/AM-90/15

Inhalation Toxicology: X. Times to Incapacitation for Rats Exposed Continuously to Carbon Monoxide, Acrolein, and to Carbon Monoxide-Acrolein Mixtures

Office of Aviation Medicine
Washington, D.C. 20591

Charles R. Crane
Donald C. Sanders
Boyd R. Endecott

Civil Aeromedical Institute
Federal Aviation Administration
Oklahoma City, OK 73125

December 1990

Final Report

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

U.S. Department of Transportation
Federal Aviation Administration

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-90/15		

4. Title and Subtitle	5. Report Date
INHALATION TOXICOLOGY: X. TIMES TO INCAPACITATION FOR RATS EXPOSED CONTINUOUSLY TO CARBON MONOXIDE, ACROLEIN, AND TO CARBON MONOXIDE-ACROLEIN MIXTURES	December 1990
	6. Performing Organization Code
7. Author(s) Charles R. Crane, Donald C. Sanders and Boyd R. Endecott	8. Performing Organization Report No.
9. Performing Organization Name and Address FAA Civil Aeromedical Institute P. O. Box 25082 Oklahoma City, OK 73125	10. Work Unit No. (TRAIS) 11. Contract or Grant No.
12. Sponsoring Agency Name and Address Office of Aviation Medicine Federal Aviation Administration 800 Independence Ave., SW Washington, D.C. 20591	13. Type of Report and Period Covered 14. Sponsoring Agency Code

15. Supplementary Notes

This work was performed under task AM-B-87-TOX-55.

16. Abstract

Laboratory rats were exposed to experimental atmospheres of (a) carbon monoxide (CO) in air, (b) acrolein in air, and (c) to mixtures of CO and acrolein in air. The toxic potency of each of the three types of environments was evaluated toxicokinetically by measurement of time-to-incapacitation (t_i) as a function of toxic gas concentrations. Regression equations were derived that relate the observed t_i's to the concentrations of individual gases.

Comparison of the combined-gas-exposure data with results obtained from the individual gas exposures indicated that there was no evidence of synergistic action, i.e., that the effect of the combination was never greater than that predicted by the sum of the two individual gas effects. Evidence did exist for an inhibitory or antagonistic effect of undefined mechanism when acrolein was present in the mixture at concentrations of lesser toxic potency than that of CO.

An empirical equation was derived that allows the calculation of a predicted t_i for combinations of CO and acrolein concentrations within the ranges utilized in the experimental exposures.

17. Key Words	18. Distribution Statement
Inhalation toxicology; carbon monoxide; acrolein; irritant gas; CO-acrolein toxicity; time-to-incapacitation; animal exposure systems.	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	26	

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INHALATION TOXICOLOGY: X. TIMES TO INCAPACITATION FOR RATS EXPOSED CONTINUOUSLY TO CARBON MONOXIDE, ACROLEIN, AND TO CARBON MONOXIDE-ACROLEIN MIXTURES

INTRODUCTION

The development of fire in an otherwise survivable aircraft accident greatly decreases the passengers' chances for survival, both from direct fire effects and from the loss of capacity for escape from the fire environment caused by breathing the toxic components of smoke. Disorientation can occur, not only due to the direct visual obscuration caused by smoke, but also because of the lachrymatory effects of irritant gases within the smoke. Concurrently, certain systemic toxicants, such as carbon monoxide (CO) and hydrogen cyanide (HCN), are being inhaled that will eventually result in physical incapacitation. Death, either from continued inhalation of toxic gases or from thermal effects, is the likely sequel.

While dose-response relationships for single gas exposures have been defined for some of the major toxic components of smoke (1,13,19,23) and for a few of the irritant gases (2,3,7,10,16,17,20,22), less information exists pertaining to the combined effects of these gases on the intact mammalian organism (11,15,18,21,25). With present technology, it is not feasible to reconstruct, or even to define, all of the complex mixtures of combustion products that occur in aircraft fires. It is possible, however, to determine the reaction of laboratory animals to simple, defined mixtures of pure gases to better understand the combined effects of these gases in the fire environment. This study was designed to investigate the combined effects of CO, a systemic toxicant, and acrolein, a toxic, unsaturated aldehyde noted for its extreme irritant properties.

CO is a colorless, odorless gas produced by the incomplete combustion of carbon-containing materials, particularly under smoldering conditions where insufficient oxygen is available to allow further oxidation to carbon dioxide. The ubiquitous distribution of such materials (such as cotton, wood, hydrocarbon fuels, and most plastics) virtually assures that some CO will be present in any aircraft fire. The classical mechanism for CO toxicity is the rapid combination of CO with the hemoglobin of the red blood cells, thereby reducing the oxygen-carrying capacity of the blood (23). Other workers (6) have presented evidence indicating that the more probable toxic action is one of CO on cellular respiration, where <u>dissolved</u> CO (in plasma) crosses into the tissues and competes with oxygen for cytochrome a_3 in the mitochondria. Whatever the actual mechanism(s), carboxyhemoglobin levels of about 50% and 70% are generally associated with incapacitation and death, respectively, in humans (5,8).

Acrolein, the other toxic gas component in this study, was briefly used as a chemical warfare agent in World War I (8), but

is probably more familiar to the public as the acrid, tear-producing odor associated with the overheating of animal fats and oils. Structurally, it is a 3-carbon, unsaturated aldehyde with the formula CH_2=CHCHO. It is a suspected thermal decomposition product of certain materials used in aircraft cabin interiors and has been identified in 91% of 120 building fires monitored in a 1978 study by the Harvard School of Public Health (26). Firefighters, in particular, have recognized the irritant and toxic dangers of acrolein, particularly in low temperature (300-400°C), smoldering thermal conditions involving polyethylene, polypropylene, vinylon, and cellulose containing materials (14).

Considerable confusion exists in the literature concerning the levels of acrolein likely to be incapacitating or lethal to humans. Predicted (10-20 ppm) levels appear conservative (8,24) when compared to experimental values for various animal species (7,20), and are apparently based on discomfort indices instead of actual physical incapacitation. Our earlier study (3) indicated that approximately 4200 ppm of acrolein was necessary to produce incapacitation in the laboratory rat in 10 minutes, and Kaplan (9) found that baboons could successfully escape from an exposure chamber after exposure to 2780 ppm for 5 minutes.

This study evolved from a need to better understand the relative toxic contribution of individual combustion gases to the overall toxicity of a mixture. No aircraft fire produces only a single combustion product; therefore, if we are ever to predict the probable physiological effect of a gas mixture, we must first obtain an understanding of how the mixture components interact to produce their effect on the experimental subject. Do two (or more) components react in such a way as to be synergistic, antagonistic, or are their toxic actions completely independent of each other? There has also been speculation that irritant gases, at low concentrations, might cause shallower breathing with a lowered minute respiratory volume, thereby exerting a "protective" effect against other non-irritant systemic toxicants such as CO, resulting in a longer time-to-incapacitation (t_i) than would be expected from the CO concentration alone.

Our earlier work with CO, HCN, HCl, acrolein, and CO-HCN mixtures (2,3,4,21) utilized a rotating cage assembly inside an animal exposure chamber to measure t_i for rats exposed to these combustion gases. Similar procedures were used in this study to relate t_i to gas concentration for rats exposed to CO and acrolein individually, and to CO-acrolein mixtures. Gas concentrations in the mixtures ranged from 1318 to 6152 ppm for CO, and from 18 to 14967 ppm for acrolein.

MATERIALS AND METHODS

Animals. Male albino rats of Sprague-Dawley origin were obtained from Charles River Breeding Laboratories, Wilmington, MA, in a weight range of 100 to 120 g. The animals were maintained on drinking water containing 1.5 g/L of sulfathiazole

for the first 4 days, then normal tap water for the remaining 4 days' isolation. The rats were fasted overnight before testing to establish equivalent metabolic states; each animal was weighed immediately prior to use.

Exposure chamber design. The animal exposure chamber utilized in this study has been previously described (3); its design is detailed in Figure 1. It differed from the chambers used in earlier studies in that its design allowed the insertion of the test animal directly into an established test atmosphere, while retaining the rotating cage assembly for the determination of t_i. Chamber construction was of polymethylmethacrylate (PMMA) with internal dimensions 50.8 cm long by 26.2 cm wide by 50.6 cm high. The two-compartment cylindrical rotating cage, 40.6 cm in diameter by 25.0 cm wide, had a plastic mesh floor (perimeter) and a perforated divider. The cage was suspended across the width of the chamber by a central axle attached to the perforated divider; the outer chamber walls functioned as end walls for the cage. Chamber access ports (10 cm by 10 cm), equipped with gaskets, were installed in each side of the chamber at the level of the rotating cage floor, to allow rapid insertion or removal of the test animals. The rotating cage was driven by a 4-rpm geared motor (Dayton model 3M098), providing a linear velocity (circumferential) of 8.5 cm/s.

Gases. CO (99.5%, CP grade) and breathing air were obtained from Big Three Industries, Inc., La Porte, TX. Acrolein was purchased from the Aldrich Chemical Co., Inc., Milwaukee, WI, as a 97% solution containing approximately 3% water and 200 ppm hydroquinone to inhibit polymer formation. This material was purified by fractional distillation and refrigerated; the acrolein was brought to room temperature and centrifuged to remove any polymer (disacryl) before use for test atmosphere generation or preparation of gas chromatographic standards.

Test atmosphere generation. The amount of acrolein required for a given experiment was calculated from the desired atmospheric concentration and the enclosed chamber volume (67.35 L). A side door was removed from the dry, empty chamber, and the calculated volume of freshly centrifuged liquid acrolein was introduced by pipet into a 10-cm glass Petri dish centered on the chamber floor. The door was replaced and the fans were turned on to promote evaporation and atmospheric mixing; equilibrium was considered to be complete when all liquid acrolein had evaporated, a period of 15 to 45 min, depending on the quantity added.

CO atmospheres were produced by direct injection of pure CO into the chamber using a gas syringe; equilibration of the CO with chamber air was complete within 1 min, although time requirements for test sampling and animal handling usually permitted a 5- to 10-min equilibration time before animal insertion.

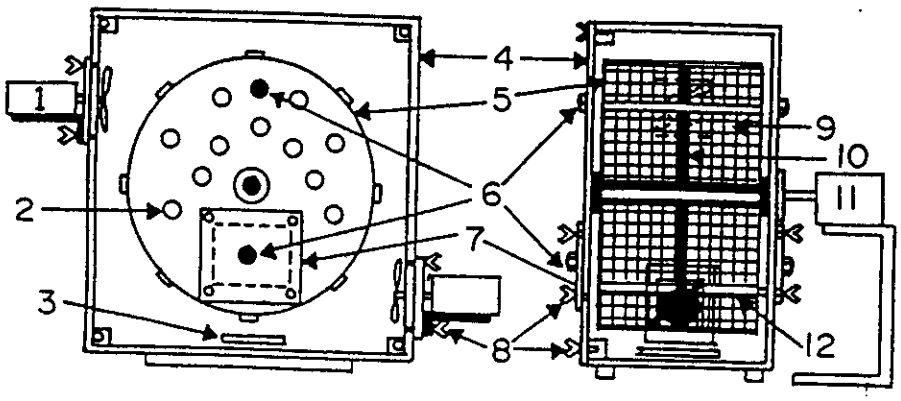

Figure 1. Animal exposure chamber

1. Mixing fan assembly, consisting of Dayton model 2M033 motor, 1/15 hp, 5000 rpm, 120 VAC, 60 Hz, fitted with a 4-bladed, 7-cm dia nylon fan.
2. Ventilation holes, 12-mm dia, cut through center divider of rotating cage.
3. Petri dish, 9-cm dia.
4. Exposure chamber walls constructed of ½-in (12-mm) thick polymethylmethacrylate (PMMA).
5. Rotating cage assembly, center divider and outer rim constructed from ¼-in (6-mm) PMMA.
6. Gas sampling ports sealed with serum vial stoppers.
7. Chamber access port for animal insertion and removal.
8. Thumbscrew fasteners.
9. Polyethylene mesh cover for rotating cage; mesh openings are approximately 7-mm square.
10. Center divider and support for rotating cage, constructed from ¼-inch (6-mm) thick PMMA.
11. Cage drive motor; Dayton model 3M098, 4 rpm, 120 VAC, 60 Hz.
12. Cross supports for chamber rims and plastic mesh perimeter.

CO-acrolein mixed atmospheres were prepared by the same techniques, except that the CO was added during the last 10 min of the acrolein equilibration period. In all cases, pre-exposure concentrations were increased by approximately 7% to allow for the expected concentration loss during rat insertion.

Carbon monoxide analysis. CO was analyzed by gas chromatography, using a Carle model 8000 gas chromatograph (GC) equipped with a thermistor detector. The analytical columns were 1/8" stainless steel, a 3-ft column packed with 40/60-mesh silica gel connected in series to a 6-ft column packed with 40/60-mesh molecular sieve 5A. The carrier gas was helium; column temperature was 86°C. Standards were prepared by making serial dilutions of tank CO with air in a 100-mL gas syringe. Sample injection was accomplished by operating a solenoid-activated gas sampling valve while manually injecting the syringe sample through the sample loop at 55 mL/min.

Chamber atmosphere samples were withdrawn through a septum in the chamber access port (from a point within 2-in of the rat's normal head position) using 60-mL plastic syringes. These aliquots were then manually flushed through the sample loop at the same 55-mL/min flow rate and injected from the loop while maintaining this flow, so that the gas pressure--and thus the concentration--in the loop would match the injection condition of the standards. CO concentrations were calculated from the peak heights of the chamber aliquots using a least-squares regression equation relating peak height and concentration of the gas standards.

Acrolein analysis. Acrolein was also analyzed by gas chromatography, using a Perkin-Elmer Sigma 2000 GC equipped with a flame ionization detector. A tandem-connected quartz capillary column was utilized, consisting of a 25-m by 0.25-mm i.d. section coated with Carbowax 20M connected to a 20-m by 0.32-mm i.d. section coated with methyl silicone. Helium was used as the carrier gas at a linear velocity of 15 cm/sec; oven temperature was 100°C.

Standards for gas chromatographic analysis were prepared by injecting known weights of redistilled acrolein into Saran gas bags containing 1000 mL of dry air, then making syringe dilutions of the "bag" standards after equilibration was complete. In application, a Saran gas bag (with septum) was flushed with room air and evacuated using 3 consecutive fill/evacuate cycles. After the last evacuation, the bag was injected with exactly 1000 mL of tank breathing air with a 1000-mL gas syringe.

Approximately 40 μL of redistilled acrolein (at room temperature) was drawn into a 50-μL Hamilton syringe, the needle was inserted into a small piece of septum to prevent leakage, and the entire assembly was weighed on a microbalance to the nearest 0.01 mg. After injecting the acrolein into the prepared Saran gas bag, the syringe assembly was reweighed and the weight of the injected acrolein was determined by difference. The acrolein

concentration in the bag was calculated at the ambient temperature and barometric pressure, and a standard curve was prepared by injecting syringe dilutions of the "bag" standard into the gas sampling loop of the chromatograph. Unknown acrolein concentrations were calculated from the peak heights of chamber samples using a least-squares regression equation relating peak height and concentration of the gas standards. The accuracy of the GC technique was verified originally by analyzing duplicate standard and test gas samples using a second, independent spectrophotometric technique (3). The differences in results obtained by the two methods never exceeded 5 percent.

The actual retention time for an acrolein sample in this system was about 5.5 min, with a slightly longer time being required for the recorder trace to return to baseline. In a static exposure system such as ours, the gas concentration slowly decreases after animal insertion due to inhalation and to adsorption on the chamber walls, animal fur, and solution in expelled body fluids. We felt that more frequent sampling would be desirable to adequately describe the area under the time-versus-concentration curve for the individual animal exposures. In this simple air-CO-acrolein atmosphere, the flame ionization detector responded only to the acrolein with no injection "artifact." This characteristic enabled us to inject samples at 4-min intervals and still allow the recorder to return to baseline between samples; no difference in peak height was found between samples injected in this manner and identical samples that were allowed to return to baseline before the next injection (about 8 min). Thus, we were able to schedule chamber sampling at 0.5, 3, 7, 11, 15, 19, 23, 27, and 31 min. A vacuum line was attached to the gas sample loop between injections to flush the loop with room air. An obvious caveat to be noted is that this type of sampling must be restricted to this, or similar simple systems, where only a single component produces a response from the selected detector; additional components should be carefully checked for possible interference.

Chamber atmosphere samples were withdrawn through a septum in the chamber access port into a 20-mL gas syringe and immediately transferred to the sample loop of the GC. The sampling syringe was flushed with room air 3 times between samples.

Animal exposure procedure. When the test atmosphere had reached equilibrium, a chamber air sample was withdrawn and analyzed for CO and/or acrolein; the concentration was then adjusted and resampled, if necessary, to reach the planned exposure level. The cage rotation and fan motors were turned off, and a weighed, fasted rat was rapidly inserted through the chamber access port. The port was immediately resealed, the exposure timer was activated, and the cage and fan motors were restarted.

Sampling of the chamber atmosphere for CO began at about 0.5 min and continued at 1 to 3-min intervals. Some staggering of

sampling times was necessary for combined CO-acrolein exposures because the samples for the different analyses were removed from the same sampling port for uniformity.

During the exposure, rat behavior such as eye-blinking, grooming, gasping, hyperactivity, and convulsions were noted and recorded along with the time of observation. Time-to-incapacitation was recorded as the elapsed time between insertion and the time at which the rat could no longer perform the coordinated act of walking in the rotating cage (i.e., when sliding and tumbling began). After t_i, a final chamber sample was removed and analyzed to provide a complete time-concentration record for the exposure period. Cage rotation was then stopped, and the rat was removed and sacrificed by insertion into a closed container of CO.

The acrolein dose/response relationship for these conditions was determined by exposing 31 rats, individually, to selected acrolein concentrations from 580 to 41550 ppm and measuring the t_i while monitoring the acrolein concentration in the chamber atmosphere. The dose/response relationship for CO alone, under the static atmosphere conditions necessitated by the CO-acrolein combined exposures, was determined in a similar fashion for 25 rats exposed to CO concentrations from 1119 to 6524 ppm.

Acrolein and CO concentrations calculated to produce 5-, 10-, and 20-min t_i's from each of the individual gases alone were combined for a series of mixed gas exposures, i.e., 5-min (CO) + 5-min (acrolein), 5-min (CO) + 10-min (acrolein),...20-min (CO) + 20-min (acrolein). In addition, 15 rats were exposed to a 10-min t_i level of CO combined with very low levels (18 to 580 ppm) of acrolein to study the irritant effect at subtoxic levels. Forty rats were exposed, individually, to the CO-acrolein mixtures, using the technique previously described for the single gas exposures. CO concentrations ranged from 1318 to 6152 ppm, and acrolein concentrations ranged from 18 to 14967 ppm.

RESULTS AND DISCUSSION

Data conversions and calculations. For all experiments, the area under the concentration-versus-exposure-time curve, for each gas, was integrated from time = 0 (i.e., at animal insertion) to t_i (incapacitation); division of the concentration*time product by t_i yielded the average gas concentration to which the animal was exposed (i.e., $C = C^*t_i/t_i$).

For each set of single gas exposures, a scatter plot was constructed displaying t_i as a function of the mean exposure concentration for the gas. A smooth curve, fitted to these points, has the shape of a rectangular hyperbola with nonzero asymptotes:

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

An equation of that form was empirically derived for each data set, using a standard nonlinear regression algorithm as a

guideline (12).

For CO, a nonlinear regression on the data set for the 25 individual exposures resulted in values of -714, -1.8, and 46350 for C_o, t_o, and K_o respectively. While adequate to predict points on the concentration-versus-time curve within the delineated concentration limits, the values for C_o and t_o are not realistic representations of their biological parameters where:

C_o is the minimum gas concentration that will cause incapacitation after extended exposure,
t_o is the minimum biological response time (shortest t_i) to an overwhelming concentration of the gas, and
K_o is an expression in ppm*min units of the mean "effective dose" of a toxic gas required to produce the measured effect (incapacitation).

Earlier work by the authors (4) indicated that 225 ppm was a practical estimate for C_o and a 1.1-min estimate for t_o gave a good fit for the higher CO concentrations on the concentration-versus-t_i curve. Using these estimates, nonlinear regression produced a K_o value of 22839. The resulting equation (Eq. 2, below) is plotted over the data points (see Table 1) from which it is derived (see Figure 2).

$$t_i \text{ for CO} = 1.1 + (22839/(CO - 225)) \qquad \text{(Eq. 2)}$$

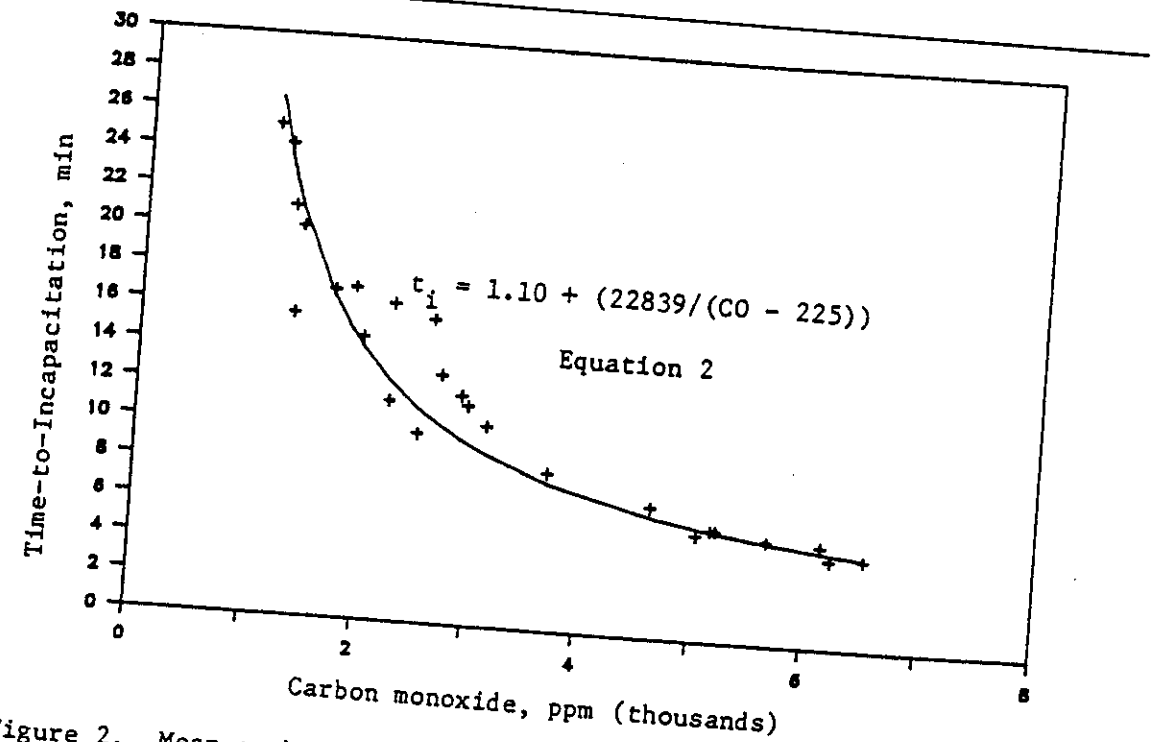

Figure 2. Mean carbon monoxide concentrations vs time-to-incapacitation

TABLE 1

DOSE-RESPONSE DATA FOR CARBON MONOXIDE

t_i, min	(CO), ppm	t_i, min	(CO), ppm
4.6	6229	11.4	2954
4.7	6524	11.9	2893
5.3	6143	12.9	2700
5.4	5666	14.6	1974
5.5	5036	15.6	1345
5.8	5207	15.7	2606
5.8	5161	16.4	2240
6.8	4626	16.9	1696
8.2	3698	17.1	1879
9.8	2511	20.1	1380
10.4	3129	21.1	1297
11.4	2240	24.3	1234
		25.3	1119

For acrolein (Acr), nonlinear regression for the 31 individual exposures produced values of 2.15, 35192, and -595 for t_o, K_o, and C_o respectively. The resulting equation (Eq. 3) is plotted in Figure 3 over the data points from which it is derived; individual values are shown in Table 2 in order of decreasing acrolein concentration.

$$t_i \text{ for acrolein} = 2.15 + (35192/(Acr + 595)) \quad \text{(Eq.3)}$$

Although this equation describes the data, the negative value for C_o does not describe the toxicological minimum "no effect" concentration. As suggested by the authors in the earlier acrolein study (3), the fact that the model equation,

$$t_i = t_o + K_o/(C - C_o)$$

could not be fit to the acrolein data by using a positive value for C_o, may indicate that the form of the equation is inappropriate for describing the toxicokinetics of acrolein.

By relating t_i to the square root of the effective concentration, $C - C_o$, and estimating values for t_o and C_o at 0.1 min and 300 ppm respectively, nonlinear regression resulted in a

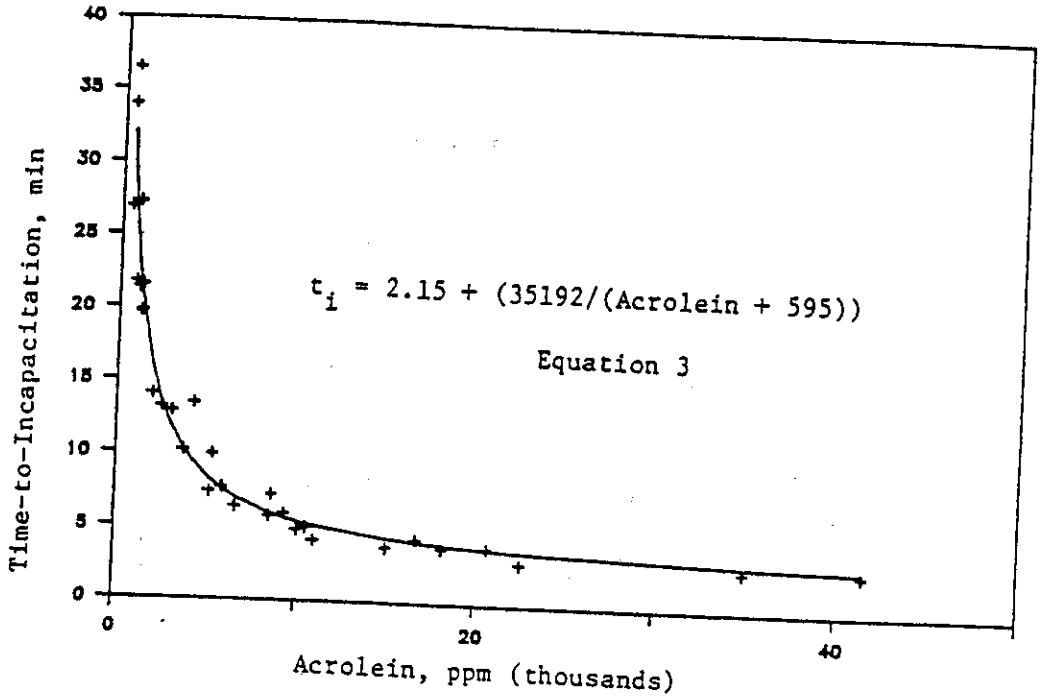

Figure 3. Mean acrolein concentration vs time-to-incapacitation

TABLE 2

DOSE-RESPONSE DATA FOR ACROLEIN

t_i, min	(Acrolein), ppm	t_i, min	(Acrolein), ppm
2.8	41550	7.7	5870
2.8	35010	10.0	5280
2.9	22590	10.2	3686
3.8	18170	12.9	3033
3.8	15060	13.2	2490
3.9	20710	13.5	4240
4.2	10990	14.1	1940
4.4	16720	19.7	1220
4.9	10060	19.8	1260
5.1	10540	21.4	1075
5.8	8505	21.6	1281
6.0	9341	21.8	900
6.4	6595	27.0	580
7.3	8630	27.3	1028
7.4	5150	34.0	580
		36.5	690

value of 589 for K_o with a residual sum of squares (RSSQ) of 190. This represents a slightly poorer "goodness of fit" than the RSSQ value of 145 obtained for Eq. 3. The mathematical relationship is expressed as,

$$t_{i\ \text{acrolein}} = 0.1 + (589/(\text{Acr} - 300)^{0.5}) \qquad \text{(Eq. 3a)}$$

and the graphical relationship of Eq. 3a to its parent data is depicted in Fig. 4. Reasonable predictions of response time for specific acrolein concentrations can be made from either Eq. 3 or 3a within the concentration limits from which they are derived.

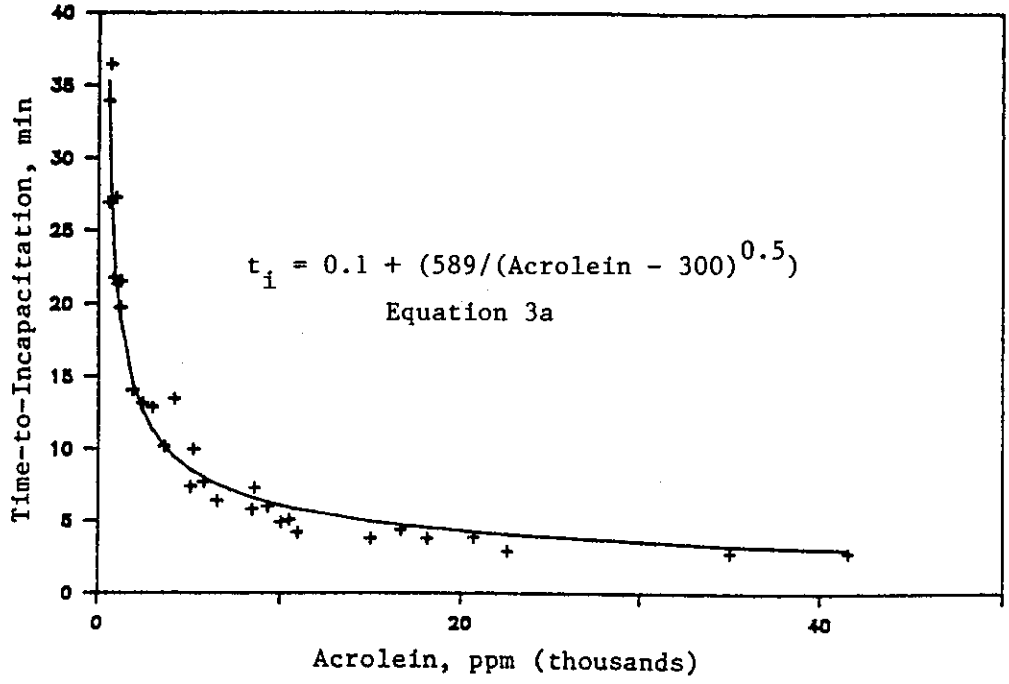

Figure 4. Mean acrolein concentration vs time-to-incapacitation

Times-to-incapacitation resulting from 25 individual rat exposures to defined mixtures of CO and acrolein are listed in Table 3 with the average concentrations for each gas to which the rat was exposed. The average concentrations were calculated as for the single gas exposure, i.e., average concentration = $(\int C\ dt)/t_i$. Two techniques were utilized to determine whether or not the toxic effects of the two gases were additive. First, for an additive effect, the reciprocal of the observed t_i for the

combined gas exposures should equal the sum of the reciprocals for the calculated t_i's for each gas.

$$1/t_{i(CO)} + 1/t_{i(acrolein)} = 1/t_{i(observed)} \qquad \text{(Eq. 4)}$$

TABLE 3

DOSE-RESPONSE DATA FOR MIXTURES OF CO AND ACROLEIN

T_i (min)	(CO), ppm	(Acrolein), ppm
4.4	3202	14967
4.7	3052	13421
5.5	1318	11310
5.8	1464	12445
6.0	5518	11283
6.7	5843	5028
6.7	6050	12416
6.8	6152	5297
7.0	2877	12344
7.4	3161	4476
8.3	5573	4761
9.1	3535	4823
9.4	3683	4922
9.7	5834	2136
10.6	1547	5196
11.0	3354	4282
11.1	2998	4174
11.4	1474	4897
12.6	5421	1830
15.0	3227	1281
17.2	3010	960
18.0	3070	1839
18.5	1321	1213
20.3	1480	1476
28.1	1463	1382

The second concept is that of the fractional effective dose. When equations 2 and 3 are rearranged as equation 1, the individual effective doses (K_o) for CO and acrolein are 22839 and 35192 (ppm*min) respectively. From the observed t_i's and calculated mean gas concentrations (C) for rats exposed to the gas mixtures, we calculated the fraction of this "effective dose" that each animal inhaled at t_i. Thus the fractional effective

dose (FED) of each gas inhaled from the mixture was equal to

$$FED = [(C - C_o)*(t_i - t_o)]/K_o \qquad \text{(Eq. 5)}$$

where C is the mean concentration for the individual gas in the mixture and t_i is the observed time-to-incapacitation. A list of the calculated values for FED(CO), FED(acrolein), and the sum of the FED's for each exposure is presented in Table 4.

TABLE 4

FRACTIONAL EFFECTIVE DOSES FROM EXPOSURES TO CO-ACROLEIN MIXTURES*

FED(CO)	FED(ACROLEIN)	FED(SUM)
0.430	0.995	1.425
0.446	1.016	1.462
0.211	1.133	1.344
0.255	1.352	1.607
1.136	1.299	2.435
1.377	0.727	2.104
1.428	1.682	3.110
1.479	0.779	2.258
0.685	1.783	2.468
0.810	0.756	1.566
1.686	0.936	2.622
1.159	1.070	2.229
1.257	1.137	2.394
2.112	0.586	2.698
0.550	1.390	1.940
1.356	1.226	2.582
1.214	1.213	2.427
0.563	1.444	2.007
2.616	0.720	3.336
1.827	0.685	2.512
1.963	0.665	2.628
2.105	1.096	3.201
0.835	0.840	1.675
1.055	1.068	2.123
1.464	1.458	2.922

*See text for definitions of Fractional Effective Doses.

From the average gas concentrations in the mixtures, we calculated the predicted t_i that would result if the animal were exposed to that concentration of each gas alone, using equations 2 and 3. These predicted t_i's are compared to the observed t_i for each CO-acrolein exposure in Table 5.

TABLE 5

PREDICTED AND OBSERVED TIMES-TO-INCAPACITATION

Time-to-Incapacitation, minutes			
Observed t_i	Predicted from [CO] (Eq. 2)	Predicted from [Acrolein] (Eq. 3)	Predicted from Combined Gases (Eq. page 20)
4.4	8.8	4.4	5.2
4.7	9.2	4.7	5.4
5.5	22.0	5.1	6.4
5.8	19.5	4.8	6.1
6.0	5.4	5.1	5.3
6.7	5.2	8.4	7.5
6.7	5.0	4.9	5.0
6.8	5.0	8.1	7.3
7.0	9.7	4.9	5.7
7.4	8.9	9.1	9.3
8.3	5.4	8.7	7.8
9.1	8.0	8.6	8.7
9.4	7.7	8.5	8.6
9.7	5.2	15.0	10.7
10.6	18.4	8.2	9.8
11.0	8.4	9.4	9.4
11.1	9.3	9.5	9.8
11.4	19.4	8.6	10.2
12.6	5.5	16.7	11.6
15.0	8.7	20.9	16.2
17.2	9.3	24.8	18.3
18.0	9.1	16.7	14.3
18.5	21.9	21.6	22.4
20.3	19.3	19.1	19.8
28.1	19.5	19.6	20.5

Logically, since the effective dose for a specific gas is equal to K_o (Eq.1), and the fraction of that effective dose inhaled from the mixture at t_i is described in Eq. 5, the sum of the fractional effective doses for each of the gases in the mixture should equal 1 if the combined toxic actions are exactly additive. For a synergistic effect, the sum of the individual FED's would, therefore, be less than 1. For an FED sum significantly greater than 1, the possibility exists for an antagonistic effect (i.e., that the presence of one gas decreases the toxic effect of the other). Another possibility is that the combined effects are "less than additive" because of differing rates of action for each of the gases at the sub-incapacitation dose levels attained at the observed incapacitation. A third possibility is simply that the toxic mechanisms for each gas are so different that each acts independently on the test animal and no interaction occurs.

The logic for Eq. 4 is based on the following relationships:

(a) Dose is proportional to the toxic gas concentration;
(b) Response time is inversely proportional to gas concentration;
(c) Therefore, response time (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 indicates that for all of the 25 combined exposures, sums of the paired FED's are all considerably greater than unity (mean FED sum = 2.28, std. dev. = 0.56, 95% confidence intervals on the mean = 2.05, 2.51). This would suggest that the combined effects are much less than additive.

Table 5 indicates that for all but two of the combined exposures, the observed t_i's were greater than or equal to the shorter of the predicted t_i's for the individual gases. When rats were exposed to concentrations of CO and acrolein selected to produce equivalent t_i's, the observed incapacitation time was not statistically different from the predicted t_i's for the individual gases at the $P_{.05}$ level. For example, the mean predicted t_i's for 5 such exposures produced the following values.

$$\text{Predicted } t_{i(CO)} = 8.5 \text{ min (s.d.=0.65)}$$
$$\text{Predicted } t_{i(\text{acrolein})} = 9.0 \text{ min (s.d.=0.45)}$$
$$\text{Observed } t_{i(CO + \text{acrolein})} = 9.6 \text{ min (s.d.=1.53)}$$

When t_i(CO) was shorter than t_i(acrolein), the observed t_i was longer than t_i(CO). Conversely, when t_i(CO) was longer than t_i(acrolein), the observed t_i was only slightly longer than the predicted t_i for acrolein alone. This could be interpreted as indicating that acrolein exerts a "protective" effect when it is present in mixtures at levels that would produce, by itself, a

longer t_i than the CO. However, when acrolein was present in a concentration that would produce a shorter t_i than the CO, the observed t_i was governed by the acrolein concentration, with little effect from the CO. A summary of these comparisons is presented in Table 6.

TABLE 6

PAIRED STUDIES -- COMPARISON OF PREDICTED TIMES-TO-INCAPACITATION (t_i's) FOR INDIVIDUAL GASES WITH OBSERVED t_i's FOR THE CARBON MONOXIDE-ACROLEIN MIXTURES

	*Predicted t_i's (min)		Observed t_i's for
	Acrolein	Carbon Monoxide	Combined Gas Mixture
EQUAL TOXICITY PAIRS	5.1	5.4	6.0
	4.9	5.0	6.7
	9.1	8.9	7.4
	8.6	8.0	9.1
	8.5	7.7	9.4
	9.4	8.4	11.0
	9.5	9.3	11.1
	21.6	21.9	18.5
	19.1	19.3	20.3
	19.6	19.5	28.1
UNEQUAL TOXICITY PAIRS	8.4	5.2	6.7
	8.1	5.0	6.8
	8.7	5.4	8.3
	8.6	19.4	11.4
	8.2	18.4	10.6
	4.4	8.8	4.4
	4.7	9.2	4.7
	4.9	9.7	7.0
	5.1	22.0	5.5
	4.8	19.5	5.8
	15.0	5.2	9.7
	16.7	5.5	12.6
	20.9	8.7	15.0
	24.8	9.3	17.2
	16.7	9.1	18.0

*Predicted t_i's for acrolein and CO were calculated from the concentrations of each gas in the combined exposures, using equations 3 (for acrolein) and 2 (for CO) respectively.

When the predicted t_i's were calculated by Eq. 4, the sum of the reciprocal response times, all of the predicted t_i's were shorter than the observed values. Linear regression of observed on predicted t_i's gave the equation;

$$t_{i(observed)} = -0.25 + 2.26*t_{i(predicted)}$$

with a correlation coefficient of 0.89.

At this stage, we attempted to identify the relative magnitudes of the toxicities for the two gases in the combined gas atmospheres. Although the sum of the individual FED's was always greater than unity, we reasoned that since incapacitation was achieved, that we might assign a weighting factor to each FED and set up a new sum equal to 1.

$$a*FED_{(CO)} + b*FED_{(acrolein)} = 1 \qquad \text{(Eq. 6)}$$

Rearranging equation 6 into the form of a straight line (y = a + bx), we obtained

$$FED_{(acrolein)} = 1/b - (a/b)*FED_{(CO)} \qquad \text{(Eq. 6a)}$$

By performing a least-squares, linear regression analysis on the data arranged as in equation 6a, we determined best estimates for the coefficients a and b of 0.17 and 0.74 respectively.

$$0.17*FED_{(CO)} + 0.74*FED_{(acrolein)} = 1 \qquad \text{(Eq.6b)}$$

The same logic was applied to forcing the sum of the reciprocal t_i's to equal the reciprocal of the observed t_i (Eq. 4) by assigning a weighting coefficient to each of the individual predicted t_i reciprocals. The modified equation was:

$$a*1/t_{i(CO)} + b*1/t_{i(acrolein)} = 1/t_{i(observed)} \qquad \text{(Eq. 7)}$$

A multiple linear regression was performed on the data using the algorithm that forces the constant term (or intercept) to equal zero. The least-squares estimates for the coefficients were 0.23 and 0.74 for a and b respectively.

$$0.23*1/t_{i(CO)} + 0.74*1/t_{i(acrolein)} = 1*1/t_{i(observed)} \qquad \text{(Eq. 7a)}$$

The calculated coefficients in equations 6b and 7a suggest that acrolein is about three-fourths as effective in the mixture as when administered alone, while CO contributes only about one-fifth of its normal potency.

A final effort to analyze the relative contributions of each gas involved examining the 10 specific exposures in which both gases were present in essentially equipotent concentrations. In each case, the concentrations for both gases were those which would produce 5-min, 9-min, and 20-min t_i's from each gas alone. Where the FED should have been 0.5 for each gas for a classical additive effect, the mean FED's for each were 1.17 with an FED sum of 2.34. This was not particularly surprising because the mean ratios of the observed t_i's divided by the t_i for each gas in the mixture were 1.15 and 1.12 for CO and for acrolein respectively. More simply, the observed t_i was always slightly greater than the predicted t_i for <u>either</u> gas alone. Although observed in all 10 cases, this slight difference between observed t_i and the predicted individual gas t_i's was not statistically significant at the $P_{.05}$ level using Student's t test.

Selection of combined gas concentrations for the preceding comparisons was limited to the concentration ranges used in defining the original dose-response curves for the individual gases, i.e., 1119-6524 ppm for CO and 580-41550 ppm for acrolein. However, one suggested mechanism for the decrease in apparent toxicity of CO in the presence of even small amounts of acrolein is that the acrolein irritates the mucous membranes, thereby making breathing painful and resulting in a slower breathing rate and a correspondingly reduced minute respiratory volume. The end effect is that it takes longer for the test animal to inhale his incapacitating dose of CO due to the irritant effect.

To examine this mechanism, we exposed 15 rats to a nominally fixed concentration of CO (3280 ppm, s.d.=180, c.v.=5.5%) plus acrolein in concentrations from 18 to 543 ppm. Since these acrolein concentrations were less than those used to define the individual gas effects, we could not relate their effects on CO toxicity on the basis of a calculated t_i or FED for the acrolein fraction. Therefore, we plotted the ratios of the observed t_i's to the calculated t_i's for the CO alone (since the CO concentration <u>was</u> within the limits of our dose-response curve) against the mean acrolein concentrations for each of the 15 low-acrolein exposures. This plot is shown in Figure 5.

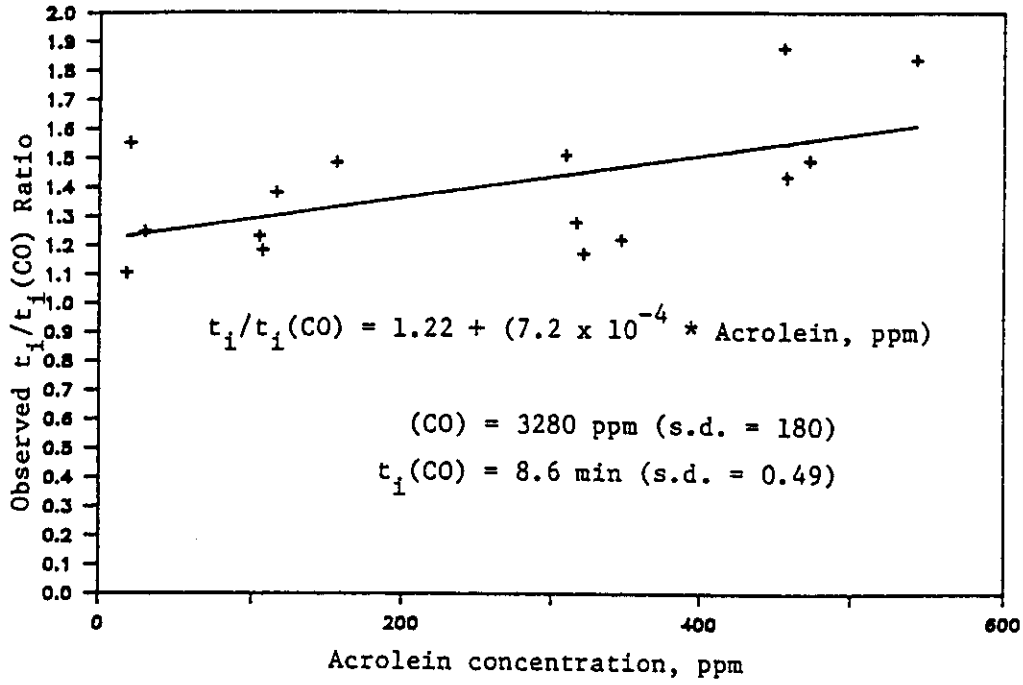

Figure 5. Observed t_i/t_i(CO) at low acrolein concentrations

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The least-squares regression indicates a slow, somewhat erratic increase in the observed t_i/t_i(CO) ratio with increasing acrolein concentration. Perhaps more significant is the increase above the mean t_i(CO) for the series (8.6 min, s.d.=0.49, c.v.=5.7%) at the lowest acrolein concentrations (y-intercept is 1.2*t_i(CO). It would appear that <u>low</u> levels of acrolein effectively increased the t_i above that normally expected from the CO concentration.

SUMMARY AND CONCLUSIONS

Laboratory rats were exposed individually to selected atmospheric concentrations of CO in air, acrolein in air, and mixtures of CO and acrolein in air. The exposure time required to produce physical incapacitation in each test animal was measured for each experimental condition.

The t_i's for exposures to the individual gas concentrations and equations describing those relationships were derived by nonlinear regression techniques. The resulting equations are:

$$t_i = 1.10 + (22839/(C - 225)) \text{ for CO, and}$$
$$t_i = 2.15 + (35192/(C + 595)) \text{ for acrolein,}$$

where t_i is in minutes and C is the toxic gas concentration in parts-per-million by volume.

The response times obtained from exposures to mixtures of CO and acrolein were analyzed with respect to the predicted response time for each gas acting alone at its concentration in the mixture. In 23 of 25 exposures to CO-acrolein mixtures, the observed t_i was equal to or greater than the shorter of the predicted t_i's for either gas in the mixture. Thus our analytical data indicates that the toxic potency of a mixture of CO and acrolein -- within the concentration ranges used -- was less than or equal to that which can be ascribed to either gas by itself.

The data do not support the hypothesis of synergism, since the sums of the paired FED's are all greater than unity. An argument for an antagonistic effect is supported by the increase in the observed t_i over that predicted for CO in cases where a "less than equipotent" concentration of acrolein is present. An argument can also be made for "no interaction" on the basis of the little or no difference between observed t_i and the predicted t_i for either CO or acrolein when they are present in equipotent concentrations.

Although the mechanism is not defined by this limited investigation, we conclude that evidence does exist for an "antagonistic-like" effect when acrolein is present in concentrations less toxic (based on individual gas toxicity) than the CO in the gas mixture, a condition that seems likely to exist in most fire conditions. The effect is probably not antagonistic in the sense that acrolein decreases the specific toxicity of CO, but that it increases the time required to inhale an effective dose of CO by decreasing the animal's minute-respiratory volume (MRV). Whether this decrease in MRV is a voluntary reaction or an involuntary neural reflex is not known. For concentrations within the ranges used in this study, we offer the following multiple linear regression equation, which may be useful for estimating time-to-incapacitation for rats exposed to atmospheric mixtures of CO and acrolein.

$$t_i = \left(\frac{0.23}{1.10+(22839/([CO]-225))} + \frac{0.74}{2.15+(35191/([Acr]+595))} \right)^{-1}$$

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