-loop timer programed to sample at 1 minute into the exposure and at 1.8-min intervals thereafter.

The HCN dose/response relationship under these conditions was determined by exposing 30 rats, individually, to mean HCN concentrations of 75 to 273 ppm and measuring the time-to-incapacitation while monitoring the HCN concentration in the atmosphere. The dose/response relationship for CO, alone, was determined in a similar fashion for 42 rats exposed to mean CO concentrations that ranged from 1437 to 5922 ppm.

HCN and CO concentrations that would produce 5-, 10-, and 20-min t_i's from the individual gases alone were combined for the series of mixed-gas exposures, i.e., 5-min [CO] + 5-min [HCN], 5-min [CO] + 10-min [HCN], ..., 20-min [HCN] + 20-min [CO]. Sixty-three rats were exposed individually to CO-HCN mixtures using the same technique described for the single gas exposures. HCN concentrations ranged from 84 to 215 ppm; CO concentrations ranged from 1332 to 6385 ppm.

Data conversions and calculations.

Average gas concentration. For both the single-gas and the mixed-gas experiments, the average concentration to which the animals were exposed was calculated in the following manner: The area under the "concentration vs exposure time" curve, integrated from time=0 to time=t_i, represents the ($C*t_i$) product; division of this product by t_i yields the average effective concentration to which the animal was exposed ($C = C*t_i / t_i$).

Dose-response modeling for individual gases. For each set of individual gas exposures, a scatter plot was constructed to display the magnitude of t_i as a function of exposure concentration. A smooth curve, fit to these points, has the shape of a rectangular hyperbola, with nonzero asymptotes:

$$(C-C_o)*(t_i-t_o) = K_o. \qquad \text{(Eq. 1)}$$

Therefore, an equation of this form was derived empirically for each data set, using a standard nonlinear regression algorithm as a guideline (8), and identifying realistic values for the three parameters: C_o, t_o, and K_o. The biological interpretation of these parameters is as follows:

- C_o is the minimum toxic gas concentration that will result in incapacitation following a lengthy exposure;
- t_o is the shortest t_i that can be produced from an overwhelming concentration;
- K_o is an expression, in units of ppm*min, of the mean "effective dose" (ED) of the specific toxic gas required to produce the measured effect when administered alone.

Dose-response modeling for the combined gas exposures. The approach utilized by the authors to devise a mathematical model for the combined effects of the two gases is based on the concept of "fractional effective doses." This concept is discussed in more detail in the RESULTS AND DISCUSSION section. For each combined-gas experiment, an average gas concentration was calculated for each of the two gases, using the technique described for the single-gas exposures. Then, using the rearranged dose-response equation (Eq. 1) derived for each gas, the "administered dose" (AD) was calculated:

$$AD = K' = (C'-C_o)*(t_i'-t_o), \qquad \text{(Eq. 2)}$$

where:

- C' is the average concentration of the specific gas in the combined exposures;
- t_i' is the observed t_i;
- C_o and t_o are the derived constants for each gas from Eq. 1.

From the above data, a fractional effective dose (FED) was calculated for each gas, in each exposure. The FED is defined as the ratio of the administered dose to the effective dose, i.e.;

$$FED = AD/ED = K'/K_o. \qquad \text{(Eq. 3)}$$

RESULTS AND DISCUSSION

Animal observations. During a typical test, the rat would walk normally on the moving surface of the rotating drum after only one contact with the shock grid. As the animal began to respond to the toxic atmosphere, its walking rate slowed and more frequent contact with the shock grid occurred, with the animal occasionally jumping back and forth from the grid to the drum surface. With continuing exposure, the rat often would allow the rotation of the drum to bring his hindquarters into contact with the grid and would cease to move his rear feet while continuing

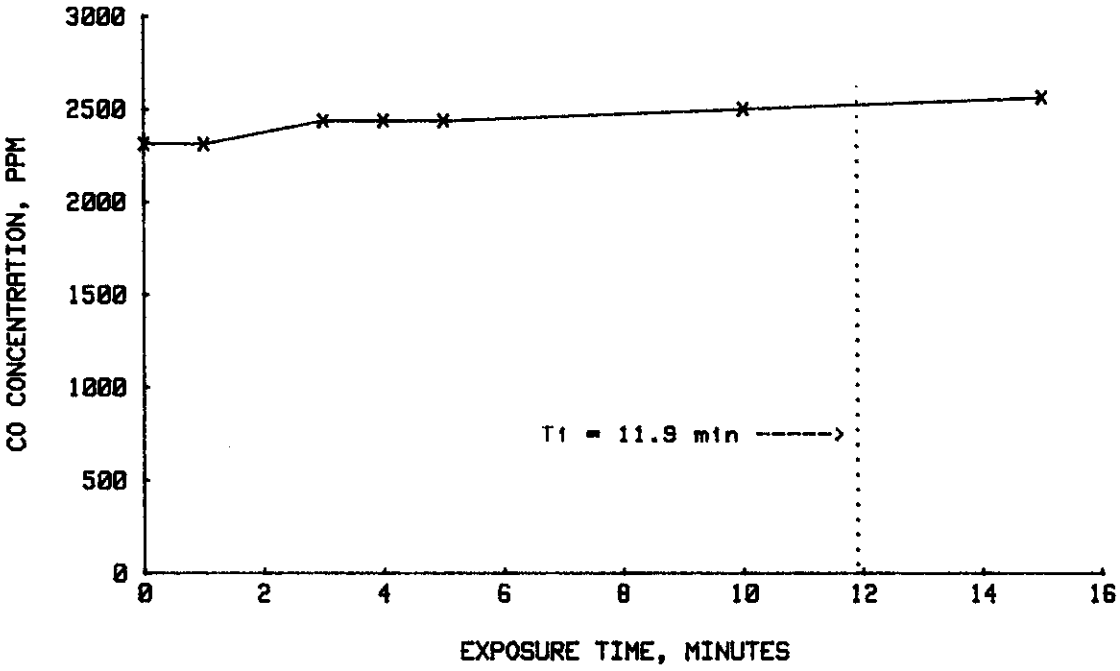

Figure 3. CO concentration vs time, for typical experiment

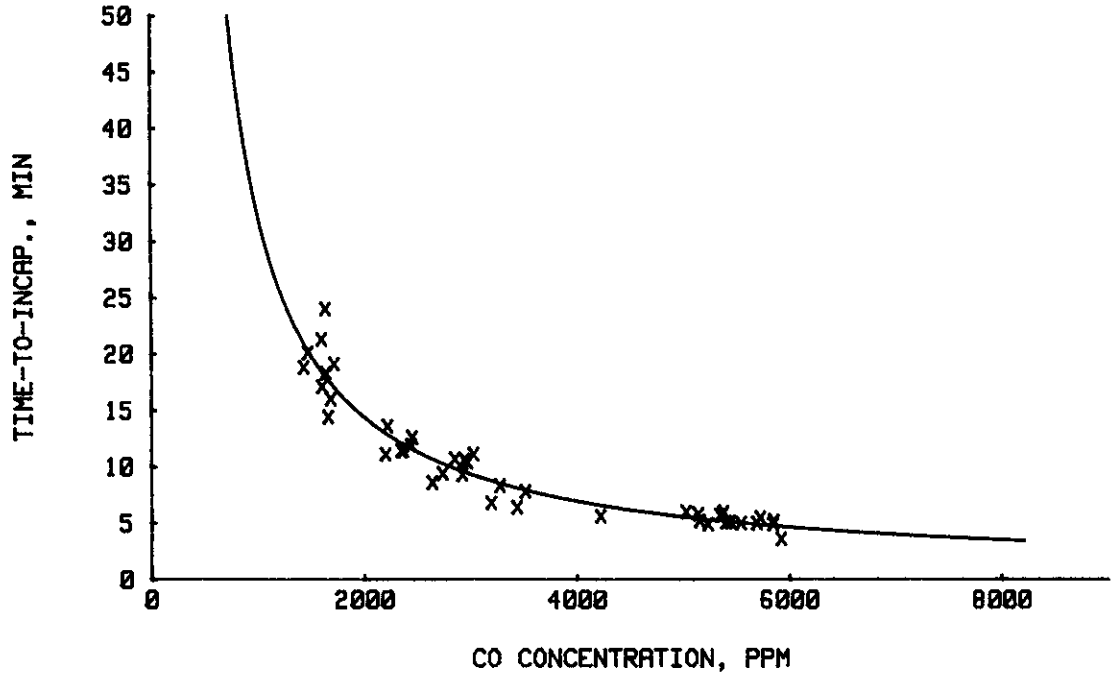

Figure 4. Scatter plot of time-to-incapacitation vs CO concentration

TABLE 1

DOSE-RESPONSE DATA FOR CARBON MONOXIDE ALONE

[CO], ppm	T_i, min	($C*T_i$), ppm*min	Pred.* T_i, min
1437	18.1	26010	20.9
1478	20.1	29708	20.3
1605	21.3	34187	18.4
1607	17.1	27480	18.4
1642	24.0	39408	18.0
1643	18.3	30067	17.9
1665	14.4	23976	17.7
1690	16.0	27040	17.4
1723	19.1	32909	17.0
2201	11.1	24431	13.0
2221	13.6	30206	12.8
2351	11.4	26801	12.1
2367	11.4	26984	12.0
2441	11.9	29048	11.6
2453	12.6	30908	11.5
2639	8.6	22695	10.7
2738	9.4	25737	10.3
2852	10.7	30516	9.8
2923	9.3	27184	9.6
2942	10.6	31185	9.5
2968	10.4	30867	9.4
3028	11.1	33611	9.2
3193	6.8	21712	8.7
3274	8.3	27174	8.5
3436	6.4	21990	8.1
3517	7.8	27433	7.9
4225	5.6	23660	6.6
5028	6.0	30168	5.5
5142	5.8	29824	5.4
5155	5.2	26806	5.4
5233	4.9	25642	5.3
5345	5.7	30467	5.2
5367	5.8	31129	5.2
5379	6.0	32274	5.2
5401	5.1	27545	5.1
5443	5.1	27759	5.1
5543	5.0	27715	5.0
5692	5.0	28460	4.9
5724	5.5	31482	4.8
5845	4.9	28641	4.8
5851	5.2	30425	4.7
5922	3.6	21319	4.7

*Predicted $t_i = 0.3+(25,017/([CO]-225))$.

to "walk" his front feet on the moving drum surface without actually changing his location. Time-to-incapacitation was not recorded until this "walking" motion ceased.

Exposures to CO alone. A plot of C vs t for a typical experiment is provided as Figure 3; for this exposure: t_i=11.9 min; integrated area from t=0 to t=t_i is 29,048 ppm*min; average CO concentration is 2,441 ppm (29048/11.9 = 2441).

The principal data from the 42 exposures to CO alone are listed in Table 1. The average CO concentration (column 1) was calculated from the integrated area (column 3) under the C vs t curve, as described under MATERIALS AND METHODS; column 2 lists the corresponding t_i's, as observed experimentally.

Figure 4 is a scatter plot of t_i vs [CO][1] for the 42 exposures conducted with CO alone. The equation for the curve that was fit to these data is:

$$([CO]-225) * (t_i-0.3) = 25,017. \qquad \{Eq. 4\}$$

A plot of this equation has been superimposed on the data points in Figure 4. The toxicokinetic interpretation of the fitted parameters in this equation is as follows: The value of the mean effective dose (ED or K_0, see Eq. 1), for exposure to CO alone, is 25,017 ppm*min; the minimal CO concentration that will produce incapacitation (C_o) is 225 ppm; and the shortest exposure time (t_o) for which incapacitation can be produced at any concentration is 0.3 min.

Exposures to HCN alone. Figure 5 shows the variation of [HCN] as a function of exposure time for a typical experiment. In this case, t_i = 7.7 min, integrated area from t=0 to t=t_i is 1,024 ppm*min, and average [HCN] is 133 ppm (1024/7.7 = 133).

The principal data from the 30 exposures to HCN alone are listed in Table 2, where the column headings are analogous to those for Table 1. Figure 6 is a scatter plot of t_i vs [HCN] for those specific exposures. The equation for the curve that was fit to these data is:

$$([HCN] - 63) * t_i = 564. \qquad \{Eq. 5\}$$

A plot of this equation has been superimposed on the data points in Figure 6. In this case, an asymptotic value of zero for the limiting time-to-incapacitation (t_o=0) gave as good a fit to the data as any nonzero value; therefore, we elected to use t_o=0 in this instance even though in other studies t_o has had values up to 0.1 min. The estimated values for the other toxicokinetic parameters are: mean effective dose (ED or K_o)=564 ppm*min and minimally effective concentration (C_o)=63 ppm HCN.

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[1]Square brackets signify concentration; [X] = concentration of gas, X, in ppm.

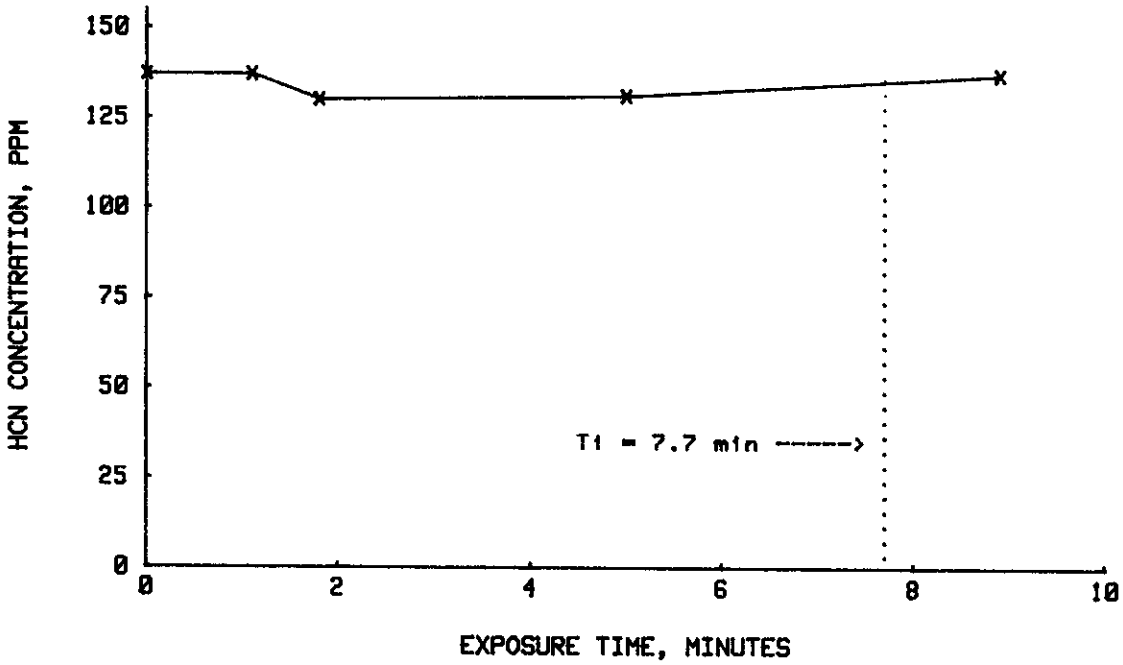

Figure 5. HCN concentration vs time, for typical experiment

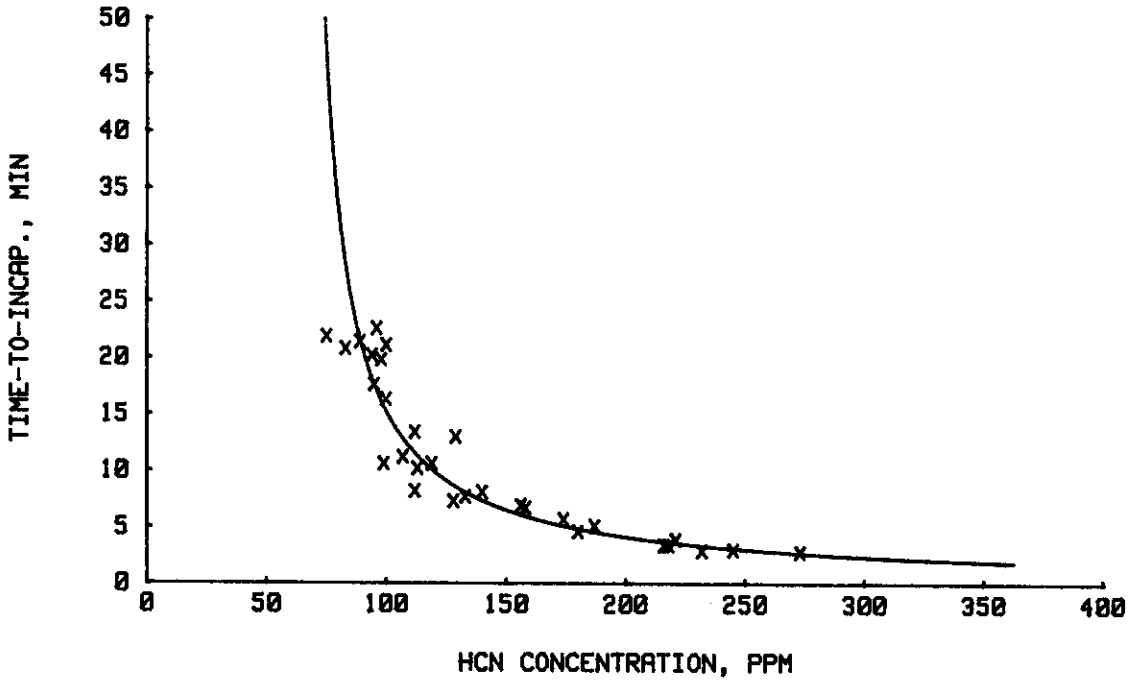

Figure 6. Scatter plot of time-to-incapacitation vs HCN concentration

TABLE 2

DOSE-RESPONSE DATA FOR HYDROGEN CYANIDE ALONE

[HCN], ppm	T_i, min	($C*T_i$), ppm*min	Pred.* T_i, min
75	21.9	1642	47.0
83	20.8	1726	28.2
89	21.4	1905	21.7
94	20.2	1903	18.2
95	17.6	1672	17.6
96	22.6	2170	17.1
98	19.8	1950	16.1
99	10.6	1049	15.7
100	21.1	2116	15.2
100	16.3	1630	15.2
107	11.2	1198	12.8
112	8.2	918	11.5
112	13.4	1501	11.5
113	10.2	1153	11.3
119	10.6	1261	10.1
128	7.3	934	8.7
129	13.0	1677	8.5
133	7.7	1024	8.1
140	8.1	1134	7.3
156	6.9	1076	6.1
158	6.7	1059	5.9
174	5.7	992	5.1
180	4.6	828	4.8
187	5.1	954	4.5
216	3.4	734	3.7
218	3.4	741	3.6
221	3.9	862	3.6
232	2.9	673	3.3
245	3.0	735	3.1
273	2.8	764	2.7

*Predicted $t_i = 564/([HCN]-63)$.

Exposures to CO-HCN mixtures. The times-to-incapacitation that resulted from the 63 individual exposures of a single rat to a defined mixture of CO and HCN are listed in Table 3, in order of increasing t_i.

The average concentrations of CO and HCN to which the animals were exposed (C', Eq. 2) were calculated as they were for the single gas exposures, and from this data the "administered dose" (AD, or K'), as defined by Eq. 2, was derived. The fractional effective dose (FED), as defined by Eq. 3, was calculated for each of the two gases and for each exposure. A listing of these calculated values, as well as the sum of the two FED's for each exposure, is presented in Table 4.

Once the administered dose is known for each gas, we also can calculate the predicted t_i that should result if an animal were exposed to that concentration of that gas alone. This is accomplished, for both CO and HCN, using equations 4 and 5 respectively, and the results are presented in Table 5.

Evaluation of CO-HCN interaction. In the viewpoint of the authors, there are at least two techniques available to us for determining whether or not the toxic effects of the two gases are additive. The first approach is based on the simple concept that the sum of the fractional effective doses, due to each gas alone, should equal unity if their combined effects are exactly additive. The second relationship is that the sum of the reciprocal t_i's, predicted for each gas separately, should equal the reciprocal of the observed t_i, for the combined gases, if the toxic effects are exactly additive. The equations describing these two relationships are:

$$FED_{(CO)} + FED_{(HCN)} = 1.0 \qquad \text{(Eq. 6)}$$

and

$$1/t_{i(CO)} + 1/t_{i(HCN)} = 1/t_{i(obs)}. \qquad \text{(Eq. 7)}$$

The logic of the FED concept can be illustrated by the following. If one administers to a subject, by injection, one-half of a lethal dose of chemical A followed immediately (or simultaneously) by an additional one-half lethal dose of chemical A, then by definition lethality should result. The end-result should be the same if the two fractional doses were three-fourths plus one-fourth, or two-thirds plus one-third, etc. This conclusion is obvious because the two doses administered are of exactly the same chemical and one would, therefore, expect their combined actions to be exactly additive. If, on the other hand, one administers two dissimilar chemicals and their combined effects achieve the defined result, then one could conclude that their actions are exactly additive provided the sum of their FED's is unity. If their combined effects are synergistic, one would expect the sum of the FED's to be considerably less than unity; if the sum is significantly greater than unity, then their combined effects must be antagonistic or "less than additive" (including the possibility of no interaction whatsoever).

TABLE 3

DOSE-RESPONSE DATA FOR MIXTURES OF CO AND HCN

Time-to-incapacitation, minutes	Gas Concentration, ppm	
	HCN	CO
2.4	197	5126
2.6	177	5202
2.8	215	5513
2.9	119	6385
2.9	129	5983
2.9	191	5391
3.0	122	5258
3.1	136	5964
3.6	120	5270
3.8	131	5445
3.8	200	3026
3.9	200	3090
4.1	94	5438
4.2	175	2489
4.2	120	5387
4.3	184	1389
4.6	174	2589
4.6	181	2816
4.6	102	5788
4.7	102	5650
4.7	89	5190
4.7	89	5492
4.8	187	1718
4.9	93	5475
4.9	184	1526
5.2	165	2761
5.3	180	1560
5.5	125	3499
5.9	157	1461
6.3	135	2565
6.5	137	2434
6.7	125	2564
6.8	150	1422
6.9	133	1593
6.9	129	2284
7.0	123	1859
7.1	125	1670
7.2	143	1706
7.2	127	2609
7.2	131	2370
7.9	122	1528
7.9	108	1594
8.1	111	1716
8.3	103	1475
8.4	92	1609
8.4	88	2812
8.4	115	1332
8.5	119	1406
8.5	84	2762
8.6	109	1592
8.7	130	1503
8.7	91	2748
8.8	86	2674
8.8	123	1514
8.9	131	1521
9.0	90	1567
9.5	92	2933
9.6	131	1498
9.6	85	2831
9.9	113	1510
10.0	108	1661
10.2	101	1570
10.9	103	1549

TABLE 4

DERIVED DATA* FOR MIXTURES OF CO AND HCN

t_i, min	Administered Dose		Fractional Effective Dose		Summed FED's
	CO	HCN	CO	HCN	
2.4	10292	322	0.41	0.57	0.98
2.6	11447	296	.46	.53	.98
2.8	13220	426	.53	.75	1.28
2.9	16016	162	.64	.29	.93
2.9	14971	191	.60	.34	.94
2.9	13432	371	.54	.66	1.20
3.0	13589	177	.54	.31	.86
3.1	16069	226	.64	.40	1.04
3.6	16648	205	.67	.36	1.03
3.8	18270	258	.73	.46	1.19
3.8	9804	521	.39	.92	1.31
3.9	10314	534	.41	.95	1.36
4.1	19809	127	.79	.23	1.02
4.2	8830	470	.35	.83	1.19
4.2	20132	239	.80	.42	1.23
4.3	4656	520	.19	.92	1.11
4.6	10165	511	.41	.91	1.31
4.6	11141	543	.45	.96	1.41
4.6	23921	179	.96	.32	1.27
4.7	23870	183	.95	.32	1.28
4.7	21846	122	.87	.22	1.09
4.7	23175	122	.93	.22	1.14
4.8	6718	595	.27	1.06	1.32
4.9	24150	147	.97	.26	1.23
4.9	5985	593	.24	1.05	1.29
5.2	12426	530	.50	.94	1.44
5.3	6675	620	.27	1.10	1.37
5.5	17025	341	.68	.60	1.29
5.9	6922	555	.28	.98	1.26
6.3	14040	454	.56	.80	1.37
6.5	13696	481	.55	.85	1.40
6.7	14970	415	.60	.74	1.33
6.8	7780	592	.31	1.05	1.36
6.9	9029	483	.36	.86	1.22
6.9	13589	455	.54	.81	1.35
7.0	10948	420	.44	.74	1.18
7.1	9826	440	.39	.78	1.17
7.2	10219	576	.41	1.02	1.43
7.2	16450	461	.66	.82	1.47
7.2	14800	490	.59	.87	1.46
7.9	9903	466	.40	.83	1.22
7.9	10404	356	.42	.63	1.05
8.1	11630	389	.46	.69	1.15
8.3	10000	332	.40	.59	.99
8.4	11210	244	.45	.43	.88
8.4	20955	210	.84	.37	1.21
8.4	8967	437	.36	.77	1.13
8.5	9684	476	.39	.84	1.23
8.5	20803	178	.83	.32	1.15
8.6	11346	396	.45	.70	1.15
8.7	10735	583	.43	1.03	1.46
8.7	21193	244	.85	.43	1.28
8.8	20816	202	.83	.36	1.19
8.8	10956	528	.44	.94	1.37
8.9	11146	605	.45	1.07	1.52
9.0	11675	243	.47	.43	.90
9.5	24914	276	1.00	.49	1.48
9.6	11839	653	.47	1.16	1.63
9.6	24236	211	.97	.37	1.34
9.9	12336	495	.49	.88	1.37
10.0	13929	450	.56	.80	1.35
10.2	13316	388	.53	.69	1.22
10.9	14034	436	0.56	0.77	1.33

* See text for definitions of derived data.

TABLE 5

PREDICTED AND OBSERVED TIMES-TO-INCAPACITATION

Time-to-incapacitation, minutes			
Observed t_i	Predicted from [HCN]	Predicted from [CO]	Predicted from Eq. 7
2.4	4.2	5.4	2.4
2.6	4.9	5.3	2.6
2.8	3.7	5.0	2.1
2.9	4.4	5.1	2.4
2.9	10.1	4.4	3.0
2.9	8.5	4.6	3.0
3.0	9.6	5.3	3.4
3.1	7.7	4.7	2.9
3.6	9.9	5.3	3.4
3.8	8.3	5.1	3.2
3.8	4.1	9.2	2.8
3.9	4.1	9.0	2.8
4.1	18.2	5.1	4.0
4.2	5.0	11.3	3.5
4.2	9.9	5.1	3.4
4.3	4.7	21.8	3.8
4.6	14.5	4.8	3.6
4.6	4.8	10.0	3.2
4.6	5.1	10.9	3.5
4.7	14.5	4.9	3.7
4.7	21.7	5.3	4.3
4.7	21.7	5.0	4.1
4.8	4.5	17.1	3.6
4.9	4.7	19.5	3.8
4.9	18.8	5.1	4.0
5.2	5.5	10.2	3.6
5.3	4.8	19.0	3.8
5.5	9.1	7.9	4.2
5.9	6.0	20.5	4.6
6.3	7.8	11.0	4.6
6.5	7.6	11.6	4.6
6.7	9.1	11.0	5.0
6.8	6.5	21.2	5.0
6.9	8.5	12.5	5.1
6.9	8.1	18.6	5.6
7.0	9.4	15.6	5.9
7.1	9.1	17.6	6.0
7.2	8.8	10.8	4.9
7.2	7.0	17.2	5.0
7.2	8.3	12.0	4.9
7.9	12.5	18.6	7.5
7.9	9.6	19.5	6.4
8.1	11.8	17.1	7.0
8.3	14.1	20.3	8.3
8.4	22.6	10.0	6.9
8.4	19.4	18.4	9.4
8.4	10.8	22.9	7.4
8.5	26.9	10.2	7.4
8.5	10.1	21.5	6.9
8.6	12.3	18.6	7.4
8.7	20.1	10.2	6.8
8.7	8.4	19.9	5.9
8.8	24.5	10.5	7.4
8.8	9.4	19.7	6.4
8.9	8.3	19.6	5.8
9.0	20.9	18.9	9.9
9.5	19.4	9.5	6.4
9.6	25.6	9.9	7.1
9.6	8.3	20.0	5.9
9.9	11.3	19.8	7.2
10.0	12.5	17.7	7.3
10.2	14.8	18.9	8.3
10.9	14.1	19.2	8.1

The logic for Eq. 7 is based on the following relationships:

(a) dose is proportional to toxic gas concentration;
(b) response time is inversely proportional to concentration;
(c) therefore t_i is inversely proportional to dose.

As a consequence, if the combined dose effect is related to the sum of the individual doses, then the combined response times should be calculated as reciprocals.

Examination of Table 4 clearly illustrates that for most of the 63 combined exposures the sum of the FED's is significantly greater than unity (mean value: 1.23; std. dev.: 0.17; $p<0.01$ that the mean is equal to 1.00). This would suggest that one, or both, of the component gases is contributing less to the combined effect than one would predict from an exact summation of their individual contributions; in other words, the combined effect is less than exactly additive.

The data presented in the first three columns of Table 5 do indicate, however, that for a large majority of the exposures (56 of 63) the response time (observed t_i) produced by the combination of gases is equal to, or less than, either of the t_i's predicted for one gas alone (mean t_i from CO alone = 12.7 min, from HCN alone = 11.0 min, mean observed t_i = 6.4 min). In other words, the presence of the second gas did increase the toxicity of the mixture beyond that attributable to the first gas alone; and even in the seven cases for which the observed t_i is larger, the magnitude of the difference (0.2 to 0.6 min) is not highly significant in light of the precision of the original dose-response data.

The fourth column in Table 5 lists the t_i calculated from the relationship expressed by Eq. 7, i.e., the sum of the reciprocal response times. If the effects of the two gases were exactly additive, the values in column 4 should equal the corresponding values in column 1; it is obvious that most of the observed t_i's are greater than the calculated (predicted) ones (mean observed t_i = 6.4 min; mean predicted t_i = 5.1 min; linear regression of observed on predicted gives a slope = 1.12, intercept = 0.61, and a correlation of 0.91). Once again, this can be interpreted as one, or both, of the gases contributing less than its theoretical toxicity to that of the mixture.

At this stage of data evaluation, an obvious conclusion would seem to be that the toxicity of an atmosphere containing either of these two gases is increased by the simultaneous presence of the other. The magnitude of the combined toxicity, however, is less than would be predicted from an exact summation of the individual toxicities. One question yet to be addressed pertains to the magnitude of fractional contribution made by each gas to the total effect.

Elucidation of degree of fractional contributions. As previously stated, there are three functional mechanisms by which the reduced toxicity of the combined gases might be explained. We have evaluated the experimental data in the following manner

in an attempt to identify the most likely of those three mechanisms.

The sum of the individual FED's (Eqs. 3 & 6) was observed to be greater than unity (Table 4); however, if each FED were to represent the real fractional contribution made by that gas to the achievement of the observed effect (and incapacitation was achieved), then the sum should equal 1.00. It occurred to us that we could modify Eq. 6 by assigning a coefficient (a weighting factor) to each of the FED's and setting this new sum equal to 1.00:

$$a*FED_{(CO)} + b*FED_{(HCN)} = 1.00. \qquad \text{(Eq. 8)}$$

Statistically-derived values for the coefficients, a and b, could then be obtained by doing a least-squares, linear regression analysis on a rearranged form of Eq. 8:

$$FED_{(CO)} = 1/a - (b/a)*FED_{(HCN)}. \qquad \text{(Eq. 8a)}$$

The least-square's best estimates for the coefficients are:

$$a = 1.05 \qquad \text{and} \qquad b = 0.57;$$

therefore, Eq. 8 could be rewritten as:

$$1.05*FED_{(CO)} + 0.57*FED_{(HCN)} = 1.00. \qquad \text{(Eq. 8b)}$$

This statistically-derived result can be interpreted as indicating that CO is contributing to the combined effect with essentially the same potency that it would exert were it present alone, while HCN is only 57% as effective in the presence of CO as it would be alone.

A similar line of logic was then applied to forcing the sum of the reciprocal t_i's to equal the reciprocal of the observed t_i, see Eq. 7. The modified equation would then be:

$$a/t_{i(CO)} + b/t_{i(HCN)} = 1/t_{i(obs)}. \qquad \text{(Eq.9)}$$

A multiple linear regression was performed on this set of data using the algorithm that forces the constant term (or intercept) to equal zero. The resultant least-square's estimates for the coefficients are:

$$a = 1.00 \qquad \text{and} \qquad b = 0.68,$$

and Eq. 9 becomes:

$$1/t_{i(CO)} + 0.68/t_{i(HCN)} = 1/t_{i(obs)}. \qquad \text{(Eq. 9a)}$$

This result also suggests that the CO is contributing an essentially undiminished potency to the combined effect while the

TABLE 6

OBSERVED AND PREDICTED TIMES-TO-INCAPACITATION

Time-to-incapacitation, minutes	
Observed t_i	Predicted from Eq 10
2.4	2.9
2.6	3.1
2.8	2.6
2.9	2.9
2.9	3.4
2.9	3.4
3.0	3.8
3.1	3.3
3.6	3.9
3.8	3.6
3.8	3.7
3.9	3.6
4.1	4.3
4.2	4.5
4.2	3.8
4.3	5.2
4.6	3.9
4.6	4.1
4.6	4.4
4.7	4.0
4.7	4.6
4.7	4.4
4.8	4.8
4.9	5.1
4.9	4.3
5.2	4.5
5.3	5.2
5.5	5.0
5.9	6.2
6.3	5.6
6.5	5.7
6.7	6.0
6.8	6.6
6.9	6.3
6.9	7.2
7.0	7.3
7.1	7.6
7.2	5.9
7.2	6.5
7.2	6.0
7.9	9.3
7.9	8.2
8.1	8.6
8.3	10.3
8.4	7.7
8.4	11.2
8.4	9.4
8.5	8.1
8.5	8.8
8.6	9.2
8.7	7.6
8.7	7.6
8.8	8.1
8.8	8.1
8.9	7.5
9.0	11.7
9.5	7.2
9.6	7.8
9.6	7.6
9.9	9.0
10.0	9.0
10.2	10.1
10.9	10.0

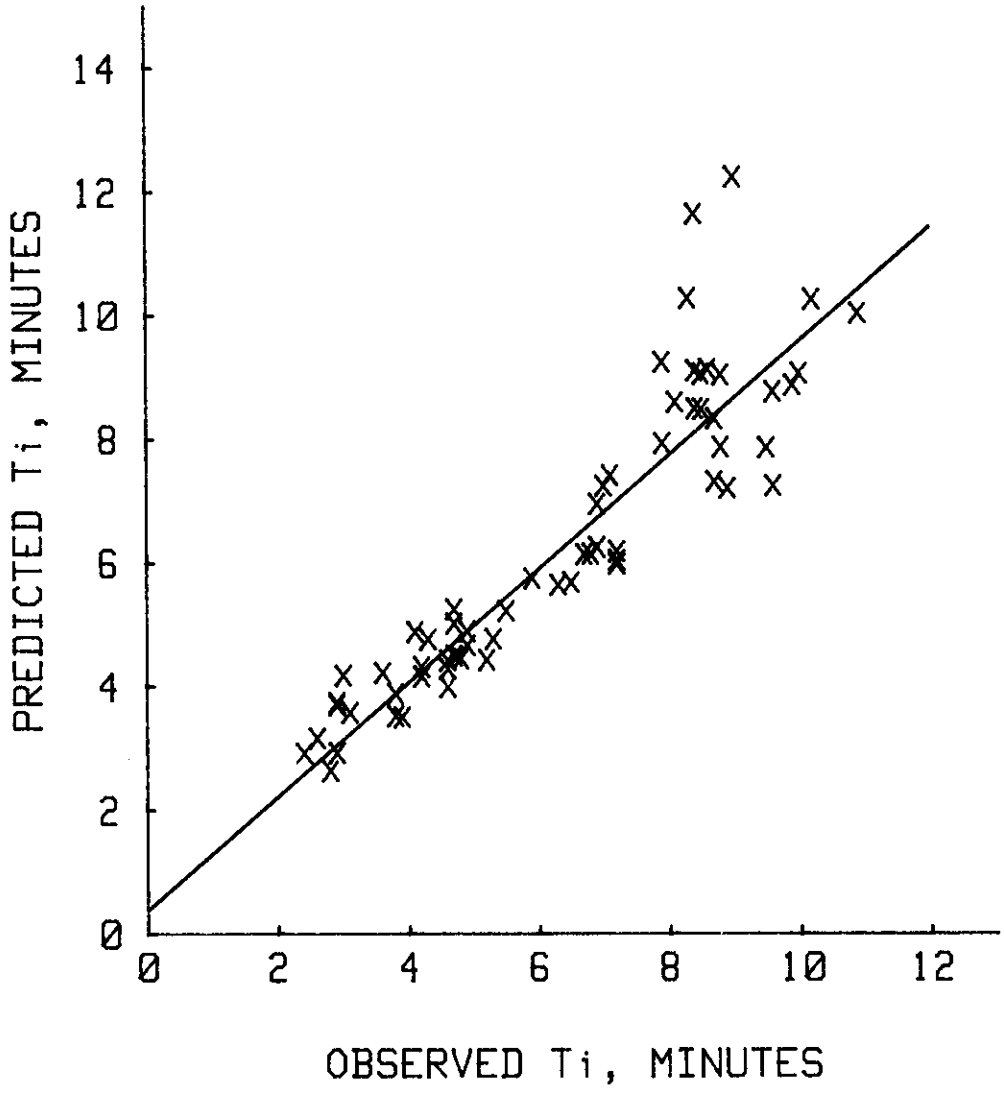

Figure 7. Observed vs predicted* time-to-incapacitation for rats exposed to CO-HCN mixtures

* Predicted t_i was determined using equation 10.

HCN is only about two-thirds as effective as when administered alone.

The real significance of the result predicted from these pieces of circumstantial evidence would be difficult to assess from the data at hand. It also would be difficult to verify experimentally except by first proposing the mechanism by which the presence of CO reduced the toxic effect of HCN, and then seeking experimental verification of the predictions based on that hypothesis. The utility of the results from the present study do not depend, however, on specific knowledge of the degree to which each gas contributes to the combined effect, but on the ability to predict t_i as a function of the individual gas concentrations.

Prediction of t_i for mixtures of CO and HCN. The statistically-derived relationship presented as Eq. 9a can be used to predict the response time that would result from a mixture of known concentrations of the two gases, provided that these concentrations are within those limits used in the original experimental exposures. Re-naming the response variable from $1/t_{i(obs)}$ to $1/t_{i(predicted)}$ and substituting the concentration-dependent relationships for $1/t_{i(CO)}$ and $1/t_{i(HCN)}$ (from Eqs. 4 and 5, respectively), Eq. 9a becomes:

$$\frac{1}{0.3+(25{,}017/([CO]-225))} + \frac{0.68}{564/([HCN]-63)} = \frac{1}{t_{i(pred)}} \qquad \text{(Eq. 10)}$$

Table 6 lists the observed t_i's along with the t_i's predicted by Eq. 10 for each of the 63 exposures. Figure 7 is a plot of the correspondence between these two sets of values, and includes the least-squares regression line that was fit to the data. The resultant equation is:

$$t_{i(pred)} = 0.38 + 0.92 * t_{i(obs)}. \qquad \text{(Eq. 11)}$$

For this regression, the correlation coefficient is 0.92 and the standard error of the estimate is also 0.92.

SUMMARY AND CONCLUSIONS

Rats were exposed, under controlled conditions, to selected atmospheric concentrations of: (i) carbon monoxide in air; (ii) hydrogen cyanide in air; and (iii) mixtures of carbon monoxide plus hydrogen cyanide in air. For each animal, and for each experimental condition, the elapsed exposure time (t_i) required to produce physical incapacitation was measured.

The response times for those experiments that utilized CO and HCN alone were plotted against exposure concentration and equations were derived by nonlinear regression techniques that

described those relationships mathematically. The resulting equations, for time-to-incapacitation, are:

$$t_i = 0.3 + 25{,}017/([C] - 225), \text{ for CO, and}$$

$$t_i = 564/([C]-63), \text{ for HCN,}$$

where t_i is in minutes and [C] is the concentration, in ppm, of the toxic gas in the atmosphere. The response times obtained from exposures to mixtures of the two gases were then analyzed with respect to the response time that would have been predicted from the action of each individual gas acting alone at the concentration utilized in the mixture.

Our conclusion from this analysis is that the toxic potency of a mixture of CO and HCN is greater than can be ascribed to the same concentrations of either gas by itself--at least for the concentration ranges utilized in this investigation. The data do not support, however, the hypothesis of synergism--that is, that the combined effect would be greater than that predicted by summing the two individual effects. The mechanistic hypothesis that is supported by the data is that the toxic potencies of these two gases are fractionally (or incompletely) additive when they are present in the same atmosphere at concentrations that fall within the range included in this investigation. With emphasis on this important caveat, we offer an equation that may be of value in estimating the time-to-incapacitation produced by mixtures of CO and HCN.

$$t_i \text{ (pred)} = \frac{1}{\left(\frac{1}{0.3+(25{,}017/([CO]-225))}\right) + \left(\frac{0.68}{564/([HCN]-63)}\right)}.$$

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