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16. Abstract

Acrolein, an organic aldehyde (CH_2=CH-CHO), is extremely irritating to the respiratory passages at very low concentrations. It is known to be present in the smoke from certain materials used in aircraft cabin interiors and could contribute, therefore, to an individual's failure to escape from a burning aircraft. In order to assess acrolein's ability to produce physical incapacitation in a mammal, laboratory rats were exposed continuously to measured atmospheric concentrations of acrolein vapor until they expired. The exposure time required to produce lethality was measured, as was the time at which physical incapacitation occurred. Incapacitation was defined operationally as loss of the ability to walk in a motor-driven wheel, which was enclosed in the exposure chamber. Dose-repsonse curves were generated by equating these two endpoints, time-to-incapacitation and time-to-death, to the atmospheric acrolein concentration via statistically derived regression equations. Experimental results suggest that the acrolein dose that will produce physical incapacitation could be 10 to 100 times greater than has been predicted in the past. The possible relationship between the effective toxic doses of acrolein for rats, and those reported for humans, is discussed.

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INHALATION TOXICOLOGY: VII. TIMES TO INCAPACITATION AND DEATH FOR RATS EXPOSED CONTINUOUSLY TO ATMOSPHERIC ACROLEIN VAPOR

INTRODUCTION

Acrolein, an unsaturated aliphatic aldehyde (CH_2=CH-CHO), is a known product of the thermal decomposition of certain materials used in aircraft cabin interiors, and a suspected component of the combustion products of others"'. Since lachrymation and the irritation of exposed mucous membranes have been reported for humans exposed to acrolein concentrations of less than 1 part per million (ppm), the presence of even small quantities of this compound in smoke from an inflight. or postcrash fire could be postulated to at. least impede passenger escape[21].

In a 1978 study conducted by the Harvard School of Public Health, and reported by Burgess[4], acrolein was identified in 91 percent of the 120 building fires that were monitored, and reached concentrations *98* times greater than the "tolerable" level in over half of these. Jacobs[12] reported. that acrolein was used as a war gas in World War I. Cases of accidental poisoning have also been reported[14], but the possibility that more than one toxic gas was involved has precluded the estimation of time-concentration effects from such data.

Inhalation studies have been conducted with several animal species. Early studies were Lewin's[15] turn-of-the-century experiments with rabbits and guinea pigs, and Iwanoff's[11] cat studies in 1910. More recent work has utilized rats, mice, rabbits, and guinea pigs. Skog[22] exposed rats for 3:)-minute periods to acrolein concentrations of 0.1 to 0.7 mg/L (45 to 310 ppm) establishing a 30-min LC_{50} of 0.3 mg/L (135 ppm). Salem and Cullumbine[17] exposed mice, guinea pigs, and rabbits to acrolein concentrations of 5,225 mg/m³ (2,330 ppm) and found the lethal doses ranged from 70,000 to 140,000 mg·min/m³ (31,250 to *62*,500 ppm·min). For the same three species, Pattle and Cullumbine[16] reported the LC_{50} to be 24.4 mg/m³ (10.5 ppm) for a 6-hour exposure. Other workers[17,1] have explored the biochemical effects on, and the adaptation of, rats exposed to extremely low levels of acrolein.

Our primary interest was in acrolein .levels that cause irritation sufficient to incapacitate within 30 min and that could be potentially lethal for persons attempting to escape from a fire situation. Our earlier work[6] with carbon monoxide (CO) and hydrogen cyanide (HCN) has shown that time-to-incapacitation (t_i), as measured with rats in a rotating cage assembly, is a useful tool for comparing the relative toxicity of combustion

gases from aircraft interior materials. A similar system was used in this study to establish t_1 as well as time-to-death (t_d) for the laboratory rat subjected to preestablished concentrations of acrolein in air. These concentrations ranged from 530 to over 40,000 ppm.

MATERIALS AND METHODS

Animals. 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 and were held in isolation for 8 days prior to use. All 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.

Rats were fasted avernight before testing in order to establish equivalent metabolic states; just before use, each animal was weighed and marked with an identifying color code.

Exposure chamber design. The animal exposure chamber utilized in this study differs somewhat from that used in previous studies; its design is detailed in Figure 1. It was constructed of 1/2-in 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 assembly, 40.6 cm in diameter by 25.0 cm wide, had a plastic mesh floor (perimeter) with a perforated internal divider. The cage was suspended across the width of the chamber by a central axle attached to the perforated divider; the outer chamber walls function as the end walls of the cage. A door (10 by 10 cm), equipped with gaskets, was installed in each side of the chamber at the level of the rotating cage floor, to allow rapid insertion or removal of test animals. The rotating cage was driven by a 4-rpm geared motor (Dayton model 3M098) providing a linear (circumferential) velocity of 8.5 cm/s. Two plastic-bladed fans mounted on opposite sides of the chamber provided uniform mixing of the chamber atmosphere.

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 amount of freshly redistilled 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 turned on to hasten evaporation and atmospheric mixing; equilibration was considered to be complete when all liquid acrolein had evaporated, a period of 15 to 45 min depending on the quantity added. A sample of this preexposure atmosphere was then taken for analysis.

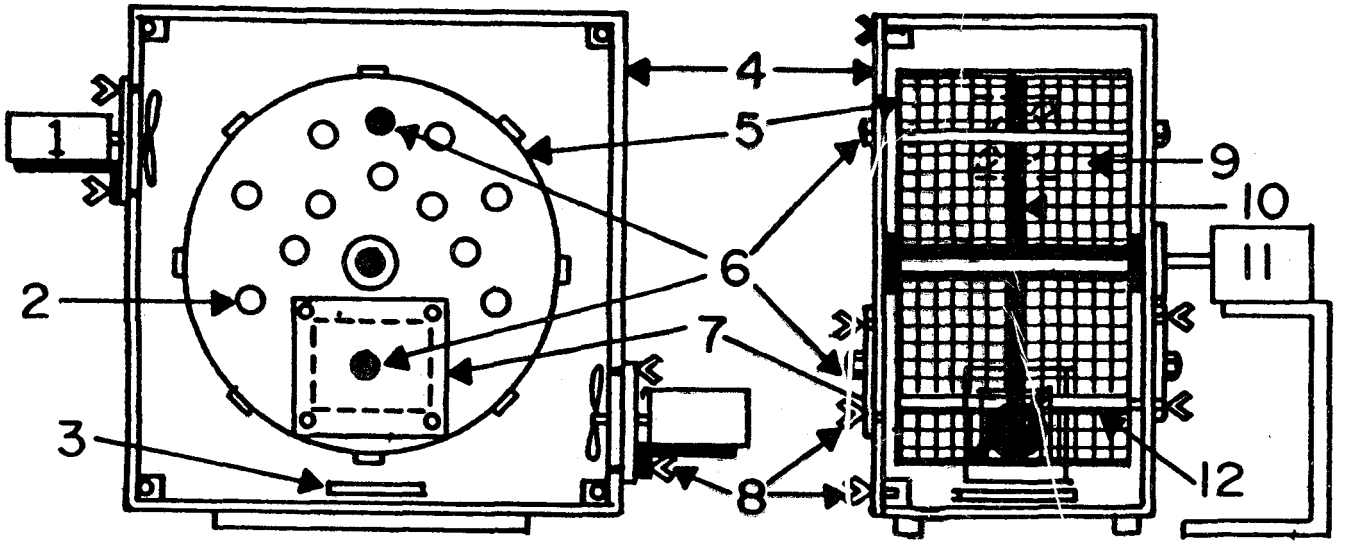

Figure 1. Animal exposure chamber.

1. Mixing fan assembly, consisting of Dayton model 2M033 motor, 1/15 hp, 5,000 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-in (6-mm) thick PMMA.
11. Cage drive motor; Dayton model 3M093, 4 rpm, 120 VAC, 60 Hz.
12. Cross supports for chamber rims and plastic mesh perimeter.

Acrolein analysis. The routine measurement of acrolein concentration in the chamber was accomplished gas chromatographically (GC). The accuracy of this GC technique was verified originally, and rechecked periodically, by analysis of duplicate standard and test gas samples using a second, independent spectrophotometric analytical technique. The differences in results obtained by the two methods never exceeded 5 percent.

The GC method utilized a 12-ft by 1/8-in column of Porapak Q, a nitrogen gas carrier flow of 20 mL/min, an oven temperature of 210 °C, and a hydrogen flame ionization detector. The retention time for acrolein under these conditions was 4.95 min.

Samples of chamber atmosphere were taken for this analysis in a glass syringe and used immediately to flush and fill a 200-microliter (μL) gas sample loop on the GC. The areas of the resulting acrolein peaks were measured with an electronic integrator and converted to equivalent parts-per-million in the sampled atmosphere by means of a standard curve.

The acrolein standard curve, consisting of three to five concentration points, was constructed daily using the following procedure. A 30- to 50-μL aliquot of freshly redistilled acrolein was drawn into a Hamilton GC syringe and the needle tip inserted into a small cork disk to prevent evaporation. The corked syringe was weighed to the nearest 0.01 mg, the cork:: disk removed, and the acrolein sample immediately injected into a plastic bag (saran film) that had been previously evacuated and filled with a known volume of air. The syringe-cork assembly was then reweighed, and the quantity of acrolein that had been added to the bag was calculated by difference, the concentration being expressed as ppm (volume/volume) at ambient temperature and pressure.

A separate gas sample bag was prepared by this procedure for each point on the standard curve. Each bag was then kneaded gently to mix the contents, and a syringe sample was removed and used to fill the 200-μL GC gas sample loop. The areas of the resulting acrolein peaks were quantified by an electronic integrator and plotted against concentration. The equation for the resulting standard curve was calculated by a linear least-squares regression, and the concentrations of unknown samples were then calculated using that equation.

The procedure that was used to confirm the performance of the GC method is based on the quantitative conversion of acrolein to its 2,4-dinitrophenylhydrazone (2,4-DNP) followed by spectrophotometric assay of this highly colored derivative". One problem associated with the quantitative Conversion of trace amounts (micromolar) of low molecular weight aldehydes to their corresponding 2,4-DNP derivatives is that the solubility of the 2,4-DNP in an aqueous medium is sufficient to prevent the reaction

from proceeding to completion. To force the reaction to completion, we employed a modification of the procedure suggested by Selim[20], which utilized a two-phase reaction medium in which the derivative is continuously extracted into a nonaqueous (isooctane) layer. The details of this analytical procedure are presented in the Appendix.

The concentration of acrolein in the chamber atmosphere was plotted as a function of exposure time for each experiment. The areas under this curve, from time=0 to t_i and to t_d, were integrated to obtain C·t products (concentration X exposure time) for both incapacitation and death, $C \cdot t_i$ and $C \cdot t_d$. An average concentration for each exposure was then calculated as the quotient of C·t divided by the appropriate response time; e.g., the average concentration for incapacitation equals $C \cdot t_i$ divided by t_i.

Animal exposure procedure. Once the test atmosphere had reached equilibrium, a chamber air sample was collected and analyzed gas chromatographically for acrolein concentration. The cage rotation and fan motors were turned off, and a fasted, weighed rat was inserted into the cage. The chamber door was immediately sealed, the exposure timer activated, and the cage and fan motors restarted.

After an elapsed time of 1 min, a second chamber air sample was withdrawn for GC analysis to determine the new equilibrium concentration. Additional samples were collected for analysis at 9- to 10-min intervals throughout the exposure, this being the time required for all major peaks from the previous injection to clear the EC column.

Time-to-incapacitation was recorded as the elapsed time at which the rat could no longer perform the coordinated act of walking in the rotating cage--the animal would begin stumbling, sliding, or tumbling. Cage rotation was stopped at t_i, and the rat was observed until visible signs of breathing ceased; this time interval (from initiation of exposure) was called t_d. A final chamber atmosphere sample was collected following the rat's death to provide a complete time-concentration record for the exposure period.

RESULTS AND DISCUSSION

A total of 22 rats were exposed, one at a time, to selected concentrations of acrolein in air. The observed response times, t_i and t_d, are listed in Table 1 along with the Ct-product calculated for each response time, and the average concentration calculated as the area under the Ct-curve divided by the response time.

Visually observed behavioral responses, in addition to t_i and t_d, can be summarized best as general observations made at different acrolein levels.

At concentrations between 500 and 750 ppm, the rats exhibited immediate agitation and rubbing of the nose (with forepaws) when inserted into the chamber, followed by normal walking in the rotating cage. Gasping was noted at about 15 min, and abnormal walking (crawling, sliding, etc.) began at about 30 min. The t_i's ranged from 27 to 36 min. Convulsions preceded death by 1 to 2 min; no cyanosis of the extremities was observed,

At concentrations around 1,000 ppm, gasping began at 3 to 6 min, becoming severe by 6 to 11 min. Walking appeared normal for the first 6 to 10 min, after which there would be alternating periods of crawling and walking--this crawling was a sufficiently coordinated type of locomotion that it was not scored as incapacitation. The t_i's occurred in the range 14 to 20 min, and severe convulsions began about 1 min before death.

At the 10,000-ppm level, gasping began by 1.5 to 2.5 min. Between 4 and 5 min the animals became hyperactive (running, climbing, frantic grooming, etc.) and occasional tremors or mild convulsions were noted during this period of heightened activity. One or more severe convulsions followed soon after the 5- to 6-min t_i, with cyanosis occurring in a few animals in the interval between t_i and t_d. Death occurred at 7 to 10 min.

In the 20,000-ppm range, the rats became hyperactive immediately on being placed in the chamber; they were gasping by 1.5 min and cyanotic by 2.0 to 2.5 min. Muscle tremors either preceded or coincided with t_i, which occurred at 3 to 4 minutes. Following incapacitation the cyanosis became more pronounced, muscular fasciculation became generalized, and sporadic whole-body convulsions preceded death, which occurred at 6 to 7 min.

At the highest concentrations, 35,000 to 40,000 ppm, gasping was severe by 1 to 2 min, and even an occasional short convulsion, with recovery, was noted prior to incapacitation. Although t_i, 2.8 min, was shorter than for the 20,000-ppm exposures, behavior was not appreciably different. Cyanosis and fairly continuous convulsive activity occurred from t_i until death--which was at 4.7 to 4.9 min.

TABLE 1

RESFONSE TIMES AND ACROLEIN CONCENTRATIONS*

t_i, min	$C \cdot t_i$, ppm·min	Acrolein Concn., ppt	t_d, min	$C \cdot t_d$, ppm·min	Acrolein Concn., ppt
2.8	116.35	41.55	4.7	194.71	41.43
2.8	98.03	35.01	4.9	170.73	34.84
2.9	65.52	22.59	5.8	130.43	22.49
3.8	69.06	18.17	6.0	108.50	18.08
3.8	57.24	15.06	8.6	128.01	14.89
3.9	80.77	20.71	7.3	150.07	20.56
4.2	46.16	10.99	9.4	102.29	10.88
4.4	73.59	16.72	7.5	124.62	16.62
4.9	49.31	10.06	9.2	91.66	9.96
5.1	53.75	10.54	6.7	70.14	10.47
7.3	63.03	8.63	10.3	88.40	8.58
7.4	38.11	5.15	9.3	47.78	5.14
7.7	45.19	5.87	13.9	80.71	5.81
10.0	52.82	5.28	20.2	105.24	5.21
13.2	32.88	2.49	24.5	57.96	2.37
13.5	57.20	4.24	15.6	65.78	4.22
14.1	27.38	1.94	18.0	34.63	1.92
19.7	24.02	1.22	30.8	36.11	1.17
19.8	24.91	1.26	28.6	34.75	1.22
27.0	15.71	0.58	36.4	20.16	0.55
34.0	19.67	0.58	50.4	26.48	0.53
36.5	25.01	0.69	56.2	36.67	0.65

*Concentrations and C·t-products are in parts per thousand, ppt.

Along with the expected decreases in t_i and t_d that were observed with exposure to increasing concentrations of acrolein, certain other response patterns became apparent. At concentrations of 1,000 ppm or less, convulsions were noted only just prior to death, while at higher concentrations, tremor and convulsions appeared before incapacitation. A surface muscle tremor or fasciculation, which differed from the whole-body convulsions, was noted at the 10,000-ppm level following periods of extreme physical activity and was a common observation at the higher concentrations, independent of physical exertion. One unexpected result was the absence of any evidence of severe eye irritation. While "grooming" motions about the nose were common on all experiments, only at the 20,000-ppm level did any abnormally rapid eye blinking occur, and then only in a single experimental animal.

All rats' eyes remained open during the exposure periods, and we observed none of the profuse lachrymation that we have noted previously in rats exposed to other irritant gases, such as hydrogen chloride, or smoke from halogen-containing polymers.

Figure 2 is a combination scattergraph of the two measured response times, t_1 and t_d as a function of act-olein concentration. In this and all subsequent figures? response times are in minutes and acrolein cancentrations are in parts per thousand (ppt). The relationship between time-to-effectand magnitude of the noxious agent is, once again, the familiar rectangular hyperbola that. we have obtained for all toxic gases studied to date and also for the measure of time-to-collapse as the result of exposure to hyperthermic atmospheres.

An equation was derived, for each response, that relates time-to-effect and concentration; this curve fitting was accomplished with the aid of a nonlinear least squares regression algorithm proposed by Marquardt[16]. When the model equation for the regression was of the form,

$$(y-a)(x-b)=k, \qquad \text{Eq. 1}$$

where y represents response time and x represents acrolein concentration, the resulting curves fit the data acceptably well, as can be seen in Figures 3 and 4. The log-log, linearized versions of these same equations are presented as Figures 5 and 6. For these two equations, the values for the parameters, a, k, and b, are as follows,

for incapacitation: a = 1.50 k = 40 b = -0.5,
for lethality: a = 3.75 k = 50 b = -0.5.

Although these equations are acceptably descriptive of the experimental data and obviously useful in predicting the continuum of response-time versus concentration relationships, and despite the fact that the form of the equation is the toxicologically meaningful one of a rectangular hyperbola with nonzero asymptotes, the negative sign of the derived values for the parameter, b, does present a modeling problem.

Equation 1 can be rewritten as follows,

$$t_r = a + k/(C-b), \qquad \text{Eq. 2}$$

where t_r is response time in minutes, and C is acrolein concentration in ppt. In the biophysical interpretation of this equation model, the parameters, a and b, represent the asymptotic values for the two ends of the hyperbola; i.e., "a" is the shortest possible response time that can be achieved from exposure to an overwhelming concentration , and "b" is the lowest acrolein concentration that can incapacitate (or kill) as the result of an

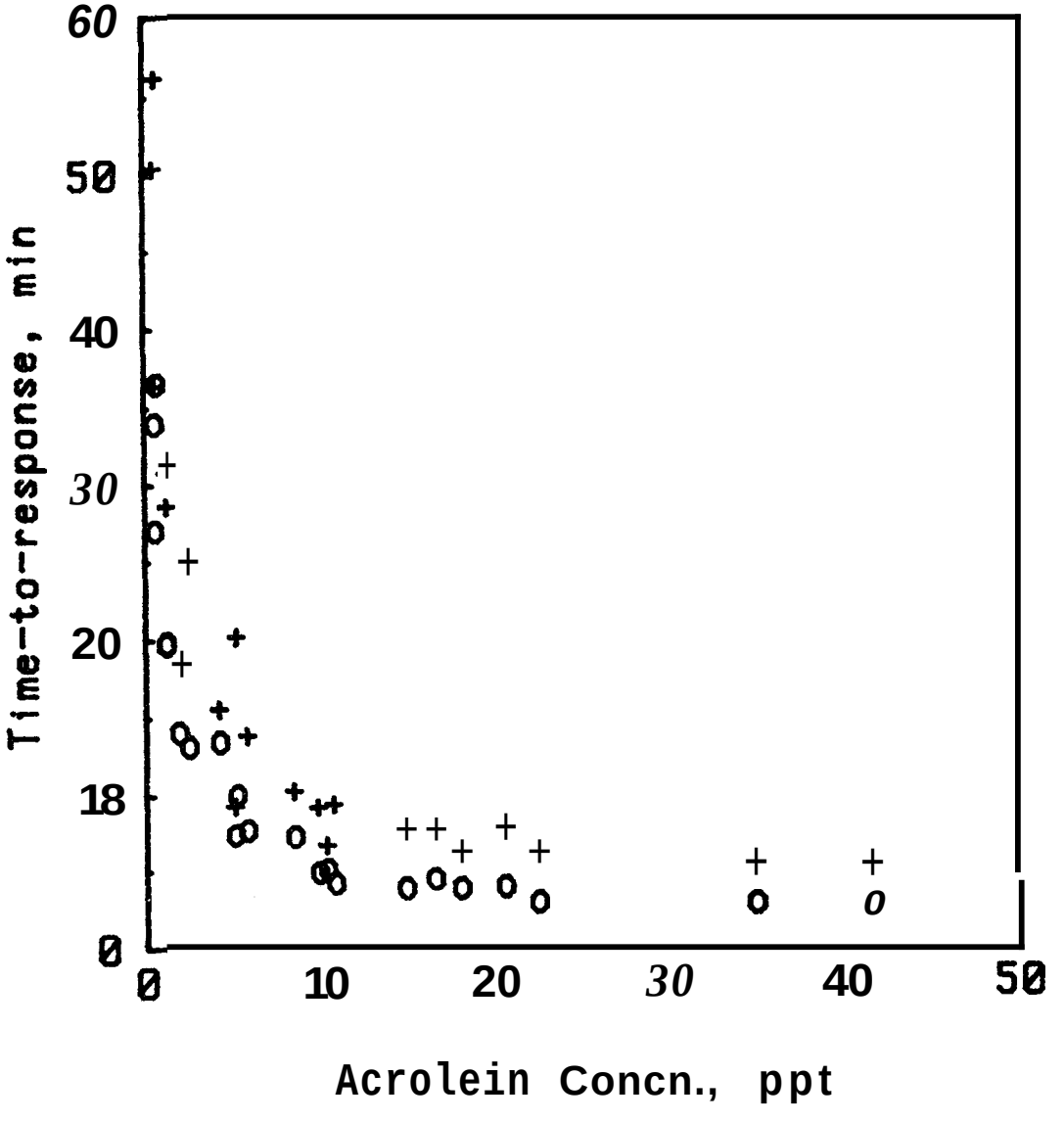

Figure 2. Times-to-incapacitation (O) and times-to-death (+) plotted against the average chamber concentration of acrolein. Each point represents one rat, exposed individually; N=22.

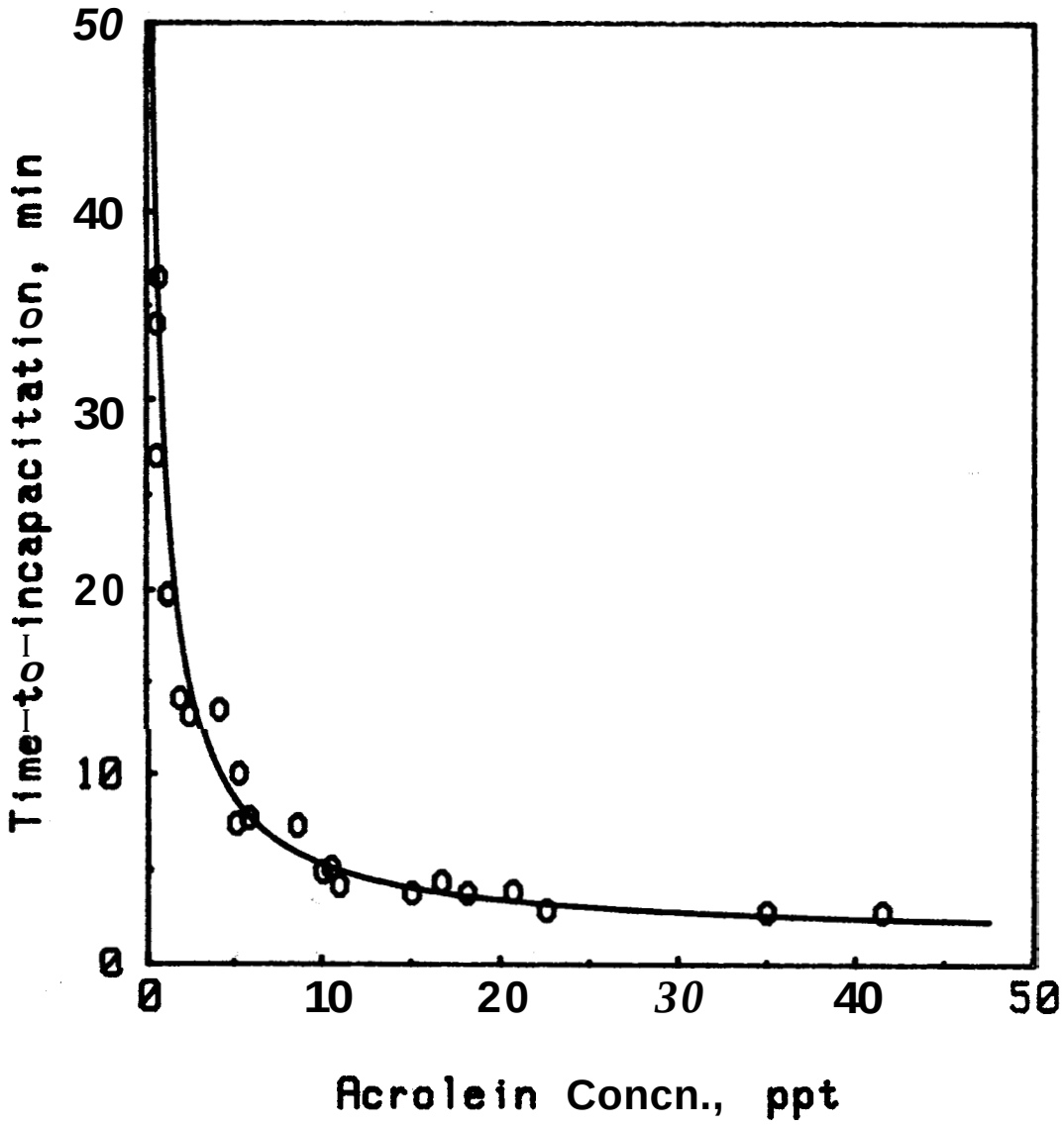

Figure 3. Time-to-incapacitation as a function of the average chamber concentration of acrolein. Fitted regression equation: $t_i = 1.5 + 40/(C+0.5)$, RSSQ = 238.

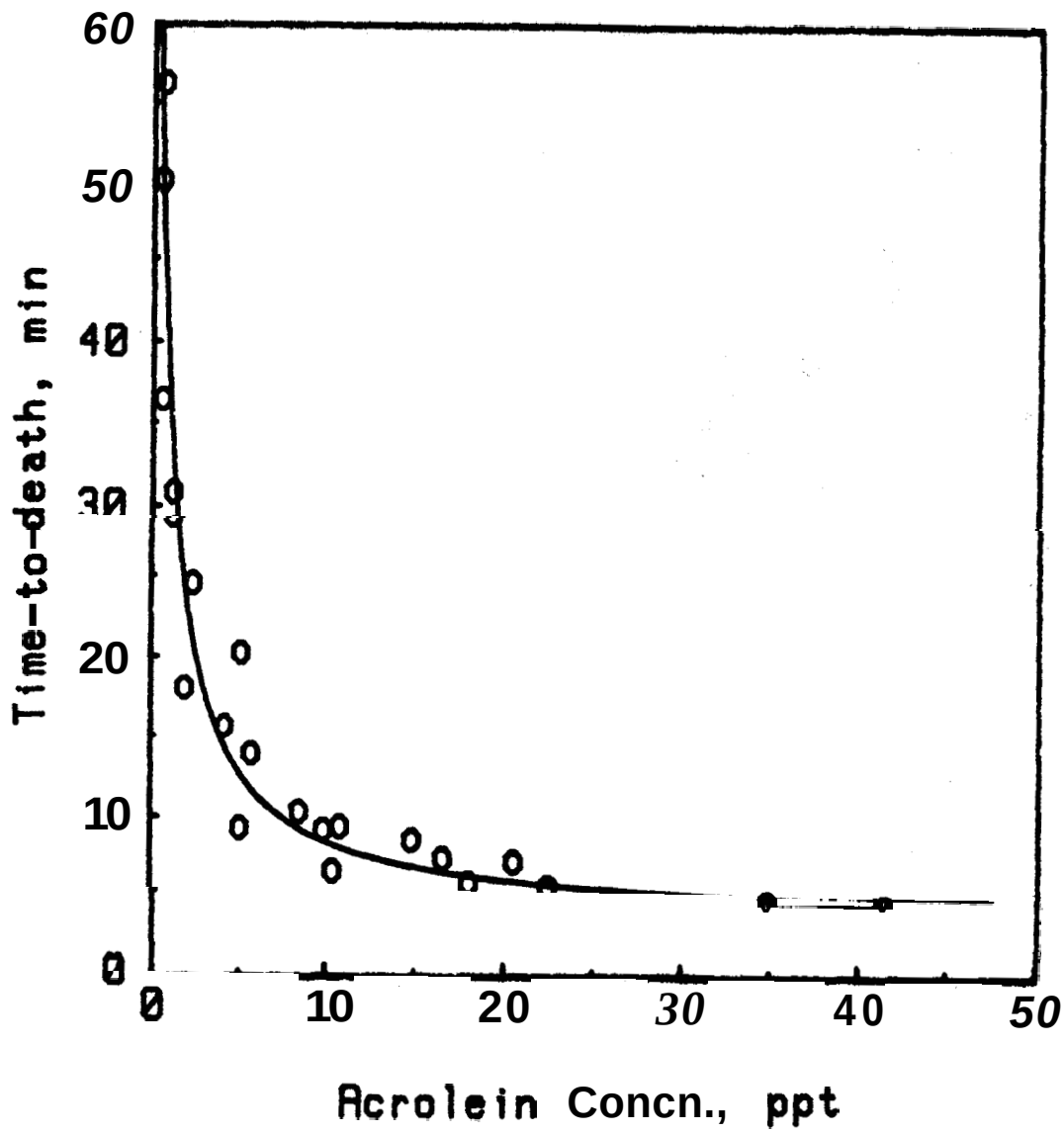

Figure 4. Time-to-death as a function of the average chamber concentration of acrolein. Fitted regression equation: $t_d = 3.75 + 50/(C+0.5)$, RSSQ = 473.

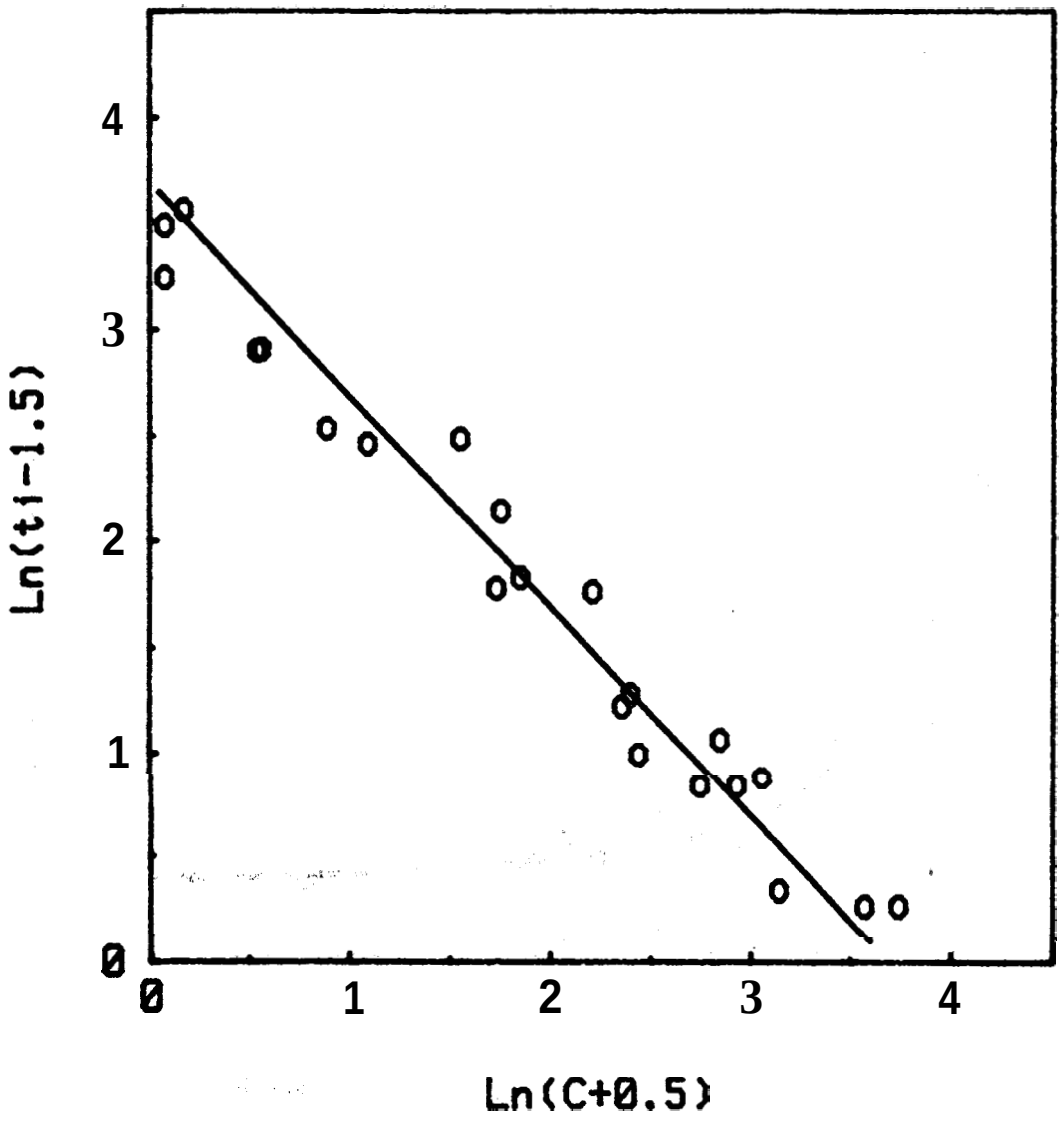

Figure 5. Loq-log plot of (t_1-1.5) versus (C+0.5), a linearized transformation of Fig. 3. Fitted regression equation: ln(t-1.5) = ln(40) - ln(C+0.5).

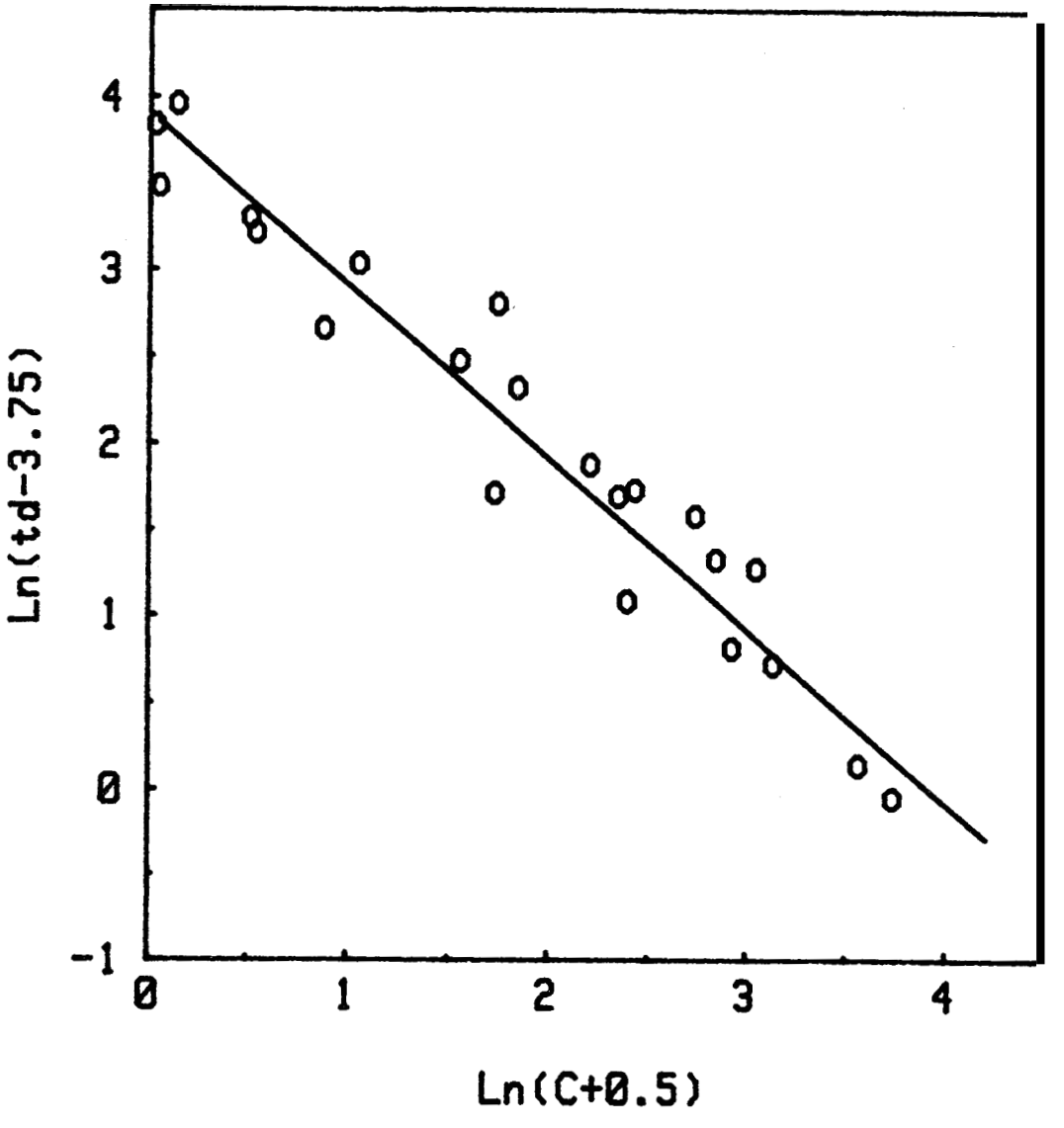

Figure *6.* Log-log plot of (t_d-3.75) versus (C+0.5), a linearized transformation of Fig. 4. Fitted regression equation: $\ln(t_d-3.75) = \ln(50) - \ln(C+0.5)$.

exceedingly long exposure time. Logically, both of these parameters should have positive values, since there could hardly be a physiological effect from a zero concentration and, correspondingly, no effect: could result from an exposure of zero minutes.

The fact that the model equation, [2], could not be fit to the acrolein data using a positive value for the parameter, b, suggests that the specific farm of that equation may be inappropriate, for describing the toxicokinetics of acrolein. A likely possibility is that acrolein is exerting its toxic effect differentially at two or more specific biological sites and, as a consequence, the overall effect on response time is a function of something other than the first power of the effective concentration, (C-b). If the Concentration is functioning at some power other than one--that is, if the effective concentration is $(C-b)^n$, where n is not equal to one--then n must have a value that lies between zero and one; otherwise the fit with the data gets worse rather than better.

If response time were related to the square root of the effective acrolein concentration, the value of n would be 0.5. The model equation would then be:

$$t_r = a + k/(C-b)^{0.5}. \qquad \text{Eq. 3}$$

Fitting this equation to the data resulted in reasonable and positive values for the three parameters (a, k, and b) and a residual sum of squares (RSSQ) that was smaller than with equation [2]. (The RSSQ is a measure of the "goodness of fit" between the data and the model equation: the smaller RSSQ representing the better fit.) The values for the three parameters in equation [3] are as follows,

for incapacitation: a = 0.1 k = 18 b = 0.3,
for lethality: a = 1.0 k = 25 b = 0.3.

Figure 7 is the plot, for equation [3], of t_i as a function of concentration, and Figure 8 is that for t_d. The corresponding log-log transformations are shown in Figures 9 and 10.

Reasonable predictions of response time for a given acrolein concentration can be made using either equation [2] or equation [3], provided one does not exceed the concentration limits utilized in the study. However, the values obtained for the coefficients in equation [3] should be more meaningful in the physiological sense. Comparison of the values from the two equations suggests, for example, that the limiting (asymptotic) response times are more likely to be 0.1 and 1.0 minutes rather than 1.50 and 3.75 minutes (for t_i and t_d respectively), and the limiting effective concentration for infinite exposures is much more likely to be 300 ppm than a negative 500 ppm.

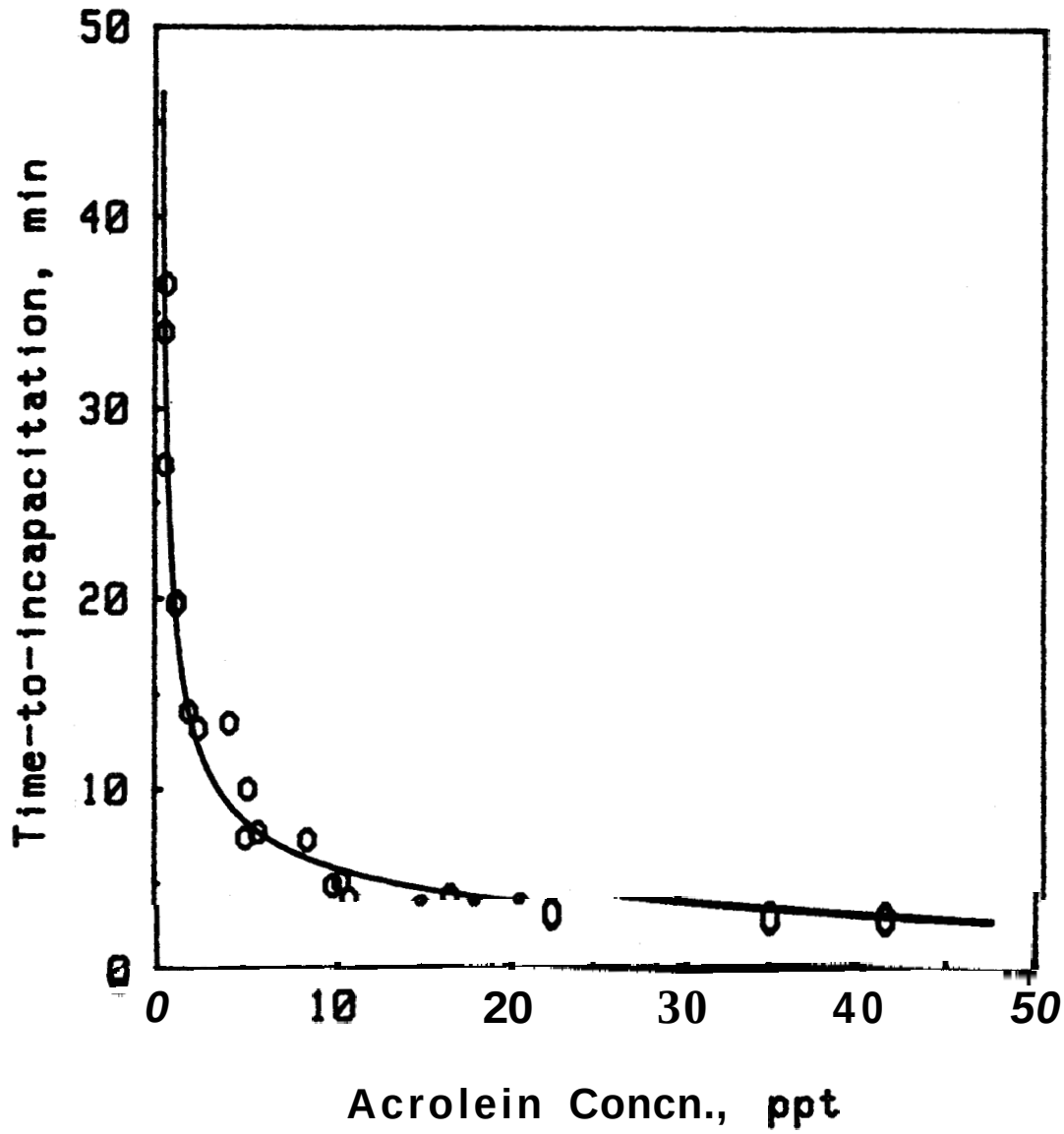

Figure 7. Time-to-incapacitation as a function of the square root of the effective acrolein concentration. Fitted regression equation: $t_i = 0.1 + 18/\sqrt{(C-0.3)}$, RSSQ = 141.

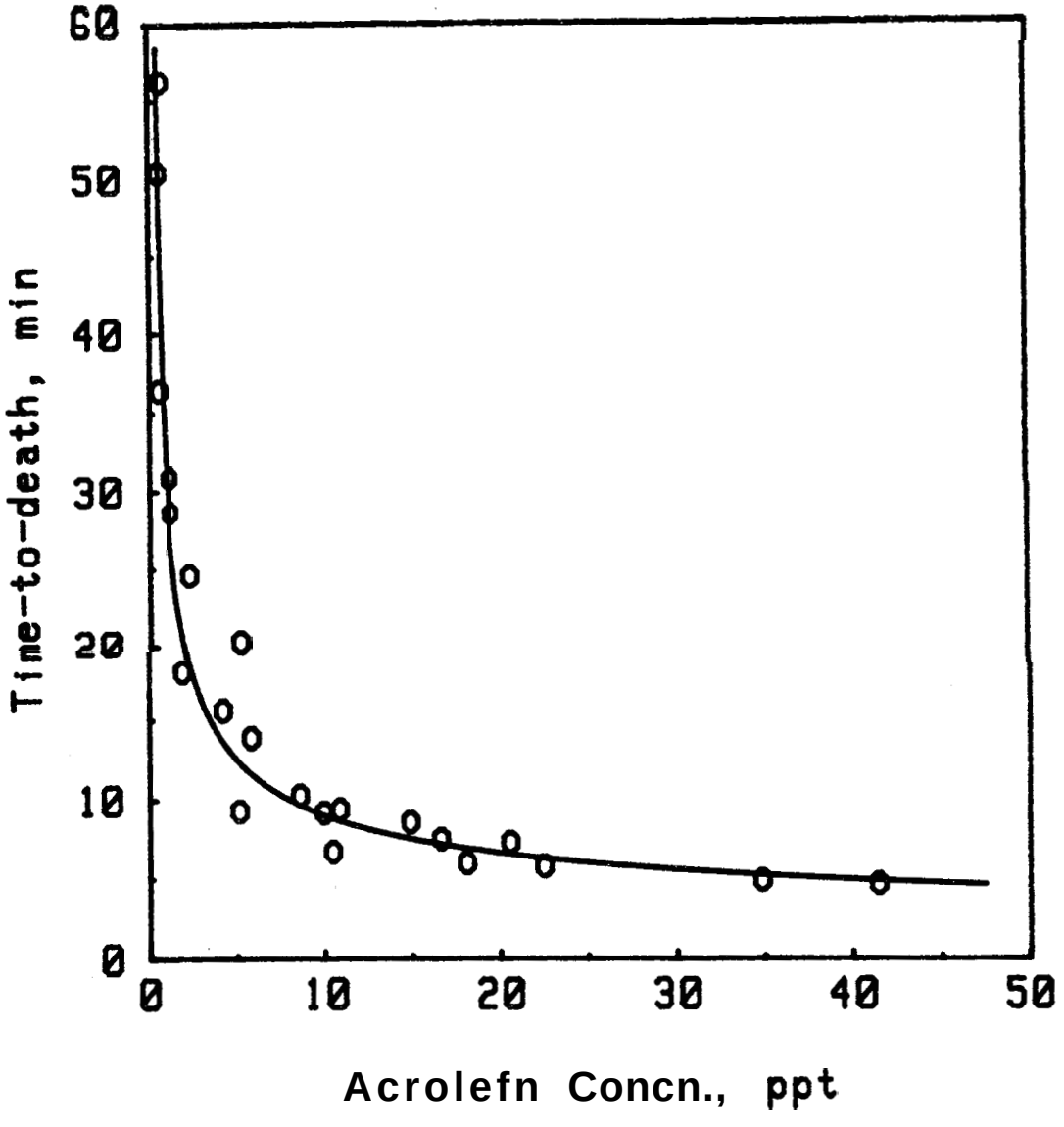

Figure 8. Time-to-death as a function of the square root of the effective acrolein concentration. Fitted regression equation: $t_d = 1.0 + 25/\sqrt{(C-0.3)}$, RSSQ = 533.

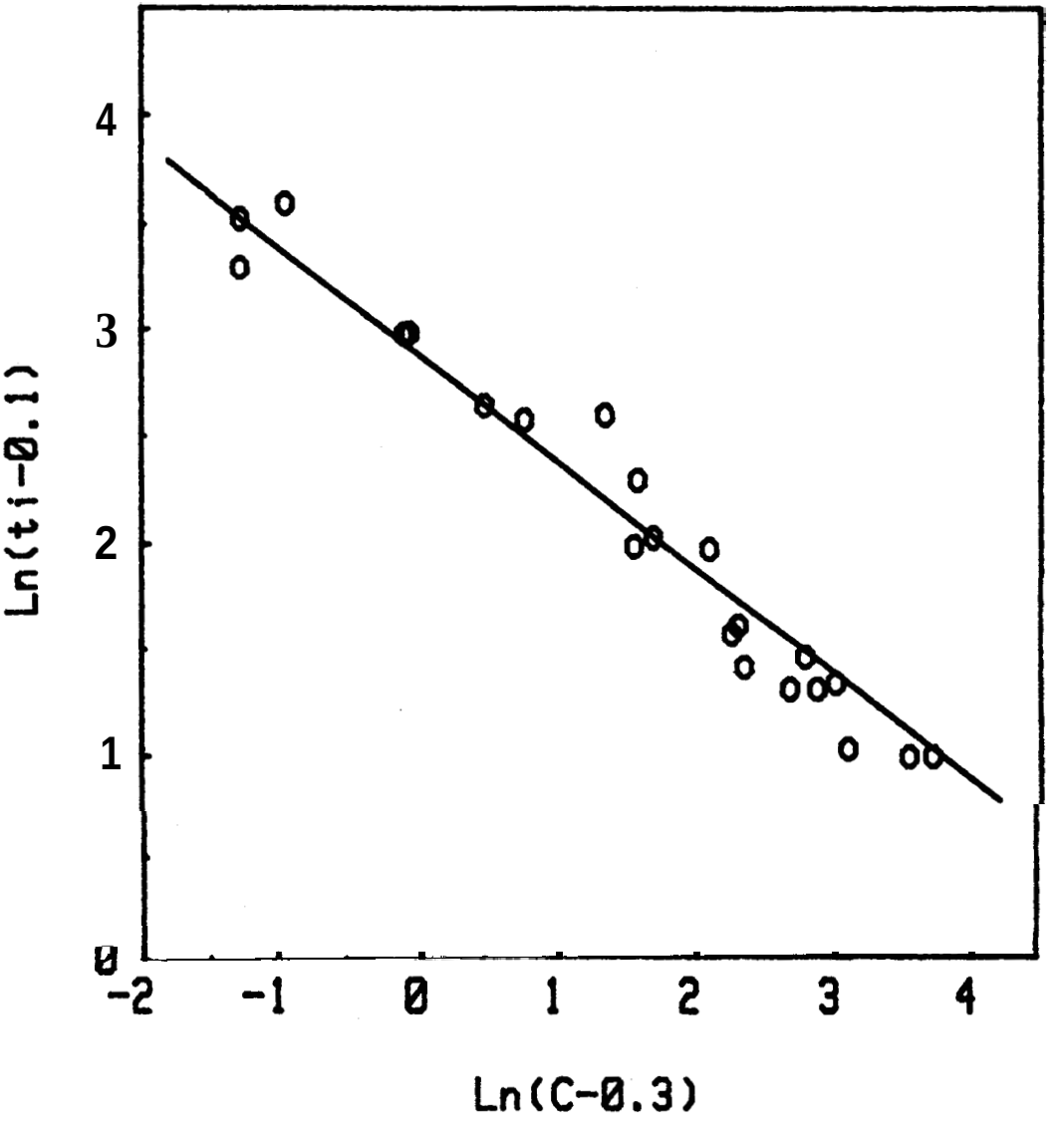

Figure 9. Log-log plot of $(t_i-0.1)$ versus $\sqrt{(C-0.3)}$, a linearized transformation of Fig. 7. Fitted regression equation: $\ln(t_i-0.1) = \ln(18) - \frac{1}{2}\ln(C-0.3)$.

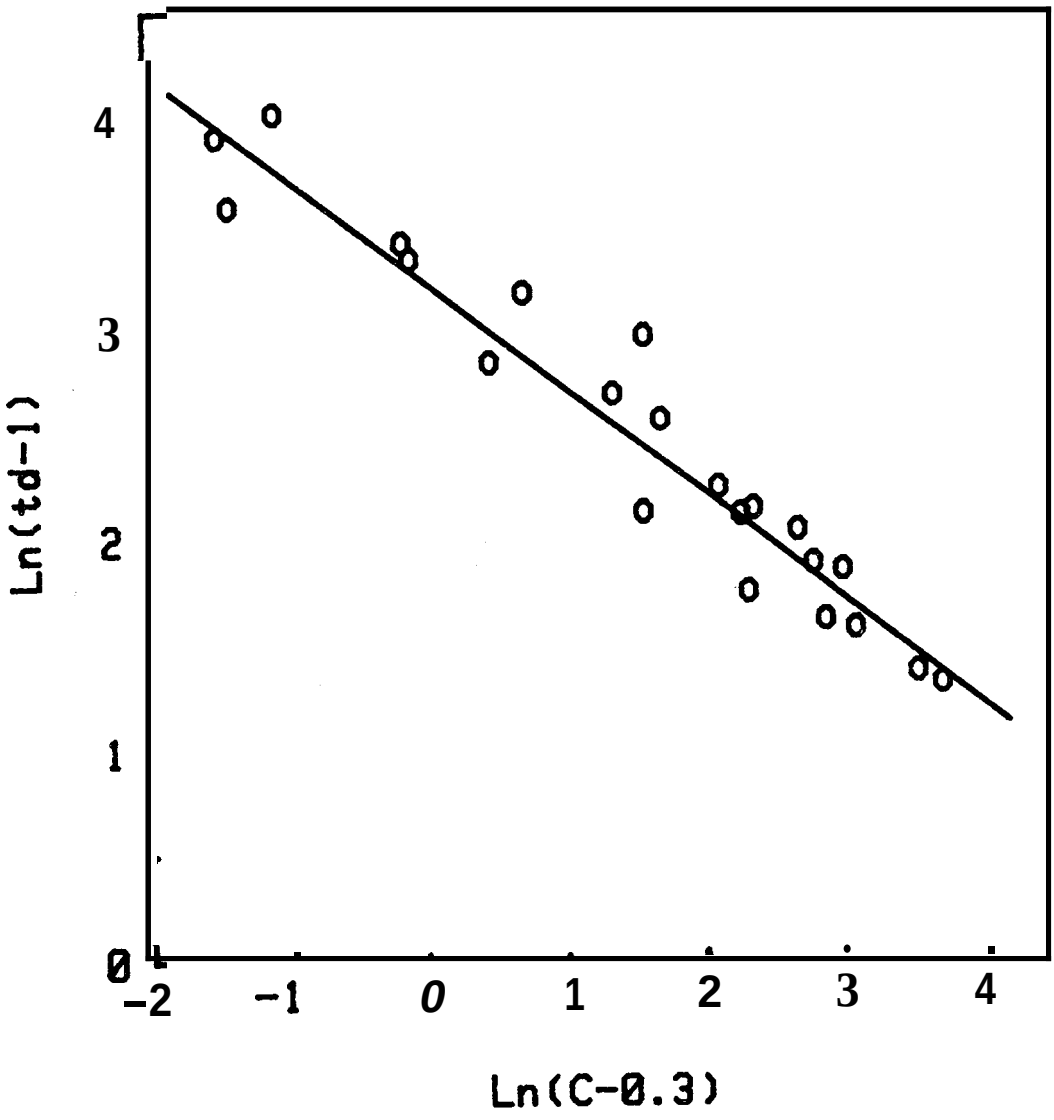

Figure 10. Log-log plot of (t_d-1.0) versus J(C-0.3), a linearized transformation of Fig. 8. Fitted regression equation: $\ln(t_d-1.0) = \ln(25) - \frac{1}{2}\ln(C-0.3)$.

We have been unable to locate in the scientific literature any other studies of the incapacitating effects of acute exposures to acrolein using rats as subjects. However, since this study was completed (in 1981) there has been a report by Kaplan et al.[13] of acute exposures using baboons as subjects. In this study, baboons were exposed to known concentrations of acrolein far 5 min and then given the opportunity to physically escape from the exposure chamber. For all acrolein concentrations tested, up to a maximum of 2.78 ppt, the subjects escaped successfully; i.e., there were no instances of physical incapacitation. If equation [3], derived for rats, can be applied to baboons, it would predict a time-to-incapacitation of 11.5 min for baboons exposed to 2.78 ppt acrolein.

Salem and Cullumbine[17] found that lethal exposure times ranged from 13 to 27 min when mice, guinea pigs, and rabbits were exposed to an acrolein concentration of 5,225 mg/m^3, which would be equivalent to approximately 2,300 ppm acrolein depending on the ambient atmospheric pressure and temperature. This is reasonable agreement with a t_d of 18.7 min predicted from equation [3] for a rat exposed to 2.3 ppt.

Since the acrolein concentration-response time relationship derived from our experimental data can predict, within an acceptable margin, the response times for three other rodent species, and, since it is not inconsistent with data obtained from baboon exposures, it would seem reasonable that it could be used to approximate human exposure responses.

There are no documented dosages for lethal or truly incapacitating human exposures; however, Alarie et al.[1] have suggested that human exposure to 20 ppm would be lethal--or at least would result in extremely severe injury to the respiratory tract. The most common statement in the literature, however, seems to be that exposure to 10 ppm or more would prove lethal for humans "in a few minutes" or "in a short time"[2,8,10]. These predictions that 10 to 20 ppm could be incapacitating or lethal in a few minutes are not reconciled easily with the reported animal experiments in which concentrations 100 times greater are required to achieve a response time of about 10 min. A possible explanation is that the acrolein toxicity previously predicted for humans is an overly conservative estimate based on (dis)comfort indices as endpoints rather than on actual incapacitation or mortality--as we suspect is also the case for hydrogen chloride gas[7].

SUMMARY AND CONCLUSIONS

Rats were exposed in the Civil Aeromedical Institute (CAMI) Inhalation toxicity assay system to selected atmospheric concentrations of acrolein vapor in air; these concentrations ranged from 530 ppm to over 40,000 ppm. The CAMI system utilires an enclosed rotating wheel that allows the measurement of a physical incapacitation endpoint as well as the traditional one of mortality. These two endpoints were measured as time-to-incapacitation (t_i) and time-to-death (t_d); the measured response times ranged from 2.8 to 56.2 min. Results were graphed as scatterplots (response time versus acrolein concentration), and regression equations were fitted to each data set using a nonlinear least squares technique. The resulting response equations are:

$$t_i = 0.1 + 18/(C-0.3)^{0.5} \text{ for incapacitation and}$$
$$t_d = 1.0 + 25/(C-0.3)^{0.5} \text{ for lethality,}$$

where response times are in minutes and acrolein concentrations, C, are in parts per thousand.

These results suggest that acrolein, an almost universal component of smoke, is not *so* incapacitating as had been previously thought. Concentrations necessary to produce incapacitation in 10 min are likely to be 10 to 100 times greater than those suggested by the scientific literature.

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APPENDIX

Spectrophotometric Assay of Acrolein As Its 2,4-Dinitrophenylhydrazone

Acrolein that was to be used in the preparation of aqueous standards was purified daily *by* distillation. Its significant volatility required special handling during the preparation of liquid standards; therefore, the approximate required volumes (0.1 to 0.2 mL) were transferred by pipet to tared, glass weighing bottles (with ground-glass stoppers) and reweighed to the nearest. 0.01 mg. These small--diameter weighing bottles were of a size that would allow them to drop freely dawn the neck of a 500-mL volumetric flask. The volumetric flask was filled to the calibration mark with deionized water and a volume of water equal to the volume of acrolein to be added was withdr-awn by pipet. The weighing bottle was held over the flask mouth, the cover was loosened, and the bottle, contents, and cover were dropped into the water. The flask was stoppered immediately and the contents mixed by repeated inversion. Working standards were prepared *by* dilution from this solution into additional 500-mL flasks; this approach allowed relatively large volumes (10 to 25 mL) of stock:: standard to be transferred, which minimized the relative magnitude of: pipetting errors. These transfers and subsequent dilutions to volume were accomplished quickly to minimize acrolein loss due to vaporization.

The 2,4-dinitrophenylhydrarine reagent. was prepared dai1y as a saturated solution in 2N aqueous hydrochloric acid (HCl); the solution was filtered immediately prior to use.

Isooctane (2,2,4-trimethylpentane), used to extract the 2,4-DNP, was chromatographic quality analytical reagent grade (Mal-1inckrodt ChromAR).

The spectrophotometric analysis of aqueous acrolein standards was accomplished by mixing, in a screw-cap glass centrifuge tube, 3 mL of the standard with 1 mL of saturated 2,4-dinitrophenylhydrazine (in 2N HCl) and adding 10 mL of isooctane. The reaction tube was capped and shaken vigorously at 5-min intervals over a 20-min period. After the final shaking, the tube was allowed to stand until the two layers Rad separated, then allowed to stand for an additional 5 min. The isooctane layer was then removed from the aqueous layer and centrifuged to remove the last trace of suspended water droplets,. Absorbance of the isooctane solution was measured in a quartz cuvette at 350 nm against a reagent blank::. The resulting absorbances were used to construct a standard curve by plotting absorbance against acrolein concentration.

Gaseous samples;, either of known concentration from the Saran bags or of unknown concentration from the exposure chamber, were obtained by withdrawing a 15-mL aliquot of the appropriate atmosphere into a 30-mL glass syringe, followed immediately by 15 mL of deionized water. The syringe needle was replaced with a cap and the syringe shaken intermittently for 30 min. All samples were kept in the capped syringes until they could be analyzed for acrolein using the spectrophotometric procedure described above for aqueous standards. Acrolein concentrations equivalent to the measured absorbances were calculated using the equation derived' from the aqueous standard curve.