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Environmental Health Effects Research Series

ANNUAL REPORT FOR CALENDAR YEAR 1972
ENVIRONMENTAL TOXICOLOGY RESEARCH LABORATORY
NATIONAL ENVIRONMENTAL RESEARCH CENTER

Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

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by

The Staff of the ETRL

J. F. Stara, Director

FOREWORD

Pursuant to environmental legislation (Sec. 103 of the Clean Air Act as amended in 1970), the Environmental Protection Agency was charged to evaluate potential toxic effects of fuels and fuel additive emissions from mobile and stationary sources and to conduct definitive toxicologic testing of individual potentially hazardous air pollutants. Because of this charge, the Agency has directed its National Environmental Research Centers to address the problems of the potential toxic effects of these emissions. The Environmental Toxicology Research Laboratory, NERC - Cincinnati, is concerned with the toxicological evaluation of emissions and potentially hazardous pollutants in laboratory animal model systems.

There is a critical need to test and clarify the potential destructive effects of environmental contamination through advanced toxicologic techniques in order to establish safe population standards. Existing methods must be used and new methods developed to evaluate and control the impact of pollution on man's health and well-being. Toxicologic research must be conducted primarily in non-human biological test systems since most chemicals found in the environment cannot be safely tested in man. Furthermore, the need for more experimental animal models with clearly defined characteristics is now well established and the utilization of such models has proven extremely successful over the years. In fact, many of the advances in biology and medicine have been derived from animal studies.

The probability of reproducing the human response in animals increases with judicious selection of the animal species. For this reason, an increasing number of mammalian species are being used in this Laboratory to develop appropriate models for toxicological evaluation of potentially harmful pollutants. In its planning, the Laboratory gives a special emphasis to certain factors which may influence susceptibility such as age-sensitivity, relevant routes of exposure, and different chemical forms of compounds found in the environment. In studying the toxic process of a disease, several "standard" animal species, e.g., mice, rats, guinea pigs, rabbits, and dogs are routinely used; others were added whenever indicated, e.g., Syrian hamsters, non-human primates and cats.

The problems concerned with the extrapolation of animal data to man are minor when compared with the great advantages of animal investigation in determining the toxic potential of various agents. The following factors favor animal experimentation: (a) strict control of exposure concentrations; (b) strict control of duration of exposure; and (c) opportunity to make a detailed biological examination of tissues and organs not possible in man. Possibly, the most cogent arguments for the use of animals in toxicological studies are: (1) they are necessary for determination of dose:effect relationships because levels much greater than those found in nature may be used; (2) they are necessary to assay potential threats of new agents which may be introduced into the environment in the future due to technological advancement.

The research approach for determining the potential toxic effects of mobile source emissions is complicated by the rapidly changing scope in fuels and fuel additives marketing, and development of new emission systems and control devices, e.g., catalytic converters. The goals of ETRL are to provide answers to these problems using a multidisciplinary toxicologic research approach outlined in Table I.

The program plans of ETRL call for conducting research in two major areas of environmental pollution:

A. Inhalation and ingestion studies of single pollutants with particular emphasis on hazardous substances (trace metals).

B. Inhalation exposure to fuel and fuel additive emissions from mobile sources, which represents a complicated bu realistic mixture of pollutants in the environment.

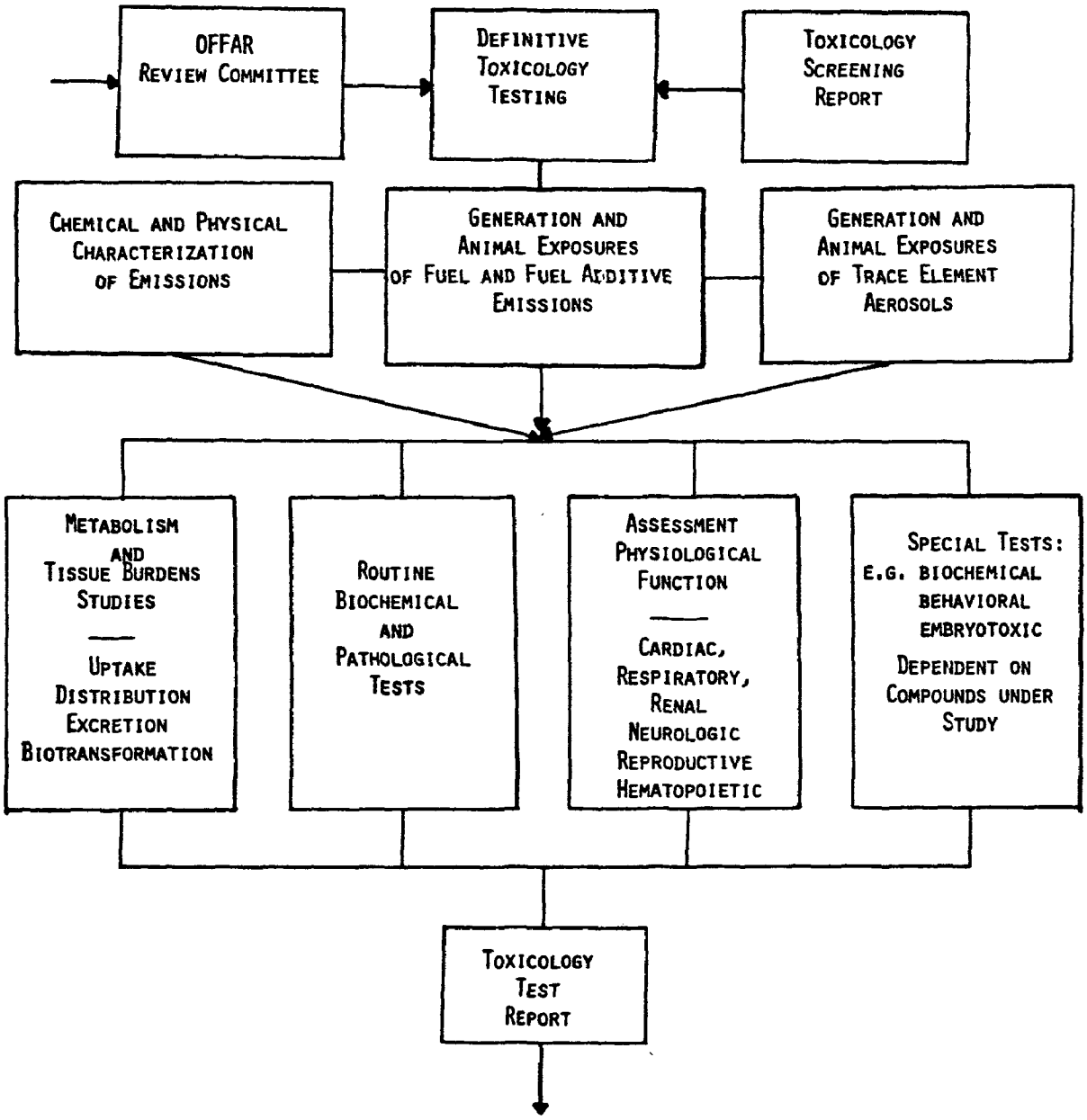
The specific studies are designed to provide data which will supplement and add to existing information on various pollutants. Such information is required for the compilation of criteria documents, which in turn serve as background material for enforcement actions and for establishment of safe, accurate and imagainative environmental pollution standards.

J. F. Stara

Table I

ETRL MODEL FOR DEFINITIVE TOXICOLOGIC TESTING

(FUEL ADDITIVES, FUELS, POLLUTION CONTROL DEVICES, TRACE METALS)



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SINGLE, INTER-MEDIA, POLLUTANT STUDIES

ORAL TOXICITY OF MMT IN RATS

D. Hysell, W. Moore, R. Miller, and W. Crocker

The paucity of information on the toxicity of MMT (per National Academy of Science request) prompted a series of studies to provide necessary data on its metabolic behavior and biological effects.

The organometallic compound 2-methylcyclopentadienyl manganese tricarbonyl (MMT) is of current interest because of its possible use as an anti-knock compound in unleaded gasoline. The compound is also marketed as a combustion improver for fuel oils under the trade name "Ethyl" Combustion Improver 2 (CI 2) and as a smoke suppressant for diesel engines and stationary jet fuel power sources. The compound is an orange colored liquid having low volatility, a distinctive herbaceous odor, and thermal stability, and is subject to rapid photochemical decomposition. The calculated manganese content is 25.2%. The proposed concentration of manganese per gallon of gasoline is approximately 0.125 g.

Eighty COBS rats, weighing 200-250 g, were divided into eight groups of 10 animals each and given a single oral dose of Mn tricarbonyl for determination of oral toxicity. The Mn tricarbonyl was diluted with Wesson Oil and given by intragastric tube. The concentrations ranged from 15 to 150 mg/kg (based on Mn content of MMT), and one group of animals serving as controls.

In animals given the high concentrations (80-150 mg/kg), deaths occurred within 24-48 hours after dosing. The progressive effects consisted of huddling, roughened hair coats, tremors, progressive weakness, labored respiration, seroganuineous nasal discharge and terminal coma. The incidence of clinical symptoms in each group essentially paralleled the mortality rate except the lowest dosage groups showed the roughened hair coat and huddling for the first 24 hours.

All deaths occurred within 6 days after exposure, and by 14 days, the survivors appeared normal. The study was terminated at this point and tissues taken for histopathological examination and Mn analysis. The mortality data are presented in Table 1.

TABLE 1. MORTALITY IN RATS FOLLOWING ORAL ADMINISTRATION OF Mn TRICARBONYL

Dose mg/kg	Mortality / Dosed	Length of survival of animals dying
15	0/10	
30	0/10	
45	5/10	2 - 6 days
60	6/10	2 - 3 days
80	6/10	<24 hr. - 3 days
100	8/10	<24 hr. - 3 days
150	10/10	<24 hr. - 2 days
Control	0/10	<24 hr. - 2 days

Other investigators had found that the oral LD_{50} varied in the rat depending on age, sex, and the medium used for dilution of the Mn tricarbonyl. The range for the male rat varied from 17-176 mg/kg, and from 9-96 mg/kg for the female rat.

Necropsies were performed on a representative number of animals dying during the study as well as selected animals euthanatized at the end of 14 days. In animals dead within 24 hr, the gross necropsy findings consisted of large sacular atonic stomachs, severely congested livers, and severely congested lungs that, on sectioning, exuded a sero-sanguineous fluid from the cut surfaces. In animals dead between 24-72 hr, the pulmonary and hepatic changes were the same. The small intestine was distended with clear watery contents and the walls appeared thin and friable. By 14 days, the organs appeared grossly normal except for the livers from animals receiving the higher dosage levels, which were a tannish yellow in appearance. At necropsy, specimens of heart, lung, liver, kidney, duodenum, and brain were collected for chemical assessment of tissue manganese. The mean Mn concentration in selected tissues for those animals that died following exposure and those that were sacrificed 14 days post exposure are given in Table 2.

Specimens of heart, lung, liver and kidney were collected for histologic preparation.

TABLE 2. CONCENTRATION OF Mn IN DIFFERENT TISSUES FOLLOWING INGESTION OF 2-METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL

ANIMALS DYING FOLLOWING EXPOSURE

TISSUE	µg/g Dry Weight					
	CONTROL	45	60	80	100	150
Duodenum		34.7	38.4	65.8	142.5	177.6
Kidney		18.3	25.3	17.4	48.0	40.0
Liver		22.8	22.9	31.6	32.0	36.5
Lung		12.0	10.2	15.4	28.1	28.6
Heart		4.00	4.83	4.67	7.84	6.06
Brain		7.18	8.68	9.66	7.53	8.16

ANIMALS SACRIFICED 14 DAYS POST INGESTION

TISSUE	µg/g Dry Weight						
	CONTROL	15	30	45	60	80	100
Duodenum	3.32	4.89	3.81	5.03	6.49	6.48	7.50
Kidney	5.81	4.64	5.62	3.16	2.62	3.59	4.99
Liver	7.05	7.14	9.11	10.52	9.55	11.33	10.82
Lung		4.40	4.87	3.67	5.56	3.13	6.39
Heart	1.86	1.83	3.77	3.00			
Brain	5.89	7.18	8.68	9.68	9.66	7.53	8.16

Microscopically, the lungs of animals dead in 24 hours showed severe congestion, perivascular and alveolar edema and alveolar hemorrhage (Fig. 2).

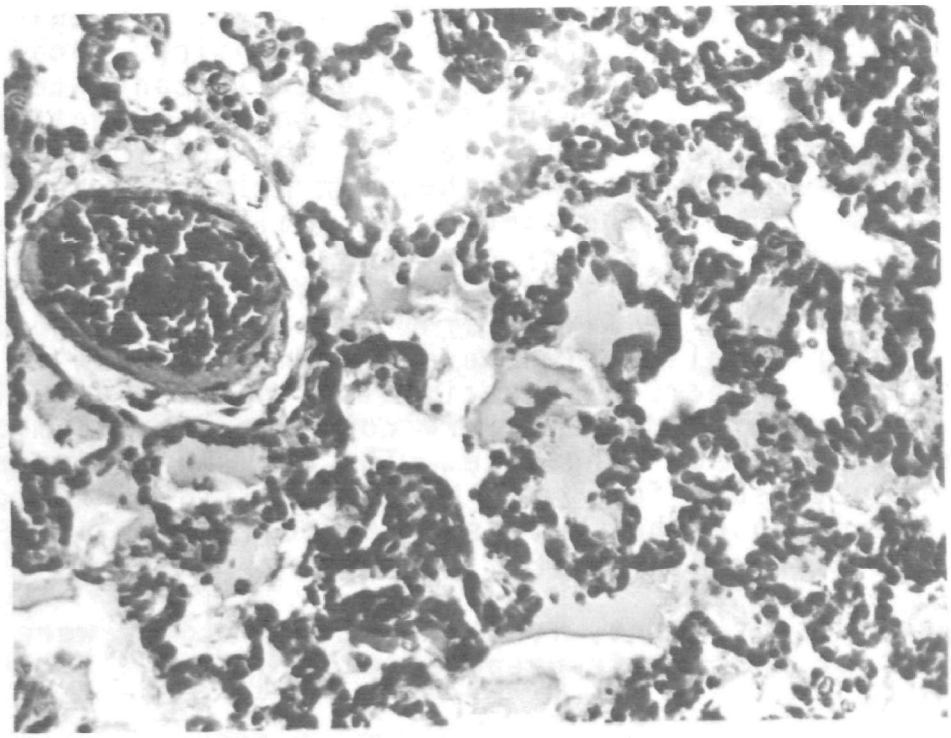


Figure 2. Microscopic appearance of pulmonary tissue from animals dead within 24 hr.

From 24-72 hr there was, in addition, a severe fibrinopurulent pneumonia with prominent macrophage infiltrate. (Fig.3)

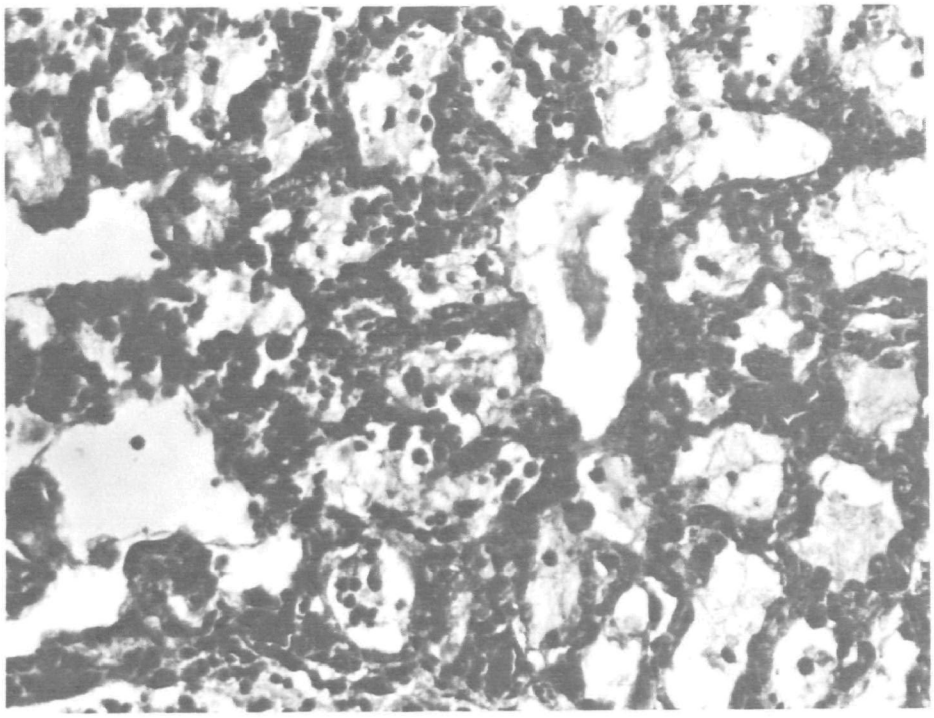


Figure 3. Pulmonary changes in animals dead 48-72 hr after exposure to MMT.

In animals surviving 14 days, the lungs showed extensive areas of consolidation, thickened alveolar septa and focal areas of alveolar macrophage activity (Fig. 4).

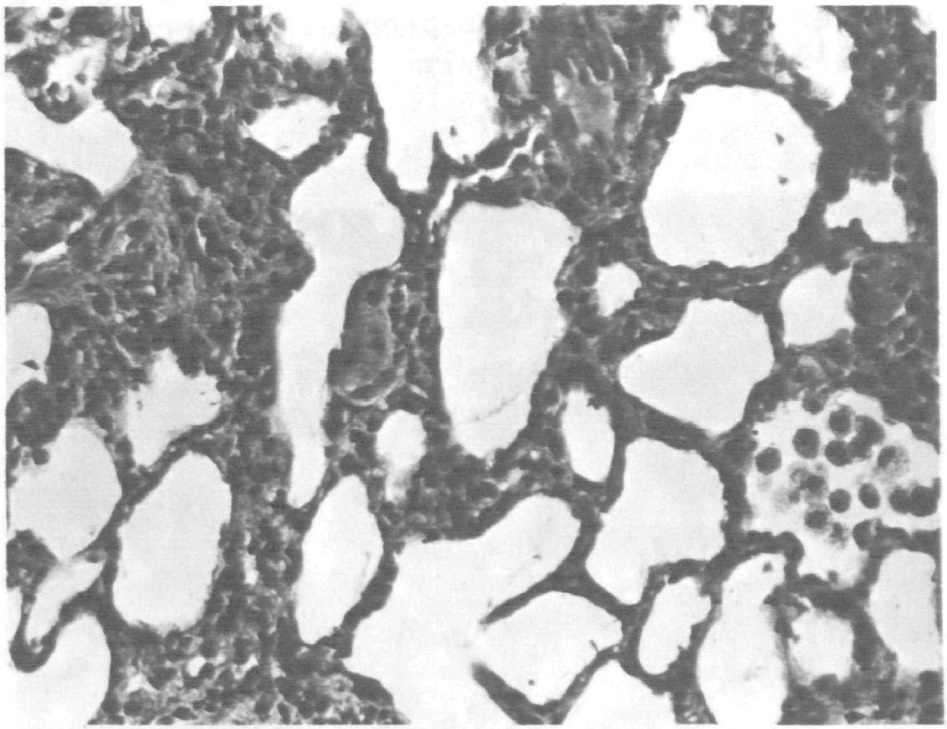


Figure 4. Chronic proliferative pneumonia at 14 days surviving MMT exposure.

The hepatic lesions progressed from acute centrolobular passive congestion at 24 hr, to hepatic parenchymal necrosis and leukocytic infiltration at 48-72 hours, and extensive cytoplasmic vacuolar change (probably lipidic) by 14 days. (Figs. 5, 6, & 7)

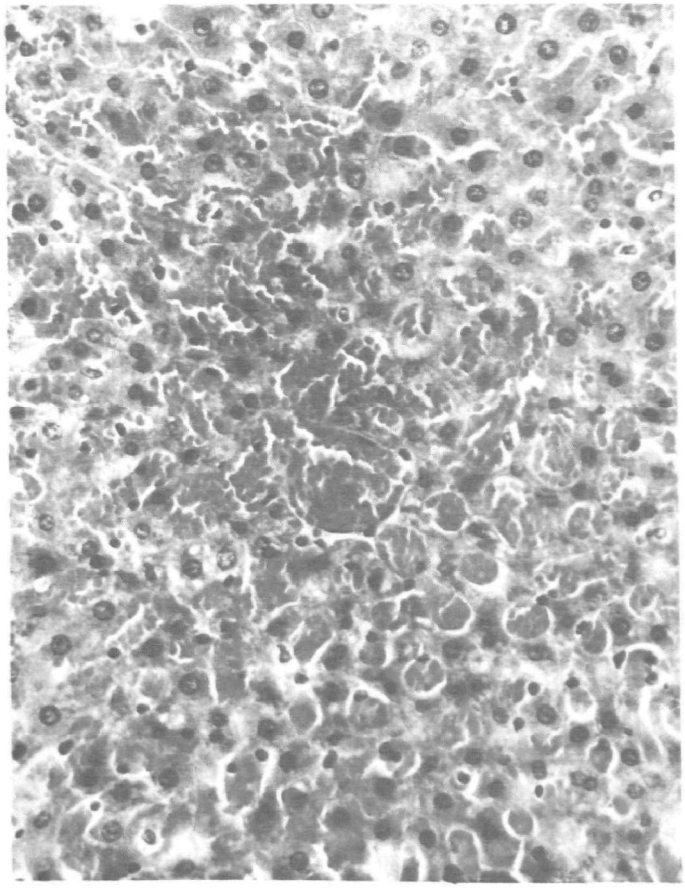


Figure 5. Hepatic changes at 24 hr.

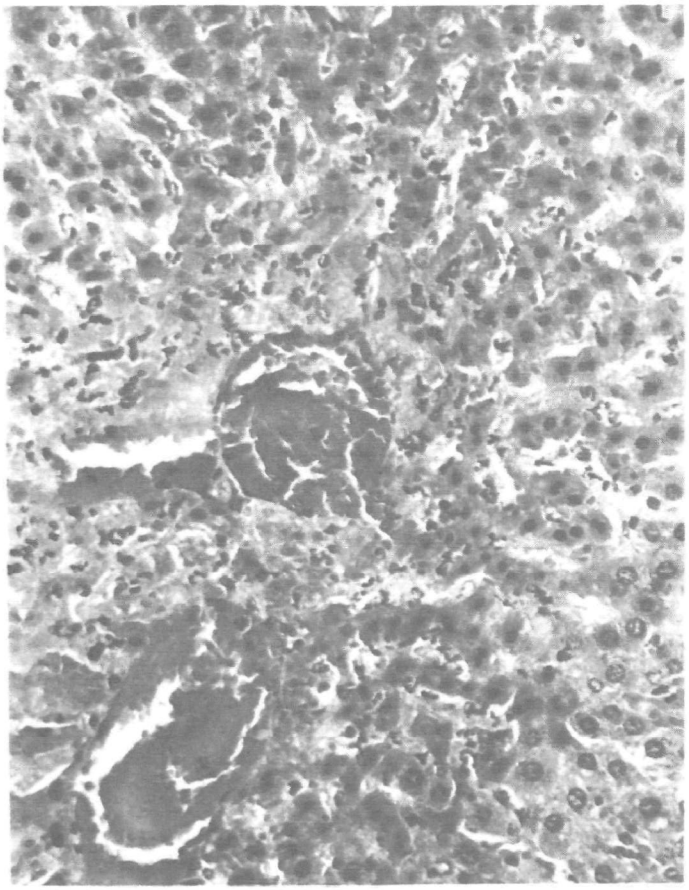


Figure 6. Hepatic changes at 48-72 hr.

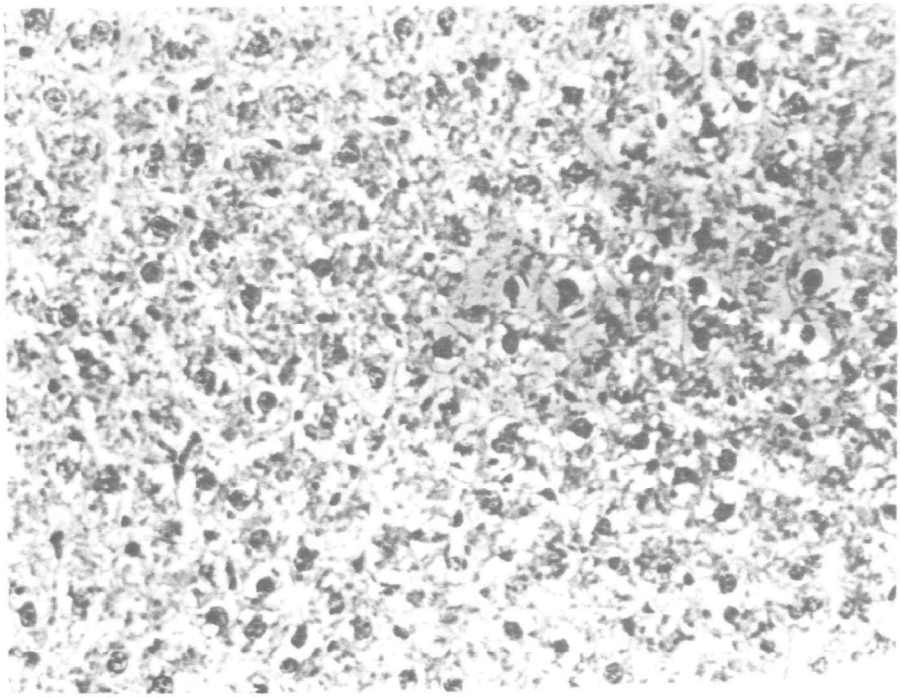


Figure 7. Hepatic changes at 14 days.

The early renal changes were hyaline droplet change and cytoplasmic vacuolation of proximal convoluted tubules plus distention of the glomerular space and tubule lumens with a material that was finely granular and stained lightly basophilic. (Fig. 8)

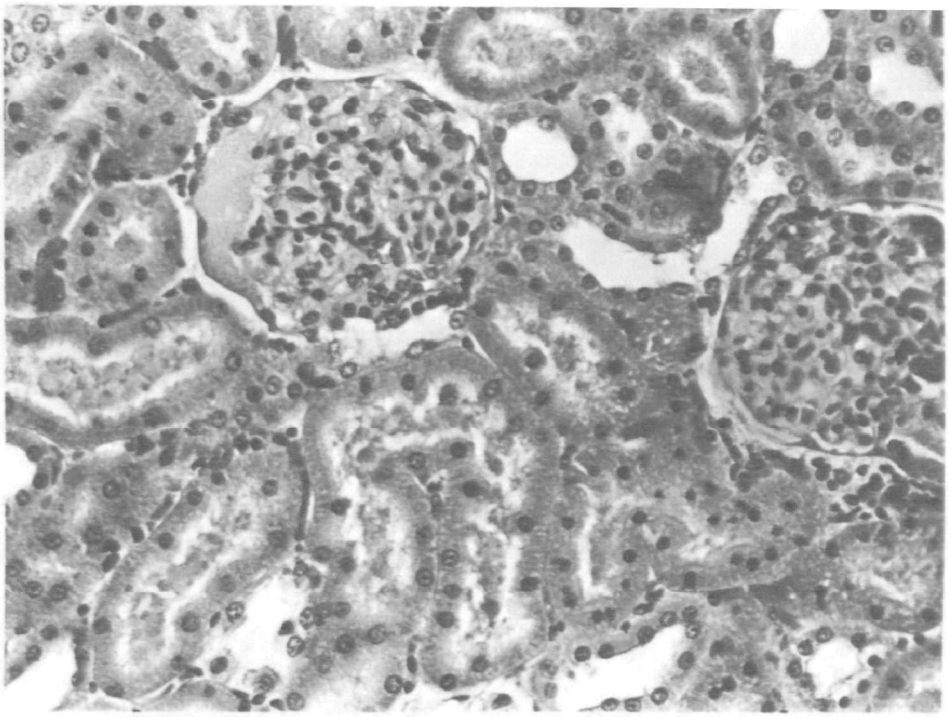


Figure 8. Renal changes at 24 hr.

By 48 hr there was severe tubular degeneration as indicated by nuclear pyknosis and cell lysis. (Fig. 9)

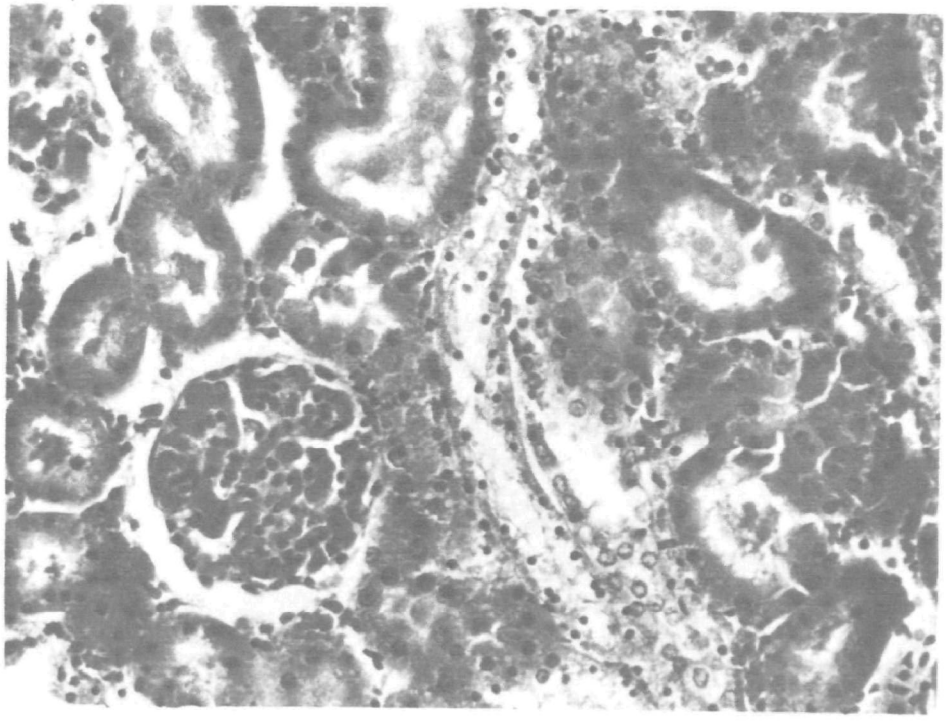


Figure 9. Renal tubular degeneration and necrosis at 48-72 hr.

In those animals surviving 14 days, no renal changes were noted.

INHALATION TOXICITY OF MMT VAPOR IN RATS

W. Moore, D. Hysell, M. Malanchuk, and J. Stara

A group of rats was exposed to MMT vapor at a concentration of 2 mg/m^3 for 4 hr (Fig. 1). Animals, in groups of six, were sacrificed immediately following exposure and at 1, 2, 4, 8 and 16 days and necropsied. No gross abnormalities were noted. Tissue specimens of heart, lung, liver, and kidney were collected for histologic preparation and microscopic examination. One eye was extirpated from each animal, prepared, and examined for determination of corneal mitotic rate. Tissue specimens of brain, heart, lungs, liver, and kidneys were collected for chemical analysis of tissue Mn.

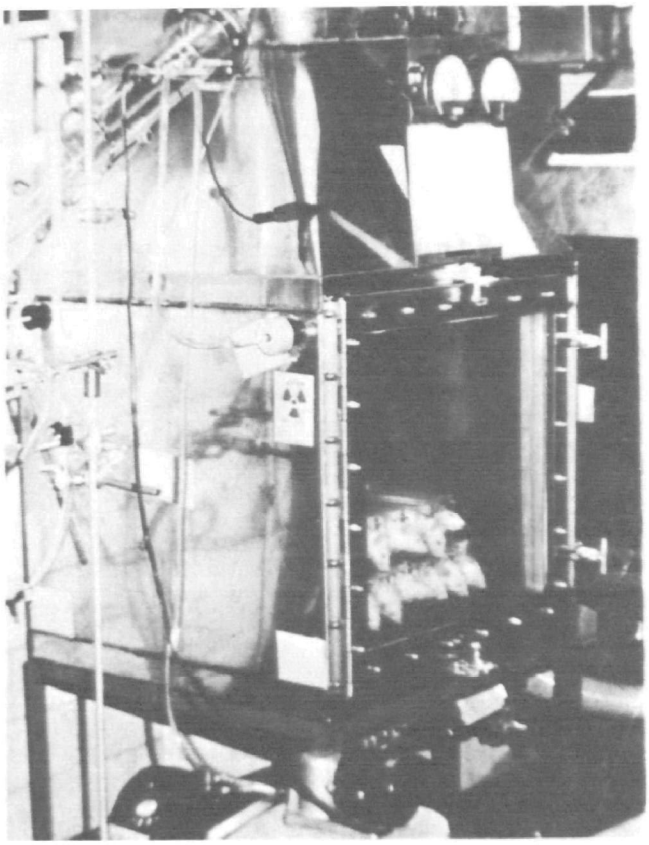


Figure 1. Aerosol exposure chamber.

Microscopically, the hearts and kidneys showed no treatment-related lesions. Pulmonary tissues showed changes

consistent with mild chronic respiratory disease of rats. No accentuation of the disease process due to treatment was apparent. Livers from control and immediate-sacrifice groups were normal. At 24 and 48 hr., there appeared to be cloudy swelling of the hepatic parenchyma. By 96 hr, there was a prominent cytoplasmic vacuolar change of the hepatocytes in several animals (Fig. 2).

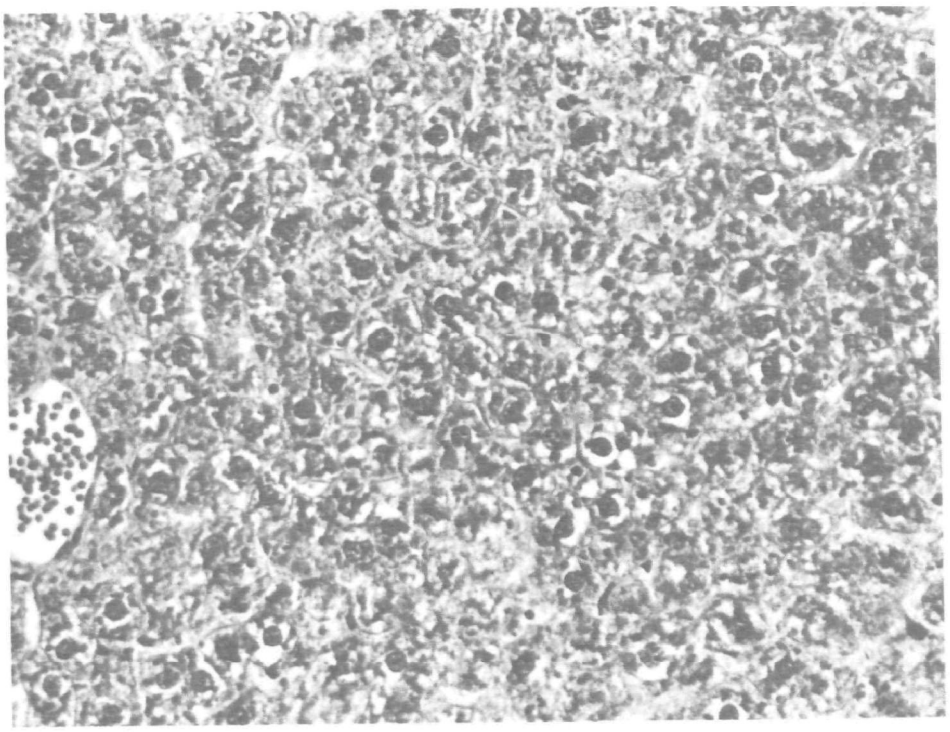


Figure 2. Hepatic changes at 96 hr in the MMT vapor exposed animals.

Morphologically, the changes were identical to the hepatic changes seen in the oral toxicity survivors euthanatized at 14 days and compatible in appearance with a lipidic degeneration of the liver. All animals sacrificed at 8 days showed the vacuolar hepatic change; by 16 days, however, the process was apparent in only two animals.

The results of the corneal mitotic rate determination are shown in Figure 3. It is not apparent that the changes in rate represent a direct ocular effect. It has

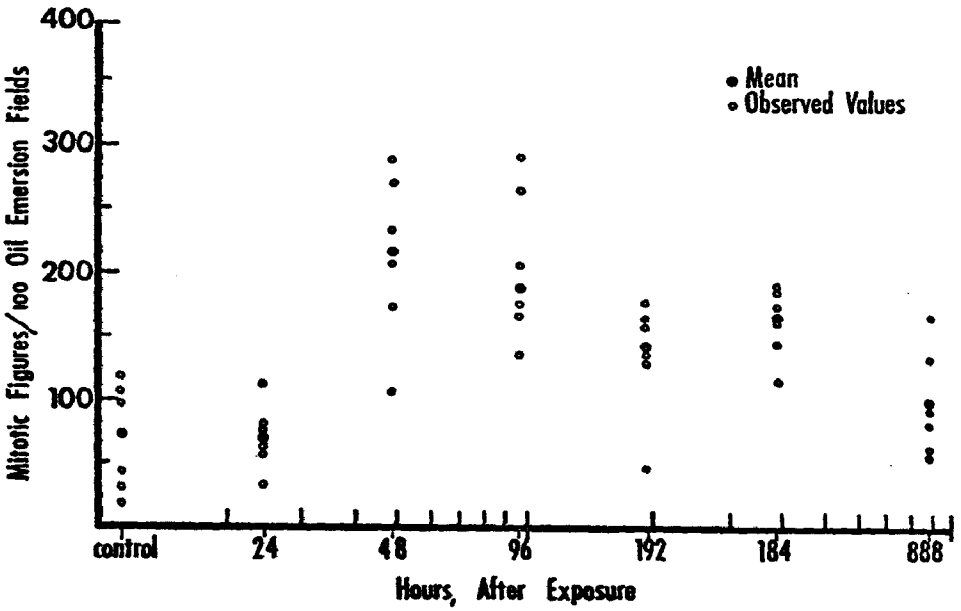


Figure 3. Corneal mitotic rates.

long been known, for instance, that partial hepatectomy results in a stimulation of corneal mitotic rate (Experientia, 24:569-70, June 15, 1968) perhaps mediated by adrenal gland function. Since the mitotic rate changes paralleled the hepatic damage, a similar physiologic response may be functioning in this case.

In the tissue analysis for Mn, there was a slight elevation in Mn levels in the lungs, liver, and kidney immediately after exposure and at 24 hr. After longer intervals of time, the Mn levels approached normal levels.

UPTAKE, DISTRIBUTION AND EXCRETION OF ^{54}Mn TRICARBONYL

W. Moore, D. Hysell, W. Crocker, and J. Stara

Through the cooperation of the Ethyl Corp., a small quantity of ^{54}Mn tricarbonyl was prepared for use in tracer studies. ^{54}Mn has a half-life of 303 days and an 0.83 MeV gamma, which makes it ideal for this type of approach. Although the values are reported as $^{54}Mn_{54}$ this should not be interpreted as to indicate that the ^{54}Mn was split off the compound. The fate of the Mn tricarbonyl molecule following absorption is not known. A further step must be the determination of the metabolic products and whether or not Mn is split off the molecule.

A group of fasted COBS rats, weighing approximately 200 gm, was given by intragastric intubation 2.5 mg (0.625 mg ^{54}Mn) ^{54}Mn tricarbonyl diluted in Wesson Oil. Whole body counts were made immediately after dosing and periodically thereafter to determine the retention of ^{54}Mn. Twenty-four-hour urine and feces samples were collected on the days the animals were counted. Another group of animals was given the same dose of ^{54}Mn tricarbonyl and then sacrificed at different intervals of time to determine the ^{54}Mn distribution in the tissues.

The percent of ^{54}Mn retained with time following dosing is shown in Figure 1. ^{54}Mn was rapidly eliminated from the body with approximately 27% remaining after 24 hr. It was evident that the retention curve is composed of several components with one component having a considerably longer half-life ($T_{1/2}$ = 24 days). Data indicated that the feces contained more ^{54}Mn than the urine (Figure 2). The urine/feces ratio varied from approximately 0.68 to 0.25; this ratio is in contrast to the normal elimination of Mn, which is primarily fecal with very little appearing in the urine. Whether the ^{54}Mn in the urine exists as a metabolite of ^{54}Mn tricarbonyl or as the ^{54}Mn salt has not been determined; however, these findings plus other evidence would suggest that a Mn metabolite of Mn tricarbonyl is present in the urine. In other studies (Kettering report) where rabbits were either injected or painted with tritium-labeled Mn tricarbonyl, nearly all of the tritium label was excreted in the urine as an acid metabolite. The presence of Mn in the acid metabolite was not determined.

The distribution of ^{54}Mn among the organs of the rat followed a pattern similar to that reported for the normal distribution of this element in animal tissues. At one day post-exposure, the highest concentrations of ^{54}Mn were found in the liver, lung, kidney, and pancreas, liver, and kidney. Smaller amounts of ^{54}Mn were found in bone, brain, testicles, lungs, and blood.

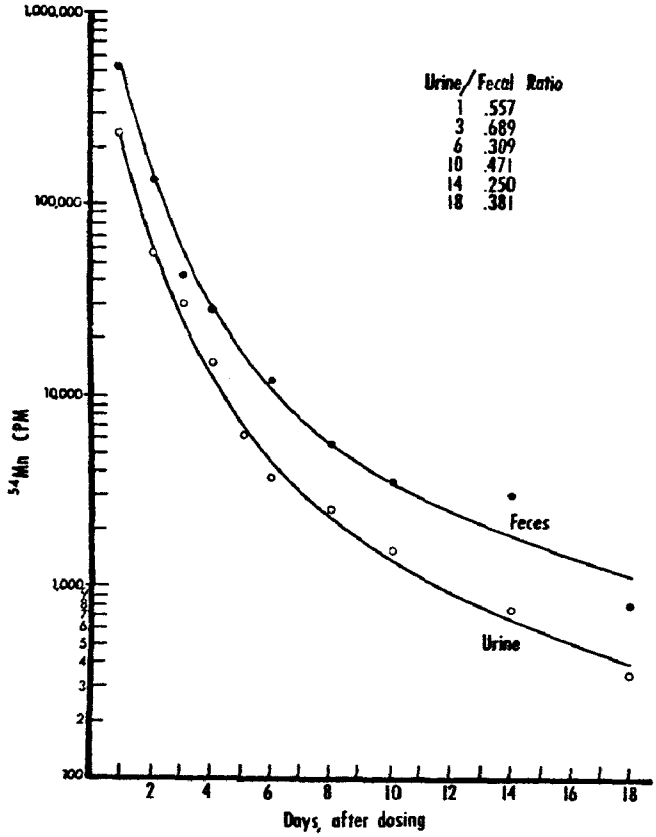


Figure 1. Whole body retention of ^{54}Mn following intragastric administration of ^{54}Mn tricarbonyl.

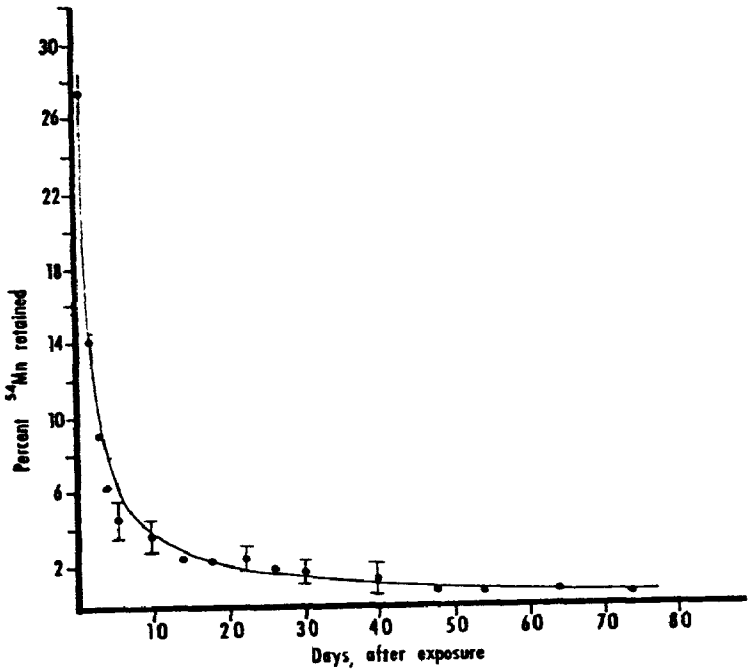


Figure 2. Excretion of ^{54}Mn following intragastric administration of ^{54}Mn tricarbonyl.

UPTAKE AND DISTRIBUTION OF ^{54}Mn IN PREGNANT RATS AND FETUSES FOLLOWING ORAL ADMINISTRATION OF ^{54}Mn TRICARBONYL

W. Moore, D. Hysell, and T. Wessendarp

An initial study on the uptake, distribution, and fetal concentration of ^{54}Mn tricarbonyl is reported. Additional studies on the level of MMT required for fetal toxicity are in process. In this study, female rats were bred and they were given a single oral dose of ^{54}Mn tricarbonyl (2.5 mg MMT). The pregnant females were sacrificed on the 20th day of gestation and the fetuses examined for abnormalities and histological changes. No gross abnormalities were seen. The fetuses and tissues from the dams were counted for determination of ^{54}Mn. The mean concentrations of ^{54}Mn for selected tissues, measured as mean count 1/min/g, are:

Maternal blood	470
Fetus	88
Maternal Placenta	173
" Bone	91
" Liver	916
" Lung	455
" Kidney	470
" Pancreas	658

EFFECTS OF MMT ON LUNG CELLS GROWN IN TISSUE CULTURE

W. Moore, M. Colvin, and J. Everts

Arrangements were made with the Eastern Environmental Radiation Laboratory to study the effects of MMT on cells grown in tissue culture. Lung cells were chosen for the study as inhalation is probably the most important route of exposure. In an effort to delineate the toxicity of MMT from Mn toxicity, the study was designed to compare MMT and $MnSO_4$ or $MnCl_2$ on primary lung cells or on passaged lung cells that were initially grown out in bottles. In this way, the effect of these compounds could be ascertained on cells taken directly from the animals as well as on cells that had gone through more than one division in tissue culture. For chromosomal analysis, only primary cultures were used.

Approximately 1-mo-old Chinese hamsters were sacrificed and the lungs removed for tissue culture. All the lungs were pooled, trypsinzed, and seeded either into roller tubes at 7×10^5 cells/tube or into bottles at 7×10^5 cells/ml. The medium consisted of Eagles plus fetal serum and antibiotics. Since no significant differences were noted in the effects of MMT on primary cells and passaged cells, only the data pertaining to the primary cells will be reported. The growth and appearance in six tubes were analyzed for each dilution using the standard procedure of scoring where 1 indicates approximately 25% of the cells affected, 2 indicates 50%, 3 indicates 75%, and 4 indicates all the cells are dead. A summary of three replicates of this study are presented in Table 1.

There were no marked differences between the effects of MMT and $MnSO_4$ although subjectivly there appeared to be additional changes in the cells exposed to MMT that did not occur in $MnSO_4$ or $MnCl_2$ exposures.

In the study for chromosomal effects, Leighton tubes containing cover slips were seeded along with the roller tubes used in the toxicity study. After 3 days, Colchimed was added to the Leighton tubes and the cover slips were harvested 4 hr later using the routine procedure for preparing cells for chromosomal analysis. The results of two replicates are presented in Table 2. Most of the aberrations consisted of chromatid type lesions.

TABLE 1. ANALYSIS OF GROWTH OF LUNG CELLS FOLLOWING EXPOSURE TO MMT OR $MnSO_4$

Compound	Dilution of Mn	Growth, days after inoculation			Comments
		1	2	3	
MMT	9.8×10^{-7}	±	3	3	Sparse cell population many granular cells, very few growing
	9.8×10^{-8}	-	2	2	Approx. half of cells growing
	9.8×10^{-9}	-	1	1	Some cells granular in appearance and rounded
	9.8×10^{-10}	-	-	-	Growth normal
$MnSO_4$	9.8×10^{-7}	±	2	3	Many dead, other cells granular, some growth
	9.8×10^{-8}	-	±	2	Some dead cells
	9.8×10^{-9}	-	-	-	Growth normal
	9.8×10^{-10}	-	-	-	Growth normal

± Slight changes in appearance
1 Approximately 25% of the cell sheet affected
2 Approximately 50% of the cell sheet affected
3 Approximately 75% of the cell sheet affected
4 All of the cells are rounded up or detached

TABLE 2. PERCENT OF ABERRANT LUNG CELLS FOLLOWING EXPOSURE TO Mn TRICARBONYL AND $MnSO_4$

Compound	Exposure dilution of Mn	Cells scored	Number of aberrants	%
MMT	9.8×10^{-7}	150	5	3.33
	9.8×10^{-8}	171	6	3.50
	9.8×10^{-9}	120	4	3.33
	9.8×10^{-10}	326	14	4.29
	9.8×10^{-11}	509	23	4.51
	9.8×10^{-12}	473	12	2.53
	9.8×10^{-13}	461	9	1.95
$MnSO_4$	9.8×10^{-7}	Cells died		
	9.8×10^{-8}	Cells died		
	9.8×10^{-9}	270	6	2.22
	9.8×10^{-10}	272	9	3.30
	9.8×10^{-11}	409	12	2.93
	9.8×10^{-12}	380	2	0.52
	9.8×10^{-13}	368	4	1.08
Control		386	6	1.55

MANGANESE EFFECTS ON FIXED-INTERVAL PERFORMANCE OF MONKEYS

M. I. Gage

Symptoms of manganese (Mn) toxicity are similar to those observed in patients who have Parkinson's Disease; notably tremor, difficulty in executing movements, and other extrapyramidal motor system symptoms. Relief from the symptoms of Mn poisoning has been obtained by the administration of the drug l-DOPA. The motor difficulties associated with Mn toxicity have been produced in the Macaque monkey and the chimpanzee by subcutaneous injection of manganese dioxide (MnO_2). A decrease in dopamine in the caudate nucleus of squirrel monkeys injected with MnO_2 has also been reported. Based on these findings, a pilot study was begun to determine if an objective, repeatable behavioral measure of motor responses can be used to estimate the toxic threshold dose level of Mn.

Four young rhesus monkeys (Macaca mulatta), one male and three female, were trained to push a round, plastic button to obtain banana flavored food pellets on a fixed-interval, 1-min. schedule of reinforcement (FI 1-min.). A pellet was dispensed for the first press made after 61.44 sec had elapsed since the last pellet was dispensed. The monkeys were given half-hour sessions daily, 5 days/wk. After performance on this schedule stabilized and the pattern of presses during the interval assumed the typical scallop shape (the frequency of responses increased as the interval approached termination), two of the monkeys were given a single subcutaneous injection of 400 mg/kg MnO_2 suspended in olive oil in the mid torso area and the other two monkeys were injected with a similar volume of olive oil. Performance continued to be observed on a daily basis after the injections. Approximately 3 mo after the manganese was administered, blood and 24-hr urine samples were analyzed for Mn and urine was analyzed for delta aminoleveilinic acid concentration.

The experiment is still in progress so only preliminary analysis of the findings can be presented. Three monkeys (the two injected with MnO_2 and the male control) showed a slight decrease in response rates on

the FI 1-min. schedule beginning about 3 mo after MnO_2 administration. The male's decrease, however, started after the decrease began in the treated monkeys. This rate decrease seemed to be progressive, but it is too soon to fully evaluate this trend. Blood and urinary Mn levels taken soon after the onset of the behavioral change were low and in the normal range in all monkeys; however, Mn levels in the treated animals were slightly higher than the levels in the controls.

In other reported experiments, the onset of observable motor symptoms of manganism began about 3 mo after MnO_2 injections in chimpanzees but began 9 mo after MnO_2 was administered to rhesus monkeys. The cause of this variability is unknown. Monkeys in the present pilot study are not displaying any obvious motor difficulties during the fourth month since MnO_2 injections. Possibly enough time has not yet elapsed for the observable symptoms of manganism to begin.

The behavioral measure of response rate on a learned operant task may be quite sensitive for evaluating performance changes due to small increases of Mn in the body. The decreases seen in the injected monkeys may represent early signs of manganism. The small sample size in the present study, however, makes it difficult to determine if the test is a good general purpose screening tool. The experiment needs to be continued until the injected monkeys display obvious abnormalities of motor behavior and needs to be repeated on a larger sample of monkeys.

EFFECT OF MANGANESE ON THE RAT VISUAL EVOKED POTENTIAL

J.P. Lewkowski, W. Moore, and J.F. Stara

The application of various computer averaging techniques has enabled investigators to observe changes in the visual evoked potential. Consequently, a wealth of information has been accumulated on the topographical distribution of various waveforms and the effects of drugs on these evoked potentials.

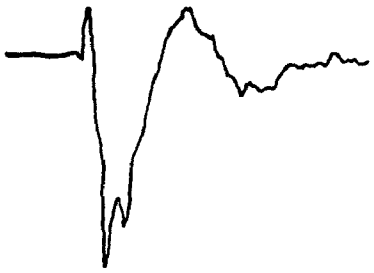
Furthermore, although some work has been done on the effects of pollutants on the visual evoked potential, few investigations have been performed on the effects of various toxicological agents such as the heavy metals. The effect of manganese is currently being tested since this cation is presently used as a fuel additive.

Preliminary experiments have indicated that the intravenous administration of low levels of manganese elicits a transient but highly reproducible change in the rat visual evoked potential. Figure 1 shows the results of one such experiment. A is the control evoked potential before the manganese injection. Immediately after the control was recorded, 1.4 mg/kg of manganese was administered. The effect 5 min. after the injection can be observed in B. However, the control waveform is elicited 10 min. after the manganese administration (C).

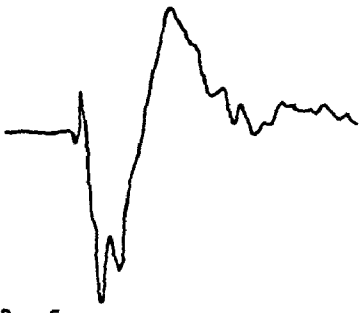
The fact that the averaged evoked response is reproducible and remains somewhat constant can be seen by comparing C and D which were recorded 10 and 30 min. after the manganese administration. C and D are also comparable to A, which was recorded before the manganese injection.

An additional 1.4 mg/kg of manganese was administered immediately after D. Again, a change in the evoked potential is elicited approximately 90 msec. after the light flash. Control evoked potential waveforms are again observed 10 min. after the manganese injection. Immediately after record G, a comparable volume of saline was administered. Little, if any, change is evident in H which was recorded 5 min. after the saline injection.

Thus, the administration of low levels of manganese has been shown to elicit a reproducible change in the rat visual evoked potential. Further work is currently underway to determine the mechanism responsible for the observed change as well as to determine the effects of various oher cations.



A. THE CONTROL AVERAGED EVOKED POTENTIAL PRIOR TO THE ADMINISTRATION OF Mn



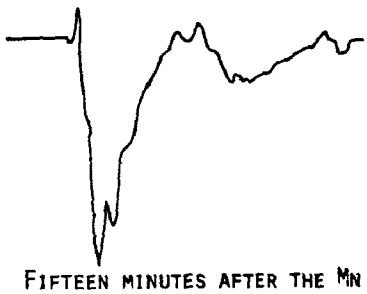
B. FIVE MINUTES AFTER THE INTRAVENOUS ADMINISTRATION OF 1.4 MG/KG

C. TEN MINUTES AFTER THE Mn ADMINISTRATION

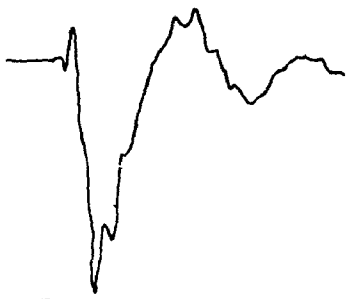
D. THIRTY MINUTES AFTER THE Mn ADMINISTRATION

E. FIVE MINUTES AFTER ANOTHER ADMINISTRATION OF 1.4 MG/KG Mn

F. TEN MINUTES AFTER THE Mn ADMINISTRATION



G. FIFTEEN MINUTES AFTER THE Mn ADMINISTRATION



H. FIVE MINUTES AFTER A SALINE ADMINISTRATION OF COMPARABLE VOLUME

Figure 1. Effect of low levels of Mn, administered intravenously, on rat visual evoked potential.

ENVIRONMENTALLY BOUND LEAD: I. BLOOD LEVELS IN RATS FOLLOWING A SINGLE ORAL DOSING

J.F. Stara, M.K. Richards, S. Neiheisel, Y.Y. Yang
W. Moore and K. Bridbord

For the process of setting meaningful environmental standards, it is necessary to obtain biological data for exposure to lead as it actually occurs in the environment. As a source of environmentally bound lead, dust samples were collected in New York's Queens Tunnel, the Los Angeles Freeway, and the immediate vicinity of the El Paso smelter. The dust samples were separated by sieving and analyzed for lead content. The fine particles were used for animal exposures since they contained higher amounts of lead (0.6-2.4%) and also because of their greater potential biological activity. Lead analyses of the dust samples were performed at four different laboratories (Table 1).

TABLE 1. LEAD CONTENT (%) IN THREE DUST SAMPLES

Site of collection	Laboratory Analyses			
	A*	B	C	D
Queens Tunnel, N.Y.	5.78	1.7	2.53	2.43
Los Angeles Freeway	3.20	1.03	1.06	1.04
Vicinity of El Paso Smelter	2.81	0.77	0.64	0.61

*Values from Lab A were discarded because of analytical error.

Gelatin capsules filled with dust from Queens Tunnel of sufficient quantity to deliver a dose of 10 mg of Pb were orally administered to a group of rats. Daily blood samples (0.2 ml) were taken from the orbital sinus up to 36 days following Pb ingestion. All samples were analyzed for lead content by the New York State Department of Health Laboratory. The mean blood levels are presented in Figure 1. Twenty-four hours after dosing, the lead blood level in the experimental group rose to an average

of 45 g/100 ml and thereafter decreased sharply so that by day 15, it did not differ significantly from controls. The mean blood levels of the control animals were approximately 10-15 g/100 ml; these agree with values reported in the literature. Similarly, tissue analyses showed increased concentration of lead in the bone and G.I. tract of the experimental animals.

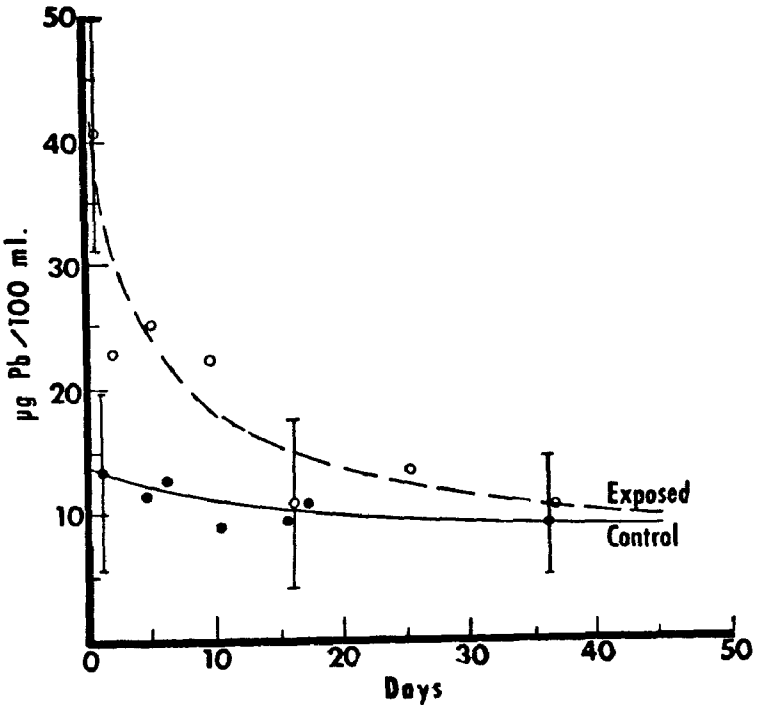


Figure 1. Mean Blood levels in rats following a single oral dose of 10 mg lead in dust from Queens Tunnel.

ENVIRONMENTALLY BOUND LEAD
II. EFFECT OF DOSE ON BLOOD AND TISSUE LEVELS OF RATS

J. Stara, W. Moore, M. Richards,
S. Neiheisel, R. Miller and K. Bridbord

The effect different dose levels have on lead absorption in rats was tested by feeding Queen's Tunnel dust at dose levels of 0.5, 1.0, and 5.5 mg lead/day in a specially-prepared diet. Figure 1 shows the fitted curves of the mean blood levels; the dose dependency is clearly indicated. An expoential equation was used in fitting a curve to the data. The lead level rose to a peak of 55 µg/100 ml on day 9 in the high dose group (5.5 mg). Peak blood levels reached 37 µg/100 ml in the medium dose group (1.0 mg) and 33 µg/100 ml in the low dose group (0.5 mg). After the blood levels reached their highest plateau, a slow descreasing trend was observed. This decreasing level may be due to such factors as growth, aging with reduced G.I. absorptive capability for lead, biological saturation of the system, or other unknowns.

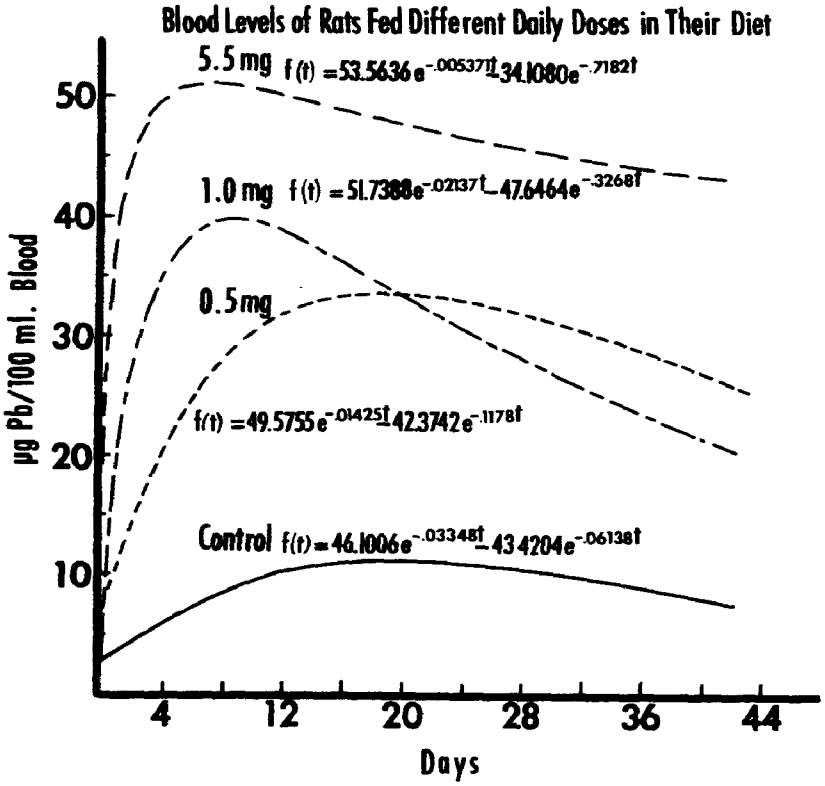


Figure 1. Fitted mean blood levels of rats fed different daily doses of lead in their diet; vehicle:dust collected in Queen's Tunnel, N.Y.

A large accumulation of lead was seen in the skeleton, and the kidney (Table 2). Other tissues such as blood, brain, liver, and lung also showed significant increases in the treated group of animals.

TABLE 2. TISSUE LEVELS OF LEAD IN RATS FED DIFFERENT DAILY DOSES

	Dose (µg Pb/g Tissue)			
Tissue	5.5mg	1.0mg	0.5mg	Control
Femur	66.1	33.6	25.7	0.75
Kidney	9.4	3.2	2.5	-
Liver	1.3	0.52	0.56	0.13
Brain	0.28	0.055	0.030	0.032

	Dose (µg Pb/organ)			
Organ	5.5mg	1.0mg	0.5mg	Control
Skeleton*	1487.0	551.0	412.0	11.9
Kidney	30.8	7.7	5.9	1.3
Liver	13.5	3.8	3.9	.98
Brain	1.0	.15	0.08	.08

*Total bone (skeleton) estimated as 7.41% of body weight (unpublished data).

ENVIRONMENTALLY BOUND LEAD: III. EFFECTS OF SOURCE ON BLOOD AND TISSUE LEVELS OF RATS

J. Stara, W. Moore, M. Richards, N. Barkley, S. Neiheisel and K. Bridbord

To determine if the biological availability of environmentally bound lead, as measured by G.I. absorption and resulting blood uptake, may vary with the source, dust samples collected from Queen's Tunnel, N.Y., Los Angeles Freeway, and the vicinity of El Paso Smelter were used in this experiment. Three groups of rats were fed a special low-lead diet for 55 days. Each group received one type of dust that was mixed in the special diet at the rate of 1 mg Pb/day. The results (Figure 1) show that the highest absorption, as measured by the blood level (45 µg/100 ml), were observed in animals fed the Los Angeles dust samples. The rats fed the New York dust had intermediate values, and the lowest values were obtained in the El Paso group of animals. A curve was fitted to the data by use of an expoential equation. Analysis of all blood and tissue data is not complete at this time.

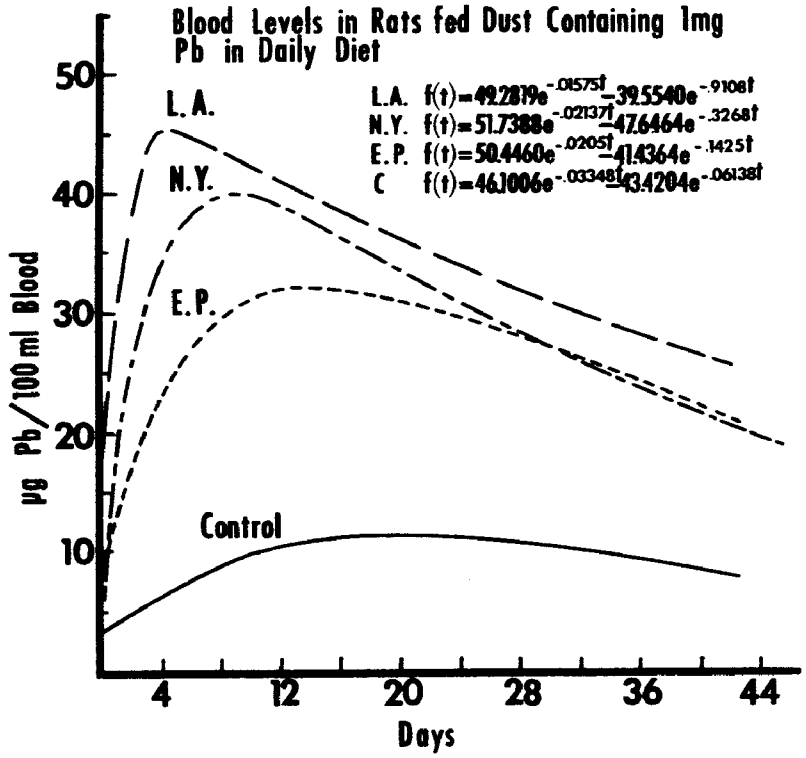


Figure 1. Fitted mean blood levels in rats fed 1 mg lead containing dust in daily diet; collection sites: Queen's Tunnel, Los Angeles Freeway and El Paso Smelter.

The uptake in tissues and resulting concentrations, however, indicated that the lead in New York dust was most readily absorbed. The tissue levels for the New York dust were, on the whole, higher than in the other two samples (Table 1). Of the organs examined, the highest concentrations were observed in the bones, kidneys, and liver, in that order.

TABLE 1. TISSUE LEVELS OF LEAD IN RATS FED DAILY 1 mg OF Pb IN DUST COLLECTED FROM THREE SOURCES

Animal Source	N. Y.*	L. A.+	E. P.‡	Control
Organ, µg Pb				
Skeleton	551.0	532.0	375.0	11.9
Kidney	7.7	6.4	5.9	1.3
Liver	3.8	2.3	2.5	.98
Brain	.15	.25	.10	.08
Tissue, µg Pb/am				
Femur	33.6	32.5	23.6	0.75
Kidney	3.2	2.7	2.5	-
Liver	0.52	0.32	0.36	0.13
Brain	0.055	0.094	0.035	0.032

* Queen's Tunnel, New York City
\+ Los Angeles Freeway
‡ El Paso Smelter

It is extremely difficult, using stable elements and standard techniques, to determine precisely the absorption of a metal like lead where the amount absorbed is very small (less than 5%). Using radioactive tracers, which permit the detection of very low concentrations in various tissues, is the most accurate method.

LEAD: PLACENTAL TRANSFER, CENTRAL NERVOUS SYSTEM EFFECTS, AND IMMUNE RESPONSE ALTERATION

Dr. W. B. Buck et al., under a contract with EPA is investigating the placental transfer of lead and characterizing the neurophysiological and behavioral effects of lead exposure in lambs. In addition, they are exploring the effect of lead on the immune response mechanism.

To investigate the placental transfer of lead, three groups of yearling ewes were fed powdered metallic lead beginning 3 weeks before breeding, continuing throughout gestation, discontinuing at parturition. All animals were then trained and tested on a series of seven visual discrimination problems (Figure 1). The results are observed in Table 1. The authors conclude that lambs from ewes fed subclinical levels of lead during gestation had statistically significant decreased performance on visual discrimination tasks in comparison with lambs from nonexposed ewes.

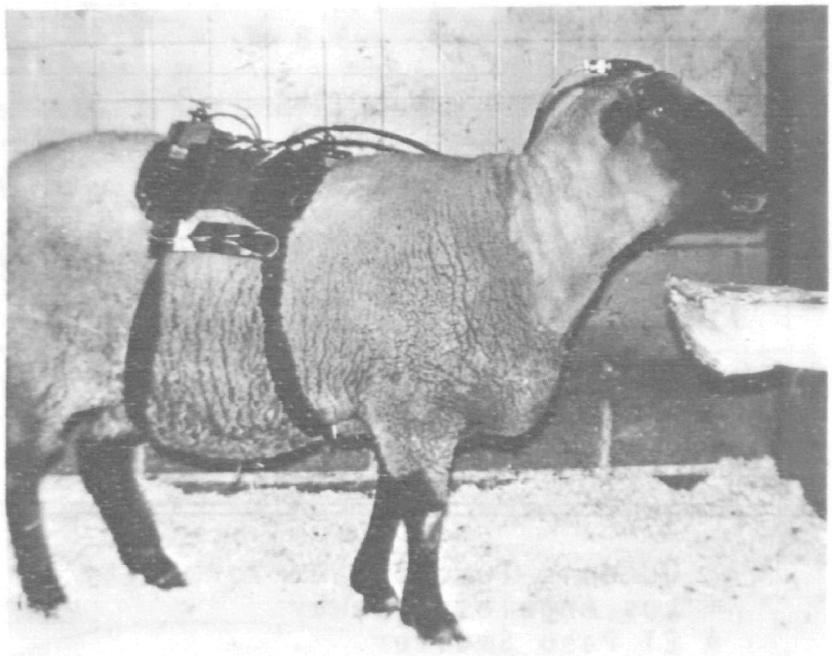


Figure 1.

Analysis of lead transfer through the placenta to the rat fetus are in progress. These data will be compared with data received in the lamb study. A determination of the effect of lead on learning and problem-solving ability is currently being investigated through the use of a modified Hebb-Williams maze. In addition, experiments to ascertain the effect of lead exposure on serum immune protein of young sheep and their immune response to bacterial antigens are also in progress. A comprehensive treatise on the effects of lead in the three areas studied will be available upon the completion of these studies.

TABLE 1. VISUAL DISCRIMINATION PERFORMANCE AND BLOOD LEAD IN LAMBS FROM EWES FED SUBCLINICAL LEAD DURING GESTATION

Prenatal Lead Exposure Group	Group Mean Days to Criterion for Visual Discrimination Problems								Blood Lead in PPM	
	Problem Number							Total for All Problems	Age (weeks)	
	1	2	3	4	5	6	7		2-4	10-12
	Problem									
	L/D[a]	○△	≡|||	▽△	▨▨	⊏⊐	○ ○			
Control[b] ($\bar{X}$ of 4 animals)	3.5	6.7	4.3	3.3	4.8	4.3	12.6	41.8	0.06	0.04
"Low" Lead[c] ($\bar{X}$ of 8 animals)	3.1	6.4	4.4	5.5	4.4	9.8	13.0	46.5	0.17	0.09
"High" Lead[d] ($\bar{X}$ of 6 animals)	3.5	8.3	4.0	6.3	5.3	16.7	29.5*	73.7*	0.27	0.14

[a]Light versus dark.

[b]The lambs in this group are from ewes which received no supplemental lead during gestation. The mean blood lead of these ewes was 0.06 ppm (6 µg/100 ml) during gestation.

[c]The lambs in this group are from ewes which received 2.3 mg lead/kg body weight daily throughout gestation. Mean blood lead of these ewes was 0.16 ppm (16 µg/100 ml) during gestation.

[d]The lambs in this group are from ewes which received 4.5 mg lead/kg body weight daily throughout gestation. Mean blood lead of these ewes was 0.30 ppm (30 µg/100 ml) during gestation.

*Significant at $P<0.05$.

GASTROINTESTINAL ABSORPTION OF DIFFERENT COMPOUNDS OF ^{115m}Cd AND THE EFFECT OF DIFFERENT CONCENTRATIONS IN THE RAT

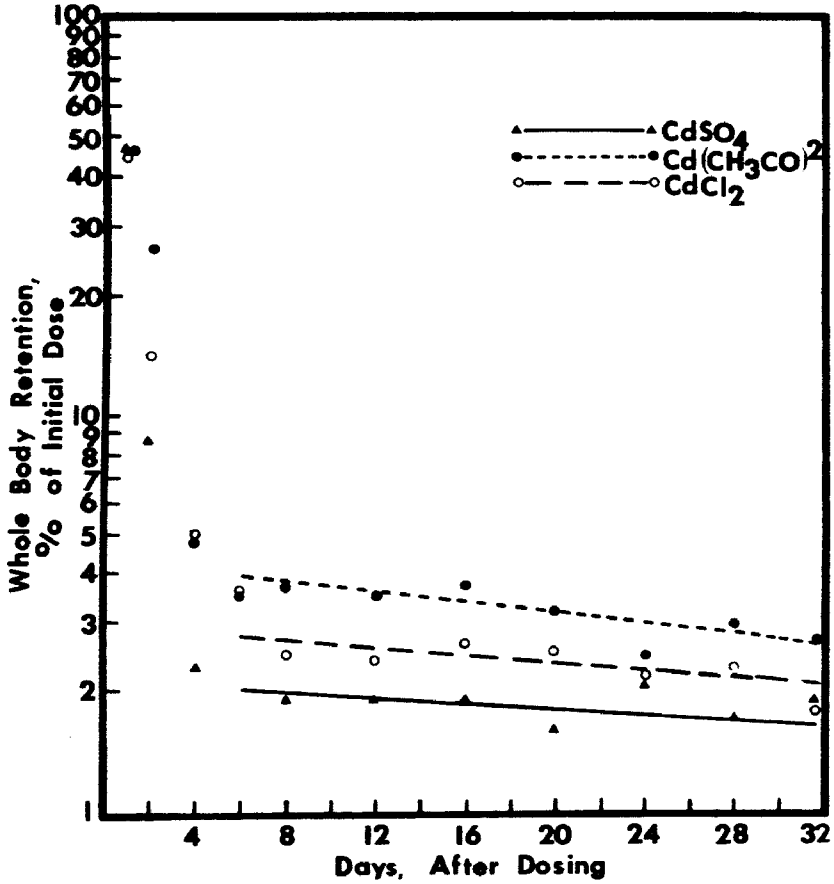
W. Moore, J. F. Stara and W. Crocker

Several studies were undertaken to determine kinetic and metabolic fate of different compounds of cadmium to provide information not currently available in the literature.

An investigation was designed to determine whether or not different chemical forms of this element influenced the absorption and metabolism of Cd following oral administration. The retention rates for ^{115m}cadmium chloride, ^{115m}cadmium sulfate, and ^{115m}cadmium acetate following a single dose given via stomach tube are shown in Figure 1.

Figure 1

Retention rates of three cadmium compounds after rats received single doses.



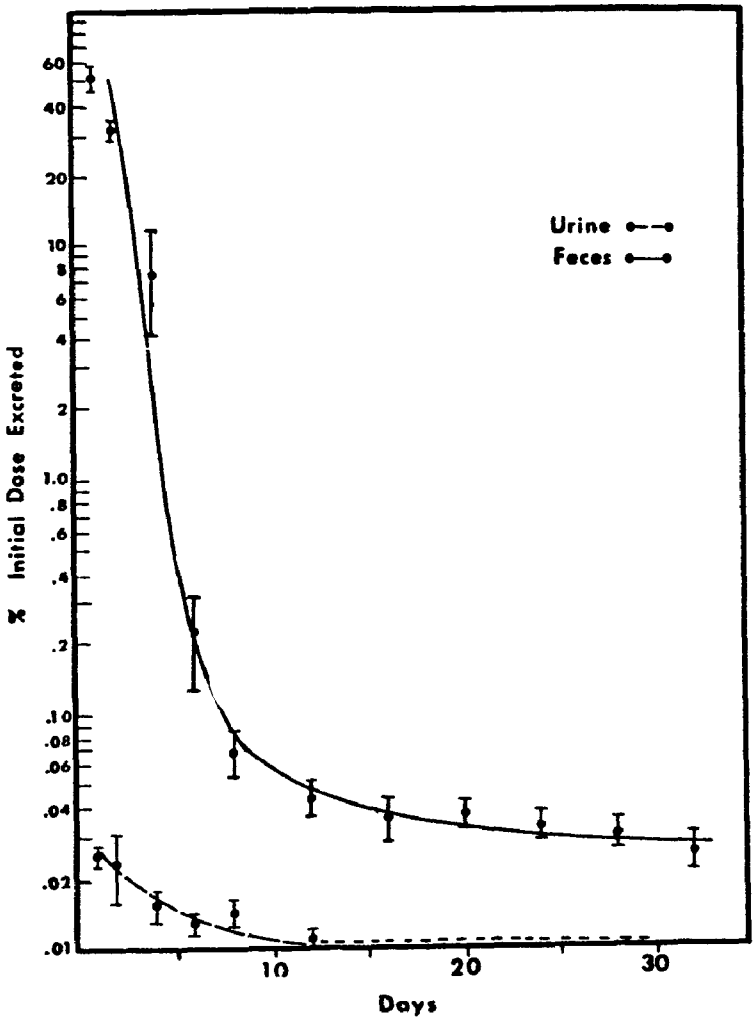
The results indicated that the type of cadmium compound did not significantly influence the G.I. absorption and retention.

of ^{115m}Cd although the amount of ^{115m}Cd retained following administration of ^{115m}cadmium acetate was somewhat higher. There was an initial rapid clearance during the first 4 days, which represented the passage of nonabsorbed cadmium through the intestinal tract. Fasting the animals for 24 hr did not significantly influence absorption or the time required for transit of the ^{115m}Cd compounds $CdCl_2$ through the G.I. tract. In the group of rats that was not fasted before dosing, 58.0% of the ^{115m}cadmium chloride was retained at the end of 24 hr and 3.01% at the end of 4 days, whereas in the fasted group, the values were 55.6% and 3.1%, respectively. Thus, after 6 days, between 2.7% and 3.5% of the ^{115m}Cd was absorbed when given as cadmium chloride, cadmium sulfate, or cadmium acetate.

Radioactive counts of the feces and urine (Figure 2) showed that the most of ^{115m}Cd was eliminated via the feces and only an extremely small amount was found

Figure 2

Percent of original dose of ^{115m}cadmium excreted in feces and urine following oral administration.



in the urine. Twenty-four hours after administration of the ^{115m}cadmium acetate, 52.1% of the initial dose was present in the 24-hr feces sample and 0.025% of the initial dose was in the 24-hr urine sample. Six days after dosing, 0.22% and 0.013% was found in 24-hr samples of feces and urine, respectively. After the first few days, there was a very low continuous rate of excretion of ^{115m}Cd in the feces during the entire period of study. At 32 days post exposure, 0.026% of the initial dose was present in a 24-hr feces sample. After a single oral dose, the only tissues containing significantly the concentration or distribution of ^{115m}Cd in the tissues.

Three different levels of $^{115m}CdCl_2$ were given orally to rats to investigate the effect of dose upon Cd absorption and body retention. The amount of Cd given influenced the amount absorbed by the G.I. tract and resulting tissue concentrations. The mean amount of Cd in the livers and kidneys (µg/g wet sample) for the three concentrations is shown in Table 1. The animals receiving the highest concentration had the greatest amount of Cd in the liver and kidney although the increase was not proportional to the increase in concentration.

TABLE 1. TISSUE CONCENTRATIONS OF CADMIUM

Amount Cd Administered, mg/animal	µg Cd/gm of Tissue, wet weight	
	Kidney	Liver
0.060	0.073	0.102
0.75	0.253	0.772
7.5	0.772	1.564

EFFECT OF DIFFERENT ROUTES OF ADMINISTRATION OF ^{115m}CADMIUM CHLORIDE UPON WHOLE BODY RETENTION IN RATS

W. Moore, J. Stara, M. Malanchuk and R. Iltis

The influence of the route of administration (intravenous, intraperitoneal, intragastric, and inhalation) on Cd retention was determined and biological half-life calculated (Figure 1). The retention curve for each of the routes of administration was divided into two components. The first component reflected the initial rapid clearance of ^{115m}Cd primarily by the G.I. tract and the second component indicated the absorption and turnover of ^{115m}Cd. Extrapolation of the second component to the intercept gave initial retention values of 93%, 91%, 41% and 2.3% for intraperitoneal, intravenous, inhalation, and oral routes, respectively. The biological half-life of the major retention component was greater than 175 days for all routes.

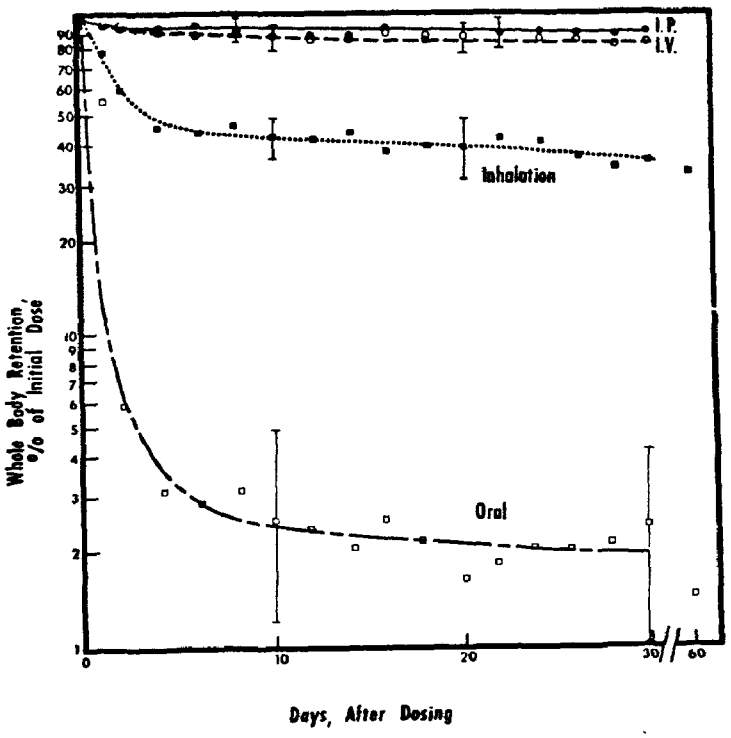


Figure 1. Retention curves indication percent whole body retention of ^{115m}Cd in rats following inhalation exposure to $^{115m}CdCl_2$ and ^{115m}CdO

RETENTION OF ^{115m}CADMIUM CHLORIDE AND ^{115m}CADMIUM OXIDE FOLLOWING INHALATION EXPOSURE

W. Moore, M. Malanchuk, R. Miller and W. Crocker

The whole body retention of $^{115m}CdCl_2$ and ^{115m}CdO following inhalation exposure is shown in Figure 1. Following inhalation exposure, considerably more $^{115m}CdCl_2$ than ^{115m}CdO was retained. Tissue values for the liver and kidney were also higher for $^{115m}CdCl_2$, which indicates greater absorption of this form of Cd by the lungs. The data indicated that approximately 54% of the initial lung burden of $^{115m}CdCl_2$ was present in the lungs 64 days post-exposure.

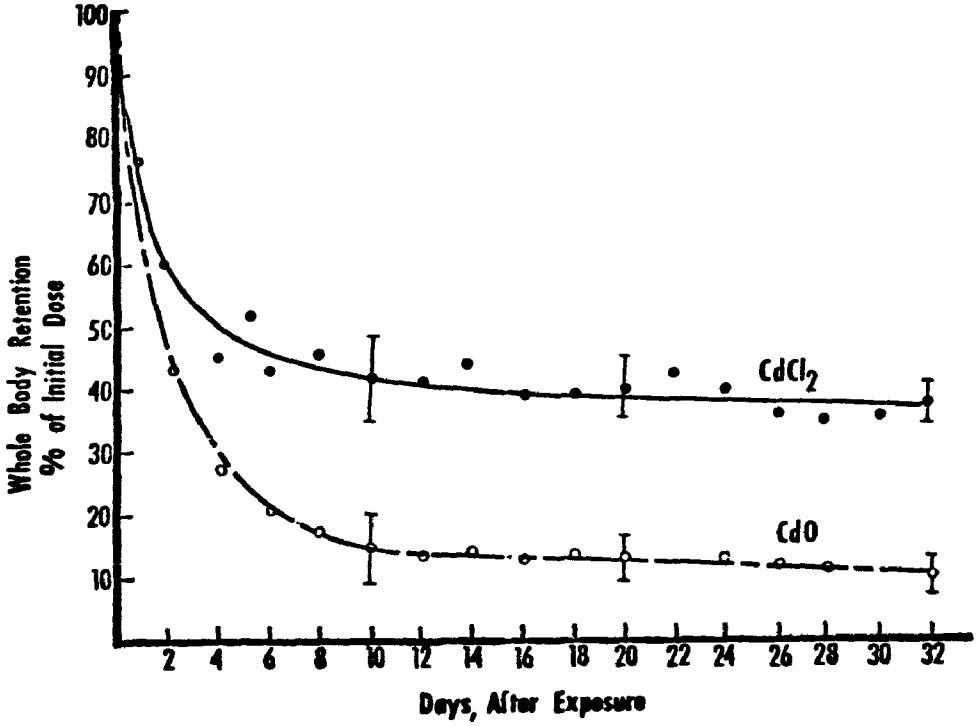


Figure 1. Retention curves indicating percent whole body retention of ^{115m}Cd in rats following inhalation exposure to $^{115m}CdCl_2$ and ^{115m}CdO.

EFFECT OF CADMIUM INGESTION ON BLOOD PRESSURE IN MONKEYS

M.J. Wiester, W. Moore and J.F. Stara

A number of investigators have theorized on the probability that environmental intake of cadmium may be intimately involved in various types of pathological processes throughout the life span of man. Cardiovascular, as well as renal, disease has been associated with cadmium exposure both in animals and man.

Presently, a pilot study is in progress that will indicate dietary levels of cadmium that will produce a pressor effect in rhesus monkeys. Parameters other than blood pressure that are observed are weight, urinalysis, and terminally, tissue cadmium levels, particularly that of the kidney cortex. In this pilot study, two rhesus monkeys are fed 100 mg/day $CdCl_2$ along with their regular diets. With this regime, we anticipate a pressor effect after approximately 2 mo. Conclusions drawn from this brief study will be incorporated in a more extensive attack on the problem using monkeys as the primary animal. The animals are monitored once a week throughout the experiment. Control levels of blood pressure were established over a period of 8 wks. before cadmium feeding. To measure blood pressure, monkeys are tranquilized with sernylan (2 mg I.M.) and chaired; pressures are read 2-3 hr. later when animals are alert. Measurements are made by use of noninvasive tail cuff method, which records systolic and diastolic pressure. This method has been verified by a series of experiments in which cuff pressures were compared with directly measured abdominal aortic pressures. The results agreed within 5-8 mm Hg (personal obsdrvations). Catherized urine specimens are taken while the animals are under the influence of the drug. In this study, no precautions have been taken to keep the animals endemically cadmium-free or to control zinc intake. A diet identical to the one used for the last 3 yr. is continued with the addition of 100 mg/day of $CdCl_2$ during the experimental period.

Since there is normal variation in blood pressure from monkey to monkey and small numbers of animals are involved, each animal will serve as its own control. The control period pressures for the monkeys are summarized in Table 1. On 12/5/72, the two monkeys were started on the cadmium contaminated diet. Analysis of the data will depend on the magnitude of pressure increase as well as the variations encountered as the hypertension progresses. Variations in

TABLE 1. BLOOD PRESSURE READINGS OF MONKEYS DURING CONTROL PERIOD

Animal		Systolic pressure, mmHg	Diastolic pressure, mmHg	Mean pressure, mmHg
Monkey #8	10-5-72	114.0	80.0	91.3
	10-13-72	115.5	65.0	81.3
	10-18-72	97.0	84.5	88.6
	10-26-72	108.5	66.5	80.5
	11-9-72	113.3	66.0	81.7
	11-21-72	106.5	70.0	82.1
	11-29-72	98.8	66.2	77.0
	12-5-72	99.5	66.0	77.2
	Mean for period	$\bar{x}$ 106.6 S.D.$\pm$ 7.4	$\bar{x}$ 70.5 S.D.$\pm$ 7.5	$\bar{x}$ 82.5 S.D.$\pm$ 5.1
Monkey #29	10-5-72	125.0	85.0	98.3
	10-13-72	138.3	91.3	106.9
	10-18-72	134.3	89.3	104.3
	10-26-72	121.0	90.3	100.5
	11-9-72	136.6	94.2	108.3
	11-21-72	113.5	71.2	85.3
	11-29-72	116.6	74.3	88.4
	12-5-72	138.7	99.7	112.7
	Mean for period	$\bar{x}$ 128.0 S.D.$\pm$10.2	$\bar{x}$ 86.9 S.D.$\pm$ 9.7	$\bar{x}$ 100.6 S.D.$\pm$ 9.6

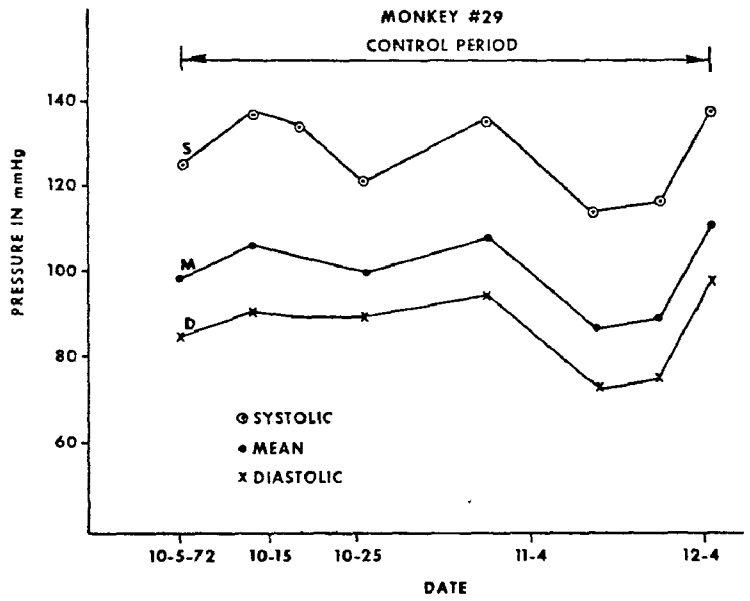


Figure 1. Variations in Tail-Cuff Blood Pressure From An Unanesthetized Monkey over 8-Week Control Period

weekly blood pressure measurements for the control period are shown in Figure 1. Experimental pressure readings will be analyzed in a classical way by observing the distribution trend of pressures versus time, which yields a definite increase in slope. If variations in weekly readings are great and the pressor effect extends into a long period of time, however, mean values and changes in standard deviation for successive segments of time will be considered.

An increase in blood pressure, as well as an estimate of renal damage (proteinuria), is anticipated as the cadmium ingestion period progresses. These changes will be correlated with time-cadmium ingestion and kidney tissue cadmium content.

EARLY BIOCHEMICAL EFFECT OF METHYLMERCURY CHLORIDE IN RATS

S. Lee, K. Butler, R. Danner, B. Johnson, L. McMillan, W. Moore and J. Stara

Several investigators have reported on biological effects of various pollutants. There still is a paucity of information concerning the effects of pollutants on biochemical interactions, however, especially of those at low, relevant concentrations of environmental pollutants where in vivo systems in nonterminal experiments have been used. Such studies are particularly valuable if repetitive testing on the same animal is desired. In addition, this type of effect evaluation has the advantage of using the same animal as its own control, and thus, allows the study of cumulative effects of certain pollutants as well as the processes of recovery from those effects. The primary objective of this series of experiments is to develop new methods or apply existing ones capable of detecting early biochemical changes in the study of low concentrations of environmental pollutants before the appearance of overt toxic symptoms.

Radiorespirometry, an experimental approach in which kinetic data on catabolism of ^{14}C-labeled substrates by a biological system can be obtained by the measurement of expired $^{14}CO_2$, was used for this purpose. This method allows dose:response measurements of substrate catabolism following exposure to selected environmental pollutants. It also may be applicable for use as a test in a rapid toxicological screening program of environmental pollutants. Of the several fates of carbon in animal systems, one of the most important, quantitatively, is its incorporation into CO_2 expired air. The measurement of CO_2 in expired air serves as an excellent indicator of overall metabolic rate. This method, however, does not provide information on the cumulative catabolism of a specific biological compound or metabolite to CO_2. This deficiency of specificity is overcome by the use of radioactively labeled compounds as substrates. Thus, the amount of $^{14}CO_2$ expired following administration of a labeled organic compound can be used in intact animals to study the metabolism of specific compounds as well as to study a particular labeled carbon atom in the compound. After establishment of the normal $^{14}CO_2$ respiratory pattern of a given labeled compound in a biological system, a variation in this pattern can be used as an indicator of

change in the physiological or biochemical state of the animal. Cary vibrating reed electrometers were employed in conjunction with ionization chambers to measure radioactivity in the expired air. The animal exposure system and associated instrumentation are depicted briefly in Figure 1.

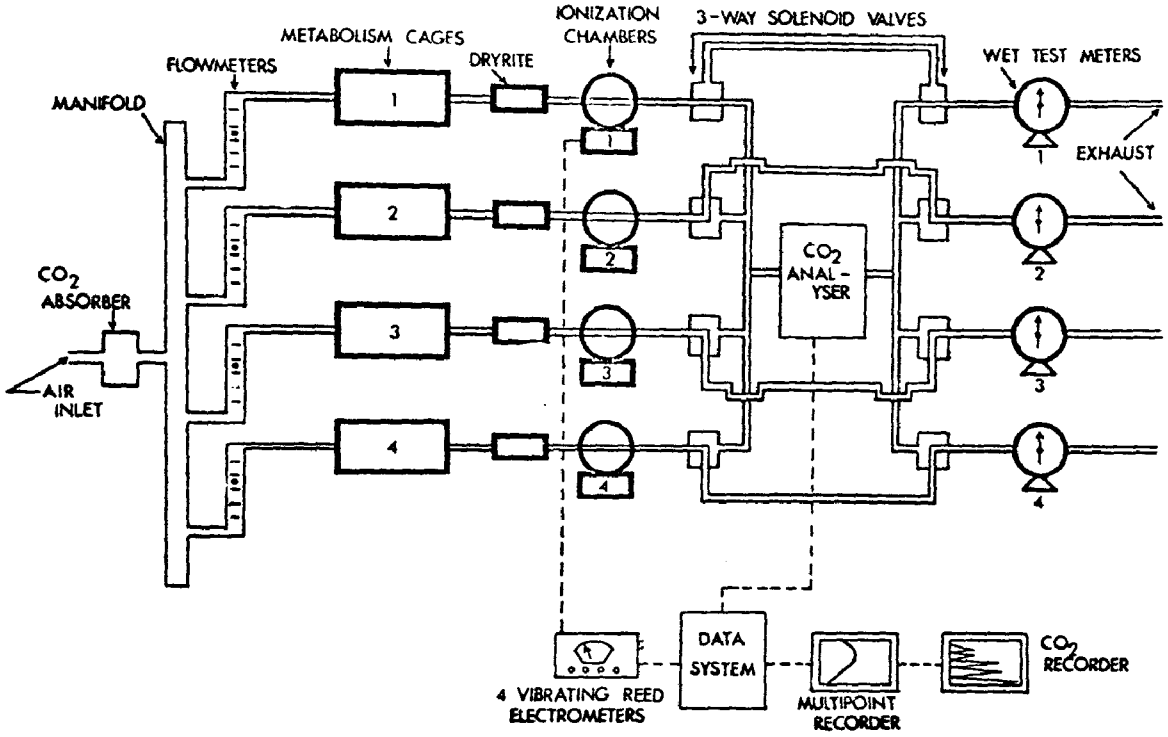


Figure 1. Diagram of Radiorespirometry Flow System

Intragastric administration of methylmercury chloride suppressed $^{14}CO_2$ output following intravenous injection of 1-^{14}C-glucose. Following the initial treatment with 0.05 and 0.10 mg/kg body weight, decreases of 6.6% and 12.1%, respectively, in respired $^{14}CO_2$ were observed. When the same treatment was repeated for each group of animals, metabolic suppression of 17.9% for the 0.05 mg group and 30.8% for the 0.10 mg group were observed. These results show that methylmercury chloride at low levels of 0.05 and 0.10 mg/kg body weight caused metabolic alteration as measured by the radiorespirometric technique. This approach demonstrated changes as early as 24 hr. after ingestion. The observed effect apparently is cumulative, since a second dose, given 1 wk. later caused an almost three-fold decrease in expired $^{14}CO_2$ when compared with the level recorded following the initial dose (Table 1).

TABLE 1. EFFECT OF REPEATED DOSES OF METHYLMERCURY CHLORIDE ON CUMULATIVE $^{14}CO_2$ OUTPUT AFTER ^{14}C-1-GLUCOSE INJECTION

Experimental Group	First Dose, % Alteration	Second Dose, % Alteration
Control	0	0
0.05 mg/kg B.W.	- 6.6	-17.9
0.10 mg/kg B.W.	-12.1	-30.8

In the concentration range used, there is no apparent dose response to a single-dose administration of CH_3HgCl on blood glutathione (Figure 2). A greater decrease in blood glutathione concentration was observed with respect to time, however, after CH_3HgCl was administered. The implication of this finding is a very broad one because numerous enzymes in our body require presence of optimum amount of glutathione for their normal functions. In vitro evidence in the literature supports this contention; however, our finding suggests the necessity of in vivo studies of specific enzymes in relation to the time of CH_3HgCl administration. With additional experimentation, including the more time-consuming "constant infusion technique" and use of ^{14}C-glucose labeled in other positions, it may be possible to demonstrate changes in relative participation of various pathways in glucose metabolism.

One must be cautious in interpretating these preliminary data. the changes reported herewith may or may not be detrimental to the animals, especially if the changes are temporary. Furthermore, the toxicity of methylmercury appears to be influenced by other factors such as the composition of the media. Ganther et al. recently demonstrated that 20 ppm methylmercury in a diet containing 17% (by weight) tuna was less toxic than the same concentration of methylmercury in corn-soya diet fed to Japanese quail. These investigators also showed that selenium content in the diet, comparable to that contained in the tuna diet, decreased methylmercury toxicity in rats. Tuna meat contains a relatively high concentration of selenium and tends to accumulate additional selenium when mercury is present.

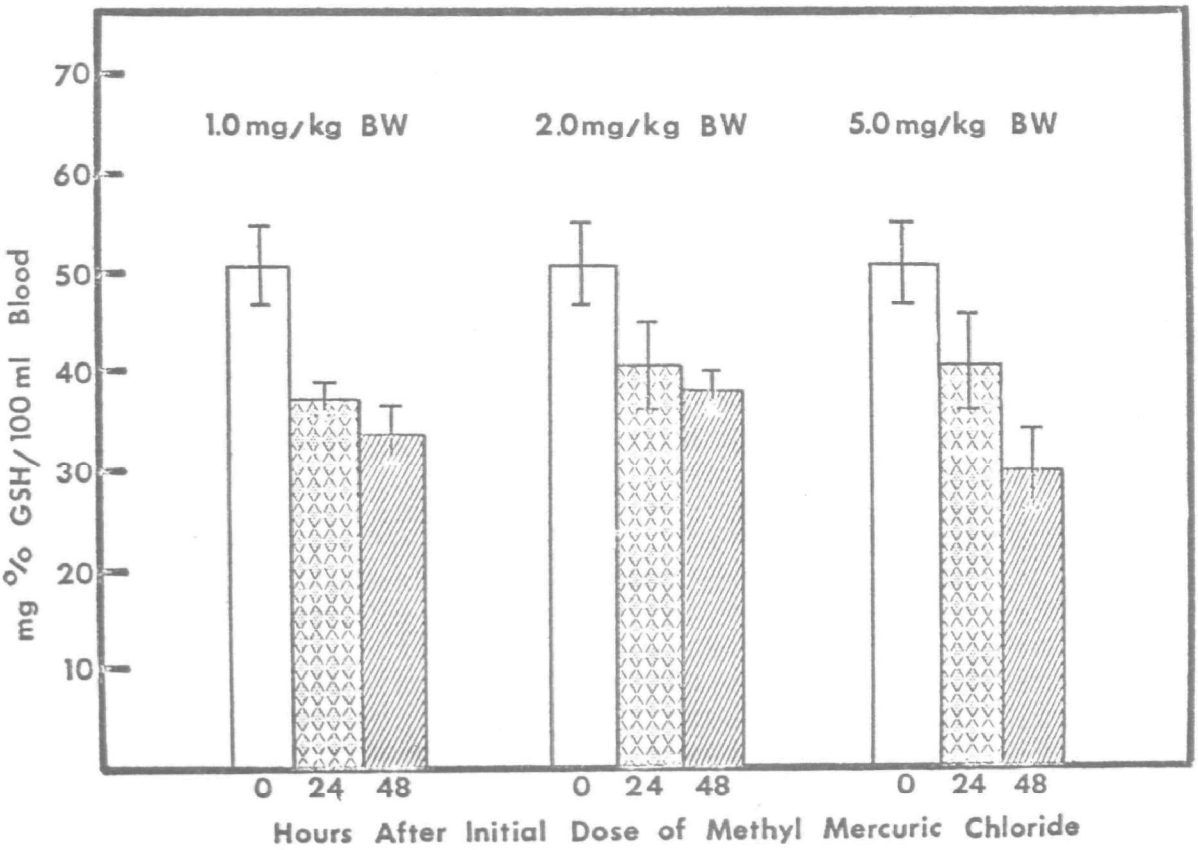


FIGURE 2. Single Dose Levels of Methylmercury Chloride and Its Effect on Whole Blood Glutathione.

These authors concluded that selenium in tuna is not a hazard in itself and that it may lessen the danger of mercury poisoning in man following ingestion of mercury-contaminated tuna.

EARLY BIOCHEMICAL EFFECTS OF O_3 AND NO_2 AND INFLUENCE OF VITAMIN E ON THESE EFFECTS

S.D. Lee, R.M. Danner, K.C. Butler,
D.B. Menzel and J.F. Stara

Trace amounts (ppm) of the air pollutants O_3 and NO_2 rapidly oxidize polyunsaturated fatty acids (Figures 1 and 2). Phenolic antioxidants retard this oxidation (Figure 3); vitamin E decreases the acute toxicity of both O_3 and NO_2. On continuous exposure to 1.5 ppm of O_3 the LT_{50} for vitamin E depleted rats was 8.2 days, compared with 18.5 days for continuously supplemented rats (Figure 4). Similarly the LT_{50} for depleted rats exposed to 33 ppm NO_2 was 11.1 days versus 17 days (Figure 5). Exposure to 0.5 ppm of O_3 also accelerated the depletion of vitamin E from erythrocytes of exposed animals in 23 days versus 36 days for unexposed animals. The polyunsaturated fatty acid content of lung tissue significantly declined in rats fed a constant fatty-acid-composition diet free of vitamin E, or exposed to NO_2, or both. The O_3 exposure decreased the oleic and linoleic acid content but increased the arachidonic acid content (Figure 6, Table 1). These changes may be complex responses of the lung to increased oxidant stress, as shown by depression of serum reduced glutathione (Figure 7) and by tissue sulfhydryl compound content, or may be related to other metabolic roles of vitamin E in the biosynthesis of polyunsaturated fatty acids.

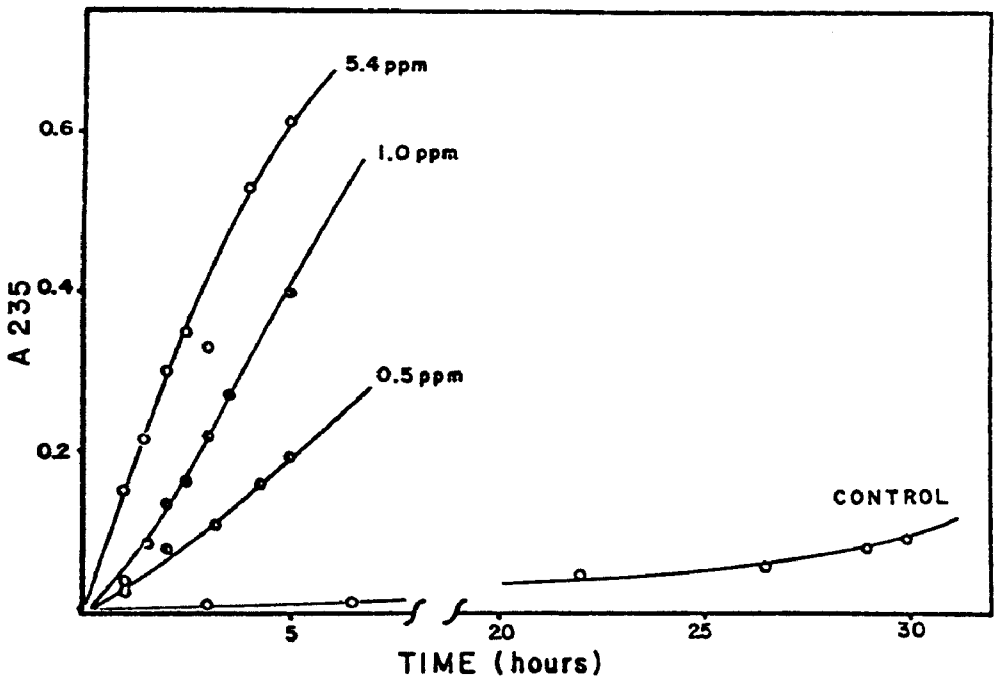


Figure 1. Oxidation of Methyl Linolenate In Atmospheres Containing NO_2: Diene Conjutgation.

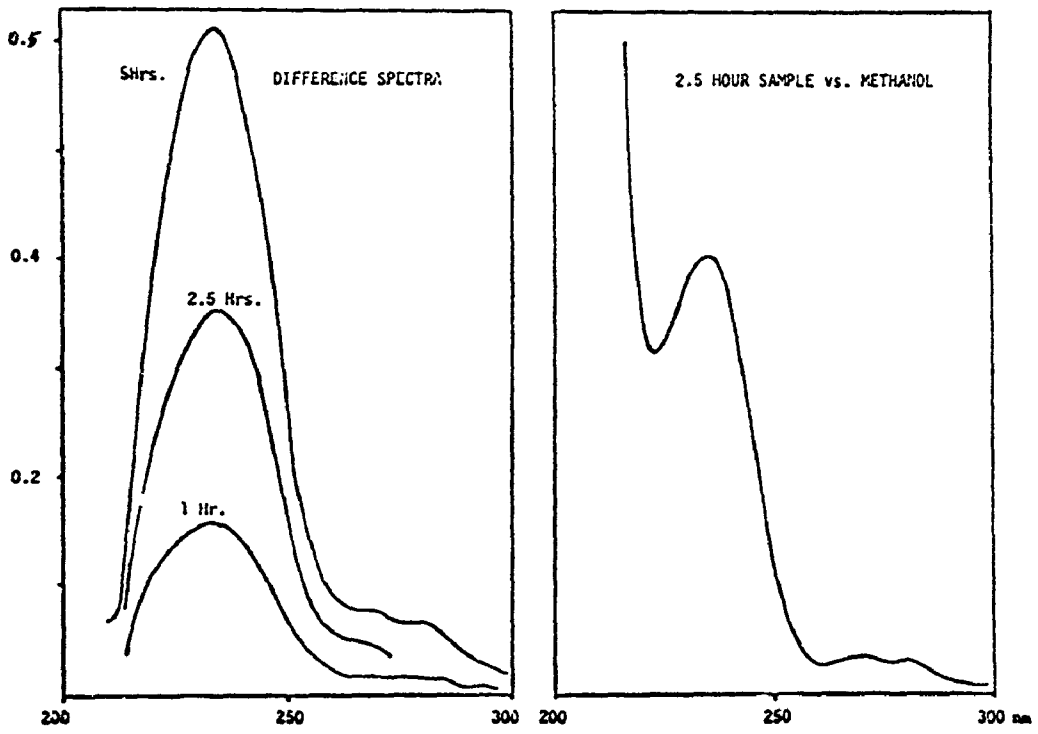


Figure 2. Spectra of Methyl Linolenate Exposed To 5.4 ppm of NO_2 (0.1 mg per ml Methanol).

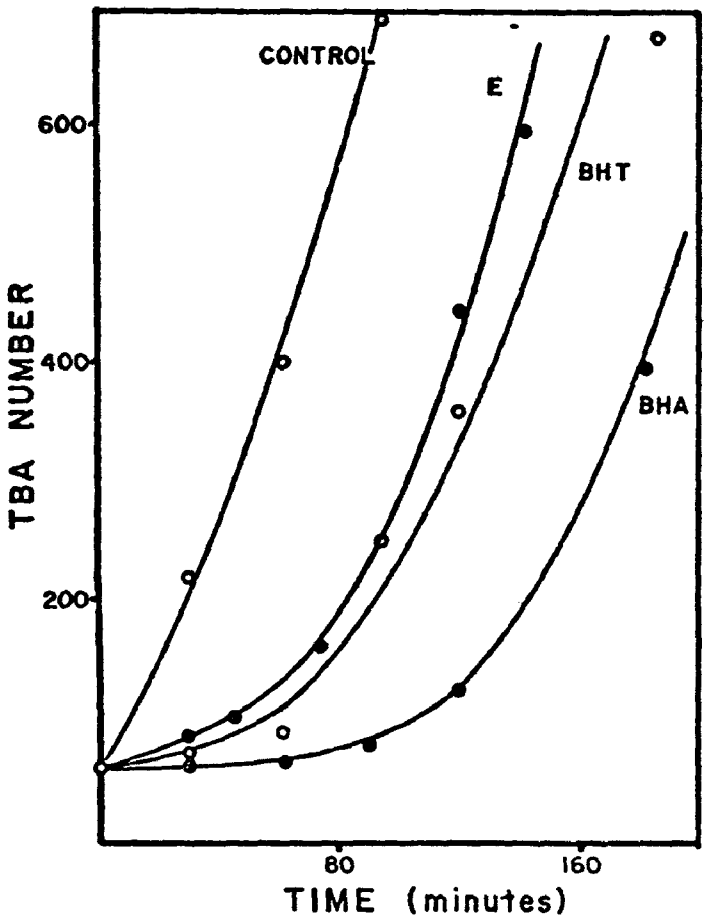


Figure 3. Oxidation of thin films of methyl linoleate in the presence of 1.5 ppm of NO_2. TBA number equals ppm of malonaldehyde.

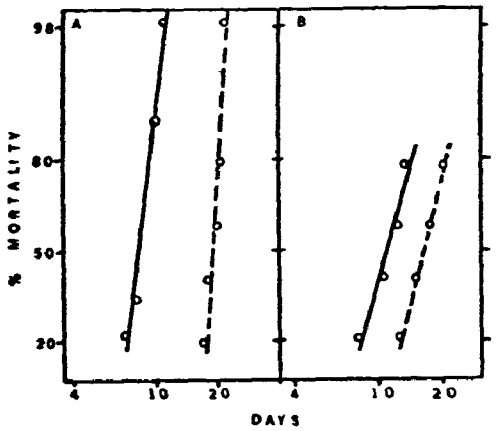


Figure 4. Mortality of vitamin E-deficient and supplemented rats exposed to NO_2 and O_3. A. O_3 exposure to 1.5 ppm. B. NO_2 exposure to 33 ppm. Solid lines represent vitamin E-deficient rats; dashed lines, supplemented rats.

Figure 5. Linoleic Acid Concentration of Lavage Lipids From Rats Exposed to 1.6 ppm Ozone or 3.0 ppm Nitrogen Dioxide.

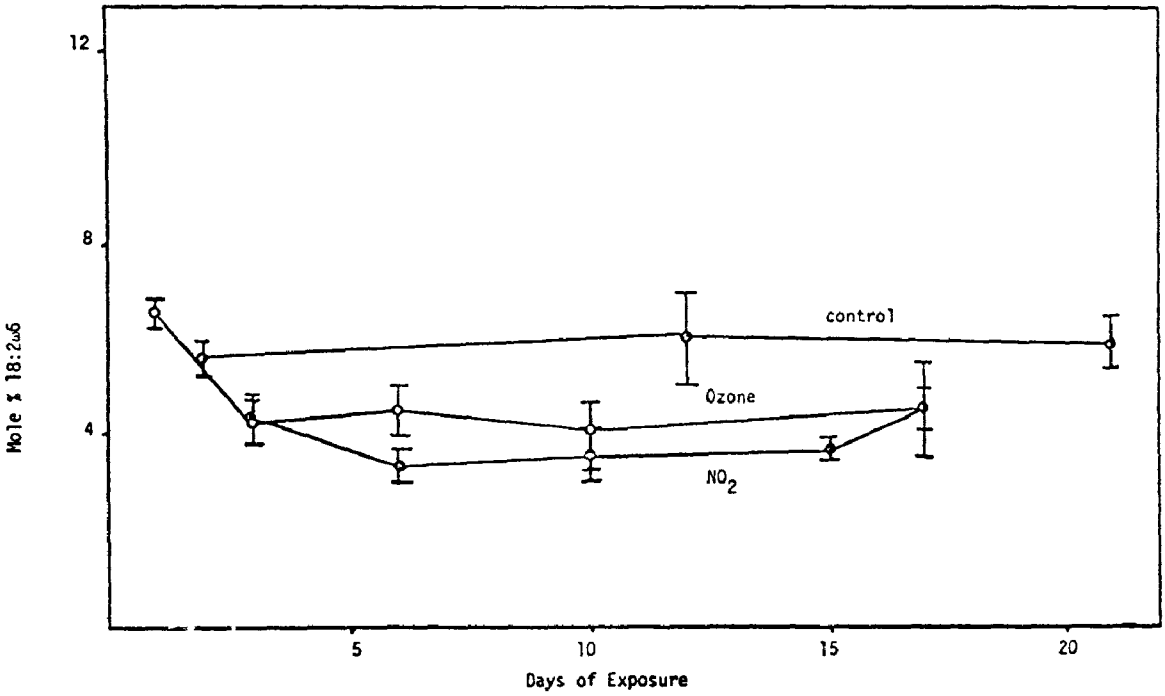


TABLE 1. TOTAL FATTY ACID COMPOSITION OF LUNG TISSUE LIPIDS FROM RATS EXPOSED TO 1.0 ppm O_3 FOR 9 DAYS*

Fatty acid+	Mole % (±SEM) of total lung tissue fatty acids≠			
	Supplemented Control	Deficient Control	Supplemented O_3 exposed	Deficient O_3 exposed
14:0	1.07 ± 0.11	1.08 ± 0.11	1.03 ± 0.11	0.83 ± 0.04
16:0	24.68 ± 0.81	25.16 ± 0.34	27.62 ± 0.65	29.22 ± 0.84
16:1	4.97 ± 0.49	4.85 ± 0.33	5.22 ± 0.67	4.34 ± 0.20
18:0	9.73 ± 0.67	9.72 ± 0.63	9.76 ± 0.83	9.73 ± 0.37
18:1	24.09 ± 1.87	23.85 ± 2.58	23.42 ± 2.99	20.70 ± 0.47
18:2	7.65 ± 0.45	7.55 ± 0.47	6.97 ± 0.47	6.14 ± 0.74
18:3	6.19 ± 0.67	6.14 ± 0.98	4.98 ± 1.06	3.43 ± 0.21
20:4	6.01 ± 0.59	6.01 ± 0.90	7.18 ± 0.93	8.87 ± 0.81
20:5	3.25 ± 0.68	3.67 ± 0.51	3.27 ± 0.86	2.21 ± 0.16
22:5	5.35 ± 1.02	4.77 ± 0.75	4.12 ± 1.26	5.80 ± 1.67
22:6	1.67 ± 0.10	2.05 ± 0.54	1.64 ± 0.23	3.50 ± 0.43

*Minor components omitted for clarity .

+The notation for fatty acids is X:Y, where X is the number of carbon atoms and Y the number of unsaturations.

≠Number of animals in each group was six.

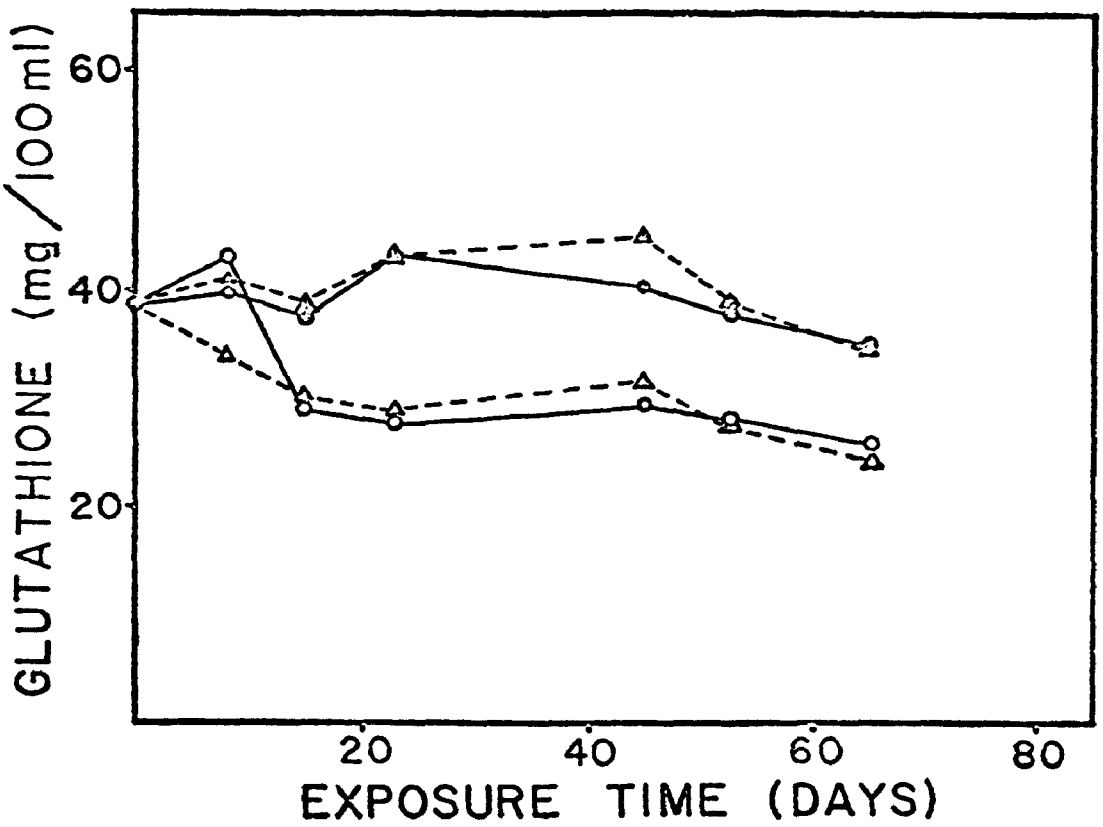


Figure 6. BLOOD GLUIATHIONE LEVELS OF RATS EXPOSED EITHER TO FILTERED AIR OR 0.5 ppm O_3 (SOLID LINES - CONTROL, INTERRUPTED LINES - O_3 EXPOSED).

ALVEOLAR PROTEIN ACCUMULATION: A SENSITIVE INDICATOR OF LOW-LEVEL OXIDANT TOXICITY

S.D. Lee, R.M. Danner, S.M. Alpert, B.B. Swartz and T.R. Lewis

Studies of response to low doses of edematogenic gases have been hampered by the insensitivity and the nonquantitative nature of the major indicators of response. Occurrence of edema in rats lungs following exposure to 0.67 ppm O_3 for 7 days is shown in Figure 1. A new and more sensitive indicator, the recovery of ^{131}I-albumin from the alveolar spaces 6 hr after its intravenous injection in rats, has been applied. Significantly increased albumin recovery was found for all concentrations of O_3 at and above 0.5 ppm, and there was no consistent histologic finding except for slight sloughing of bronchial epithelium at 2.5 ppm (Figure 2). Application of these methods to studies of steroid effects revealed increased sensitivity to O_3 following administration of methylprednisolene sodium succinate. In addition, animals treated with steroids before exposure to 0.25 ppm O_3 became tolerant to subsequent O_3 challenge, whereas animals given preexposure but no steroids did not.

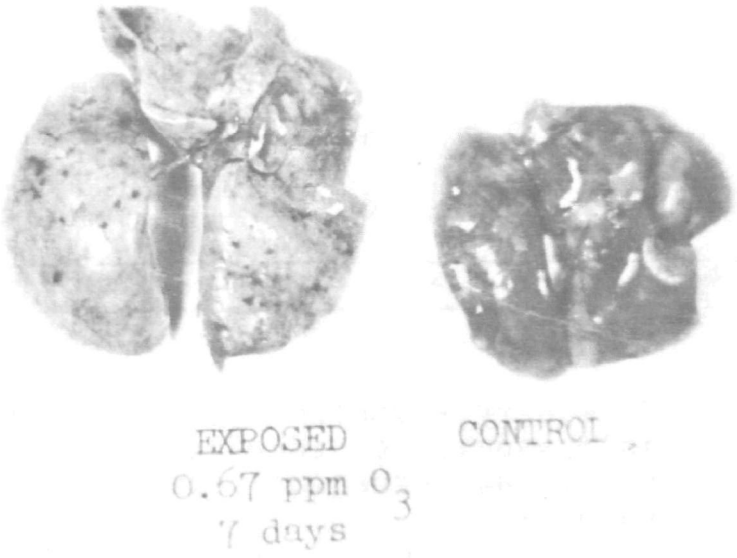


Figure 1. Excised rat lung exposed to 0.67 ppm O_3 compared with control.

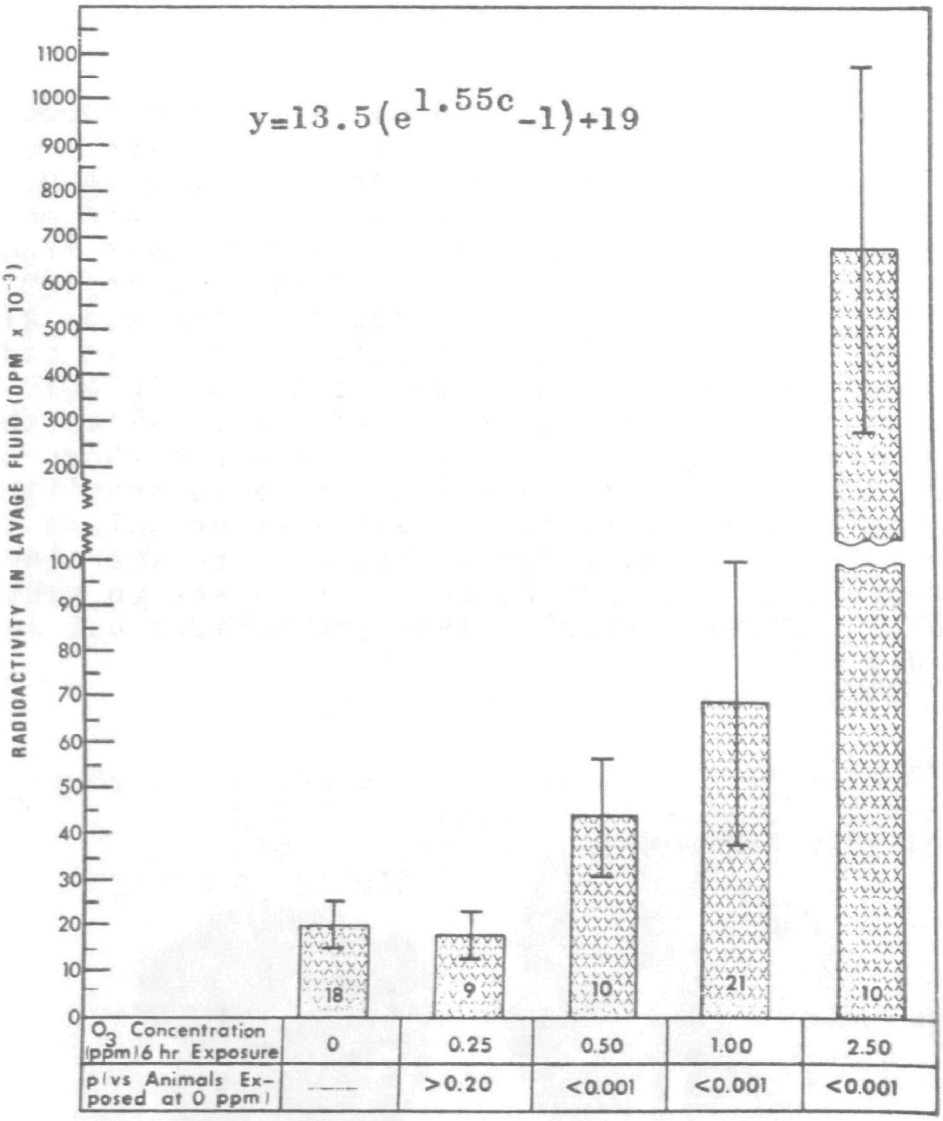


Figure 2. Pulmonary alveolar protein accumulation in response to ozone exposure (values ± 1 SD-No. of animals per group on columns).

CHRONIC EXPOSURE EFFECTS OF OZONE IN BEAGLES

J. Stara, T. Lewis, K. Campbell
B. Johnson and D. Coffin

The biochemical effects of acute ozone intoxication have been well documented. The effects of chronic ozone exposure on biological systems has received little attention, however. As a result, a study was designed in which female beagle dogs were exposed for 18 mo to air containing 1 to 3 ppm of ozone. The original protocol called for the assessment of the pulmonary and hematological effects of chronic O_3 exposure. Before the study was completed, however, several additional parameters (cardiovascular physiology, immune competency, neurophysiology, neurochemistry, and pathology) were investigated to evaluate the biological effects of O_3 more completely.

Ozone was found to have a significant effect on total lung capacity, nitrogen washout, and functional residual capacity. The hematology studies indicated that the mean corpuscular volume and red blood cell fragility both decreased with increasing exposure to ozone. The immune response has been shown to be depressed in those animals exposed to ozone.

Recent electromicroscopic studies of the dog's lung tissues, performed by Dr. G. Freeman, *et al.* under a contract with EPA, indicate the earliest response in the appearance of macrophages near the respiratory bronchiolarductal region and in adjacent alveoli. The number of macrophages increased with increasing ozone exposure. The dogs exposed to the higher levels of ozone also developed squamous metaplasia and stratification of cuboidal cells in the bronchiolar epithelium. In addition, thickening of bronchiolar walls, narrowing of alveolar openings, and a higher proportion of mucus-forming cells were noted.

Another recent report, completed by Dr. R. J. Stephens *et al.* under a contract with EPA, also indicates that the epithelium of the terminal airways and proximal alveoli are greatly changed by ozone exposure. In addition, Type 2 cells appeared to be metabolically altered and large accumulations of grid-like material appeared in the alveoli. The investigators contend that the reduction in clearance rate that results from the morphological and structural changes in the terminal airways may be an additional factor related to the accumulation of the grid-like material.

Final report on this study is expected to be issued when pathology and neurophysiology data are collected and analyzed.

INFLUENCE OF EXPOSURE PATTERN ON TOXIC RESPONSE TO NITROGEN DIOXIDE

K. Campbell and L. Hall

The probable influence of exposure pattern on associated toxic response was investigated because of its importance to the modeling and interpretation of bioeffect studies that are designed to determine relative toxic hazards of engine emissions when various fuels and fuel additives are used. Two experiments have been conducted to date to examine the comparative magnitude of selected toxic responses resulting from inhalation exposure to NO_2 delivered in three different patterns: continuous, varied and intermittent.

In Experiment A, rats and hamsters were exposed for 5 days to NO_2 in a constant-level pattern (31 ppm) and in a varied-level pattern (18 hr at 25 ppm and 6 hr at 45 ppm per day, mean concentration 26 ppm); the total dose, in ppm-days, was 126 for varied level and 149 for constant level. Timed mortality and pre- and post-exposure body weight were recorded. The data indicated that hamsters were more susceptible than rats to these exposure conditions, and further, that hamster lethality response was more severe in magnitude and time in the varied than in the constant-level exposure, even though the average concentration and "total (CxT) dose" were similar. It is suggested that (1) this exposure-pattern influence resulted from the high peak exposure levels used, even though for short periods, and (2) in this CxT condition, peak concentrations are more determinant than other co-factors (duration, average concentration, or "dose"). There was no mortality and negligible weight change among the room-air control animals. Principal results for Experiment A are shown in Table 1.

TABLE 1. EXPERIMENT A:
VARIED-LEVEL AND CONSTANT LEVEL EXPOSURE OF
RATS AND HAMSTERS TO NITROGEN DIOXIDE

Effect	Exposure Pattern	
	Varied Level	Constant Level
Total mortality		
Rats	40	30
Hamsters	100	70
Est. LT_{50}, hrs.		
Rats	72	68
Hamsters	28	46
Rel. body-weight change, %		
Rats	+ 9	- 5
Hamsters	- 7	-18

In Experiment B, mice, rats and hamsters were exposed to 37 ppm of NO_2 for 15 days at a constant-level in an uninterrupted pattern and in an interrupted pattern (8 hr NO_2 and 16 hr clean air per day); total exposure doses were 555 and 185 ppm-days, respectively. Timed mortality, initial and terminal body weight, terminal lung weights, and gross lung pathology in rat lungs were recorded. In terms of total mortality and lung:body weight ratios, the data indicated again that hamsters were more susceptible than rats. Mice appeared to be more sensitive than hamsters, so that the species would rank in decreasing order of NO_2 sensitivity: mice, hamsters, rats. Further, it was evident that altering exposure pattern did indeed influence the magnitude of toxic response, in this case to a higher degree than would be projected by a simple CxT "total dose" relationship. Mortality in animals exposed continuously for as little as 3-5 days was far greater than for those receiving 15 days, 8 hrs/day interrupted exposure. Similarly, disproportionately greater lung weight increases and gross lung pathology was observed in the continuous-exposure than in the interrupted exposure group. Analogous relationships of response severity to exposure pattern (interrupted cf. continuous), with equivalent total (CxT) doses, have, incidentally, also been observed in plants (Episcia cupreata) exposed to automotive engine emissions.

Results for Experiment B are summarized in Table 2. It is generally supposed that the reason for the reduced toxicity accompanying interrupted exposure pattern is that during intervening nontoxic periods the subjects' defense and repair mechanisms are permitted to effect a degree of recovery, or to develop a tolerance, or both, that is denied by continuous, constant insult.

TABLE 2. EXPERIMENT B:
INTERRUPTED COMPARED WITH CONTINUOUS EXPOSURE OF MICE, RATS AND HAMSTERS TO NITROGEN DIOXIDE

Effect	Exposure Pattern					
	Interrupted			Continuous		
	Mice	Rats	Hams	Mice	Rats	Hams
Total mortality, %	0	0	0	85	40	72
LT_{50}, hours	NA	NA	NA	76	NA	51
Body weight change, %	+3	-8	-1	34	-24	+2
Relative change in lung: body weight ratio, %	58	20	37	371	97	146
Relative gross lung abnormality		$\pm$			++	

The data from both studies suggest that altering exposure patterns (variations in concentration or significant interruptions in exposure) would likely affect the variability and patterns of biologic responses in test-exposure systems; this implies the need for control criteria in generating and processing fuel emissions atmospheres and for adequate atmosphere characterization to define the exposure. Study B data imply that use of a protocol involving an interrupted exposure pattern would require much higher concentrations, or greatly extended experimental periods, or both, to achieve detection and to permit comparisons of toxic response. It might also be inferred that for some reported toxic responses based on continuous exposure a "safety-factor" could exist because, and to the extent that, "real-life" exposures tend to simulate the interrupted-exposure pattern situation.

HEXACHLOROPHENE TERATOGENICITY

C. Kimmel, W. Moore, D. Hysell, and J. Stara

Pregnant animals were anesthetized on day 7 and approximately 73 mg of hexacholorphene suspended in water and Tween 80 was inserted into the vagina. Next a gauze plug was inserted and the vagina partially closed with silk suture. On days 8, 9, and 10, additional doses (for a total dose of 300 mg/kg) were given intravaginally, and on day 11, the plugs were removed. Animals were killed on day 20, and examined for teratological changes. The hexachlorophene-Tween 80 mixture produced a significant number of resorptions and malformations (Table 1).

TABLE 1. TOXIC EFFECTS OF HEXACHLOROPHENE AFTER INTRAVAGINAL ADMINISTRATION

Treatment	No. of maternal animals	Maternal mortality	No. of implants	% dead or resorbed	% malformed
Hexachlorophene*	12	2/12	123	33	40
Starch or water† control	12	0/12	158	8	4

*The hexachlorophene was administered as a 45% solution in distilled water + Tween 80. Dosage = 90 mg., or 300 mg/kg.
†Controls were treated with a starch paste in water + Tween 80, or with water + Tween 80 alone.

An example of the gross external malformations together with a normal fetus (for comparison) are shown in Figure 1.

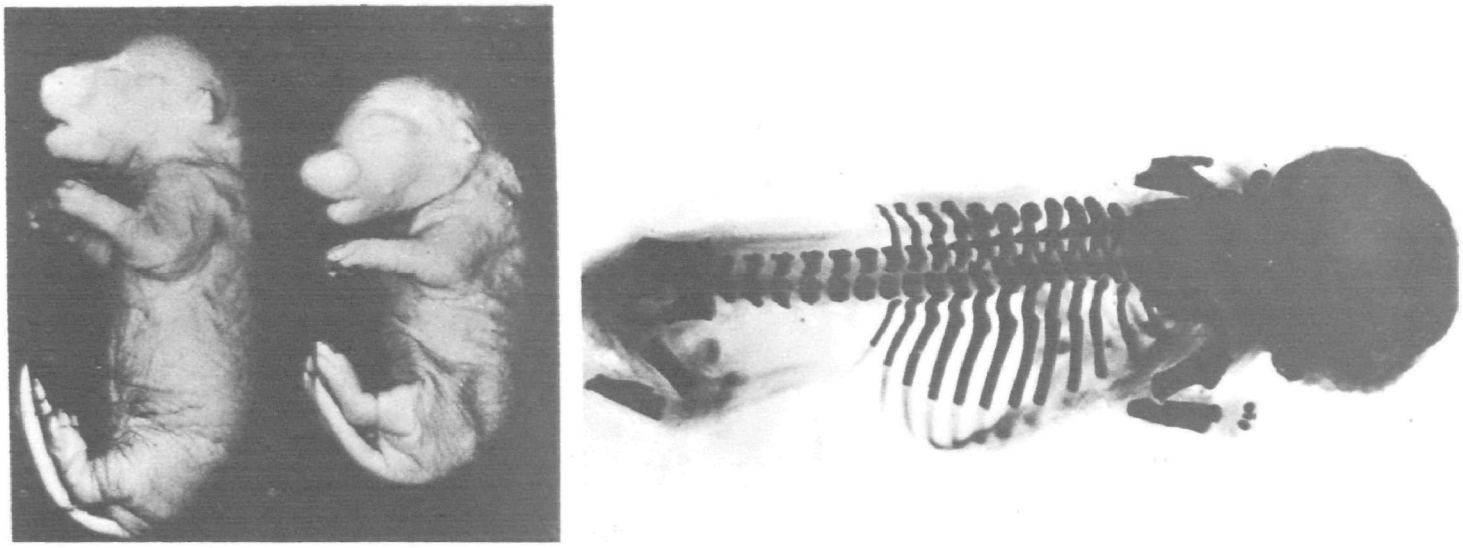


Figure 1. Abnormalities found following vaginal treatment of pregnant rat. A. Normal fetus B. Abnormal fetus showing microophtholmia and small size C. Skeletal defects, wavy ribs.

The microscopic abnormalities noted consisted of ocular malformations ranging from microophthalmia to anophthalmia with intermediate changes including dysplasia of lens, retina, and optic nerve (Figures 2, 3 and 4).

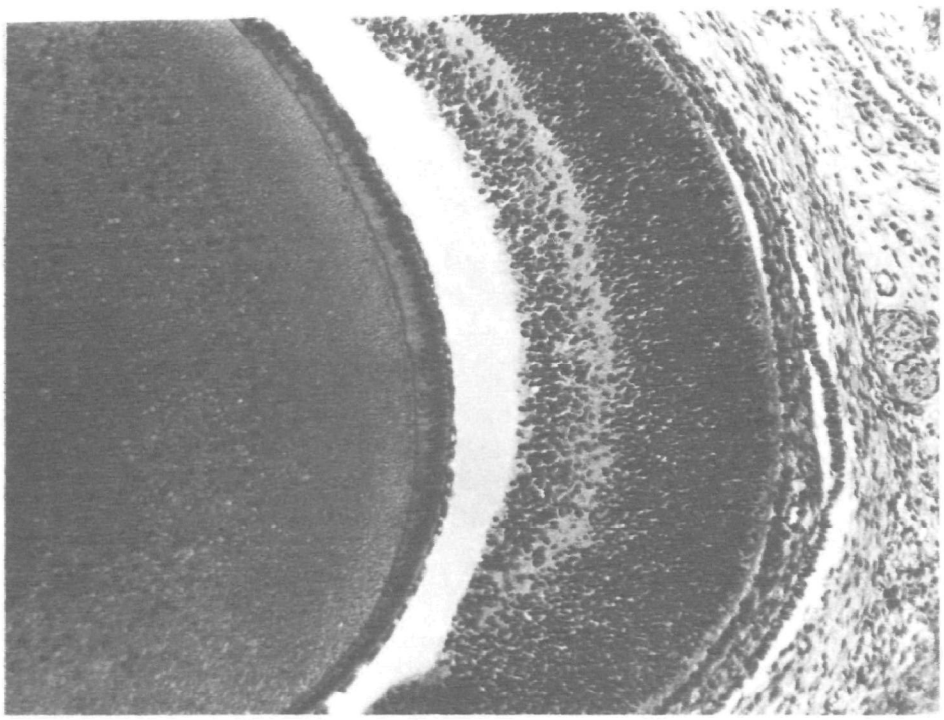


Figure 2. Normal fetal eye showing lens, retina, and optic nerve.

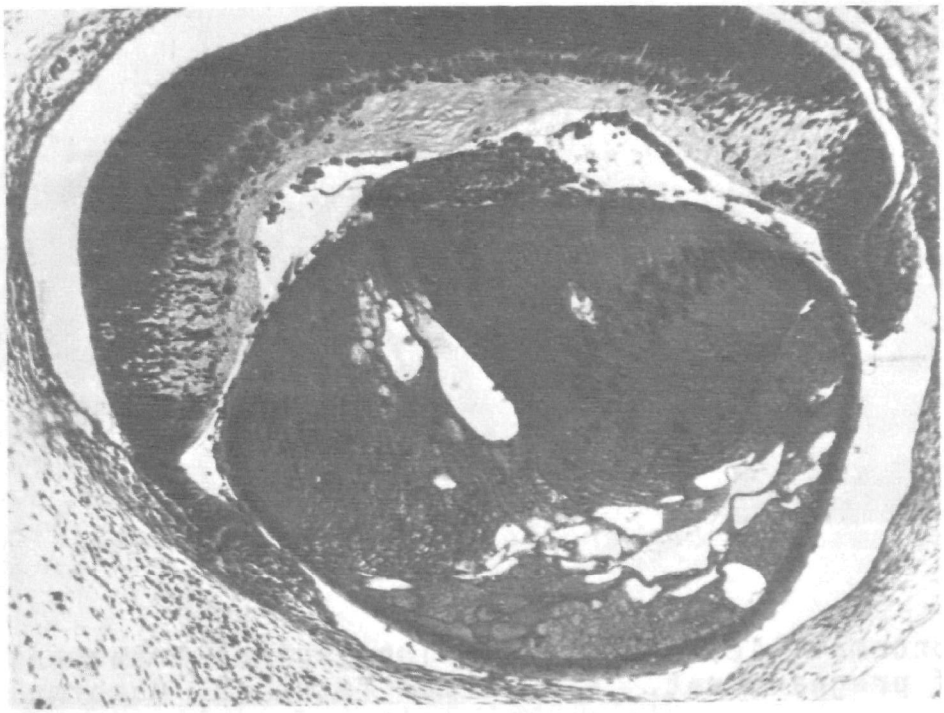


Figure 3. Fetal eye showing dysplacia of lens and retina and microophthalmia (same magnification as Figure 2).

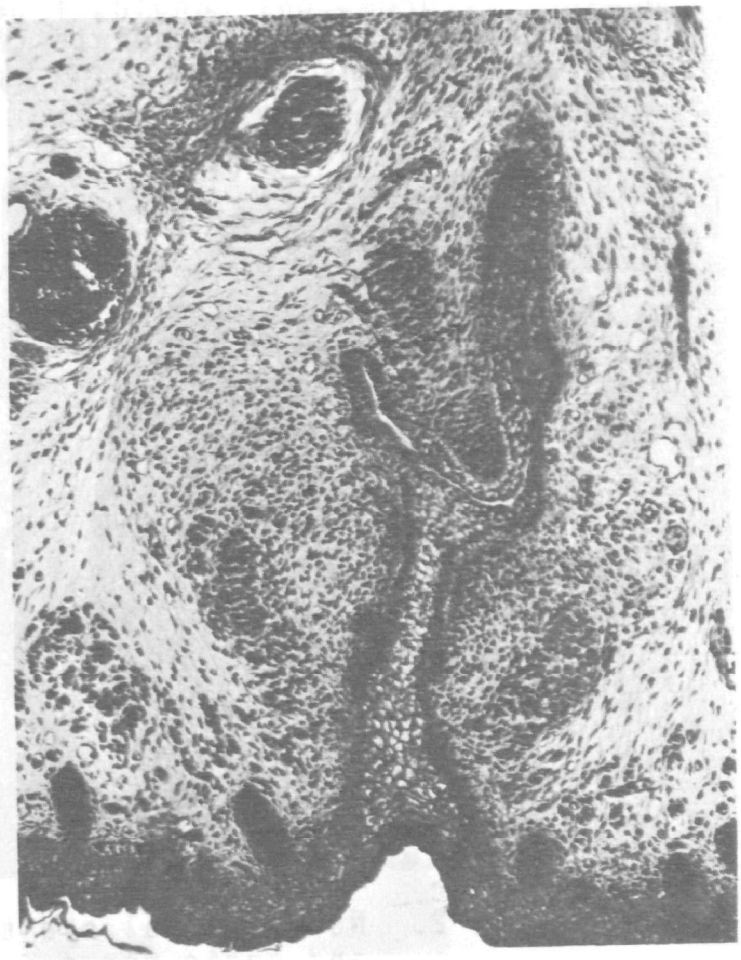


Figure 4. Anophthalmia. The only vestige of ocular development is the orbital cleft.

Cleft palate was also noted microscopically. The vaginal infections seen late in pregnancy (days 12 to 16) in hexachlorophene-treated animals were associated with gram-negative bacilli. Control animals did not exhibit any noticeable infection (Figures 5 and 6).

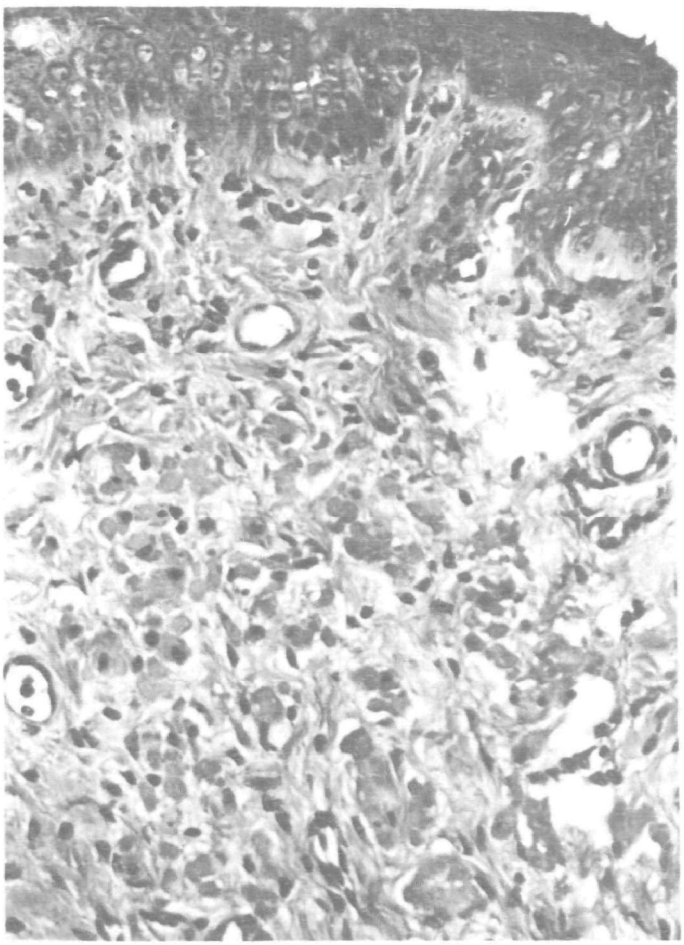


Figure 5. Normal rat vagina.

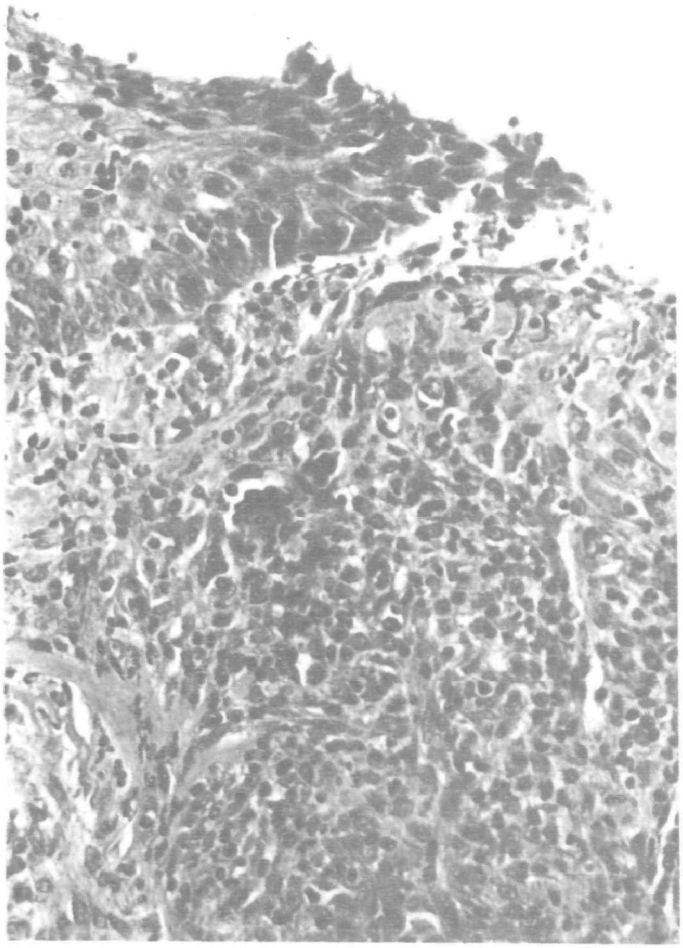


Figure 6. Hexachlorophene-treated vagina showing severe epithelial ulcer and subacute connective tissue response.

TOXICOLOGIC ASSESSMENT OF MOBILE EMISSIONS (TAME) STUDIES

TOXICOLICAL ASSESSMENT OF MOBILE EMISSIONS (TAME): OVERALL STUDY APPROACH AND OBJECTIVES

J. F. Stara

Clean Air Act Amendments of 1970 have charged the EPA with the determination of possible health hazards related to the use of various fuels and fuel additives. The results of these studies are to be used as guidelines for the possible regulation of fuels and their additives.

The objects of this project were:

1. To provide a comparative study of the chemical and physical nature of the emissions of a fuel composition with and without the presence of a fuel additive.

2. To assess comparatively the potential toxicological hazards resulting from the use of the fuel and fuel additive.

3. To develop a working test system and a definitive toxicologic model by which harmful biological effects can be satisfactorily evaluated.

Such a comparative study necessitates a high degree of control, consistency, and repeatability in all three major systems: generation and delivery, pollutant characterization, and bioeffects determination.

The need for strict evaluation of fuel additives is a necessity in light of the number and widespread use of additives in all types of modern fuels. The major classes of fuel additives are:

A. AUTOMOTIVE

1. Anti-Knock
2. Anti-Oxidants
3. Metal Deactivators
4. Detergents and Dispersants
5. Ignition Improvers

B. DIESEL

1. Ignition Improvers
2. Detergents & Dispersants
3. Dyes
4. Anti-Oxidants
5. Smoke Suppressants
6. Anti-Static

C. AVIATION

1. Anti-Knock
2. Metal Deactivators
3. Anti-Oxidants

D. JET FUELS

1. Anti-Static
2. Smoke Suppressants
3. De-Icers

E. TURBINE JET FUEL

1. Smoke Suppressants
2. Anti-Static

To ensure that satisfactory information can be submitted to EPA Headquarters, the following investigative steps have been taken as a part of ETRL standard-procedure for definitive toxicologic evaluation of an additive such as methyl manganese tricarbonyl (MMT):

1. Ingestion exposure of the additive

2. Vapor inhalation exposure

3. Simple combustion exposure primarily to provide aerometry data of what chemical forms can be expected to be found in the automotive emissions

4. Simple combustion in mixture with fuel

5. Exposure of large numbers of animals to the complex, whole, automotive engine emissions of the reference fuel and the fuel with additive mixture at the proposed or marketed concentration.

The basic design of the toxicological study of complex, whole engine emissions, which represent realistic atmosphere found in the environment, is as follows: A fuel additive is the test variable, although different fuel types, engine types, and operating modes, or emission control devices are also planned to be studied. Precise characterization and quantification of the atmospheric pollutants is an integral part of each experimental design. During the past calendar year the following full-scale automobile exhaust experiments were conducted:

TAME A. A control study using a reference gasoline without additives. Carburetor settings were provided and sealed at the factory.

TAME B. Another control study using updated engine specifications (maximum idle vacuum).

TAME C. A control experiment to evaluate the repeatability of A and to serve as a baseline for D.

TAME D. A test study operating all systems as a replicate of C with the exception that 0.37 g/gal. of an anti-knock compound, MMT, was added to the reference fuel.

TAME E. A control study for D and F using reference fuel and no additives.

TAME F. A test study in which 0.25 g/gal. MMT is added to the fuel.

DESIGN AND SYSTEM PERFORMANCE FOR MOBILE EMISSIONS BIOEFFECT STUDIES AT ETRL

R.G. Hinners, J.K. Burkart and R. Iltis

During 1972, six studies were conducted exposing animals to the exhaust emissions from a 1972 Chevrolet 350 C.I.D. automobile engine, which was operated for approximately 2400 hours. The existing exhaust dilution and exposure chamber system (Figures 1-3) was utilized for a series of multi-disciplinary studies with several biological models to assess comparative toxicity and emissions characterization resulting from control fuel (Indolene) and a pertinent test additive compound (MMT).

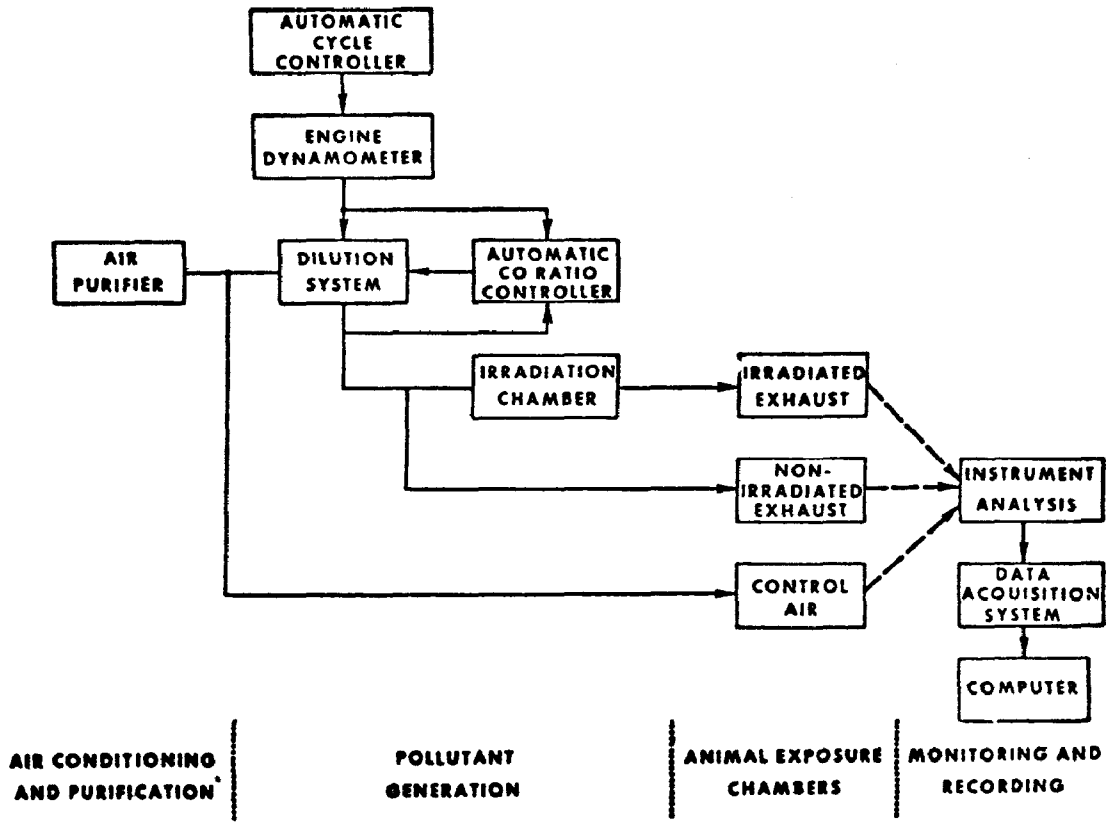


FIGURE 1. TOXICITY ASSESSMENT MOBILE EMISSIONS (TAME) FLOW DIAGRAM

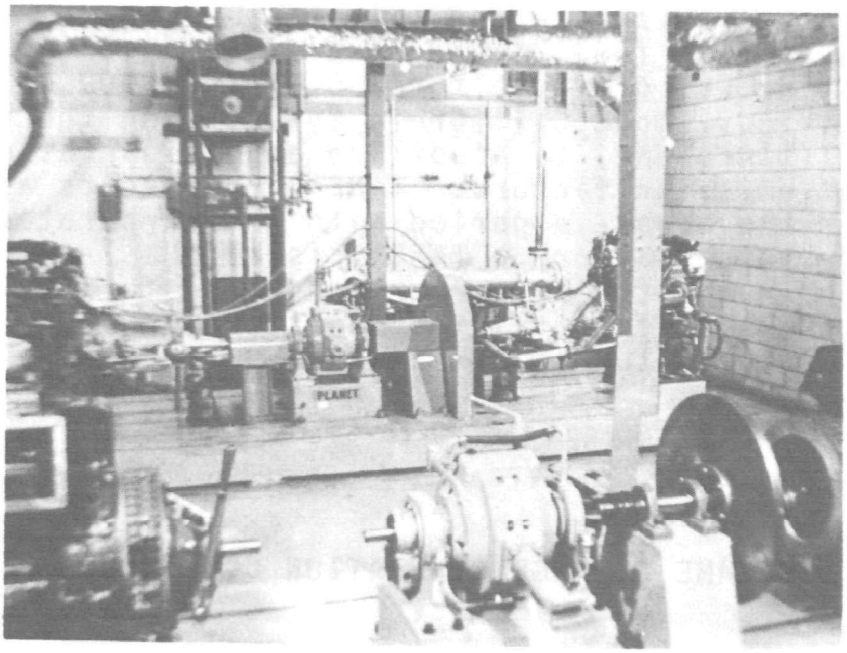


Figure 2. Engine-dynamometer room

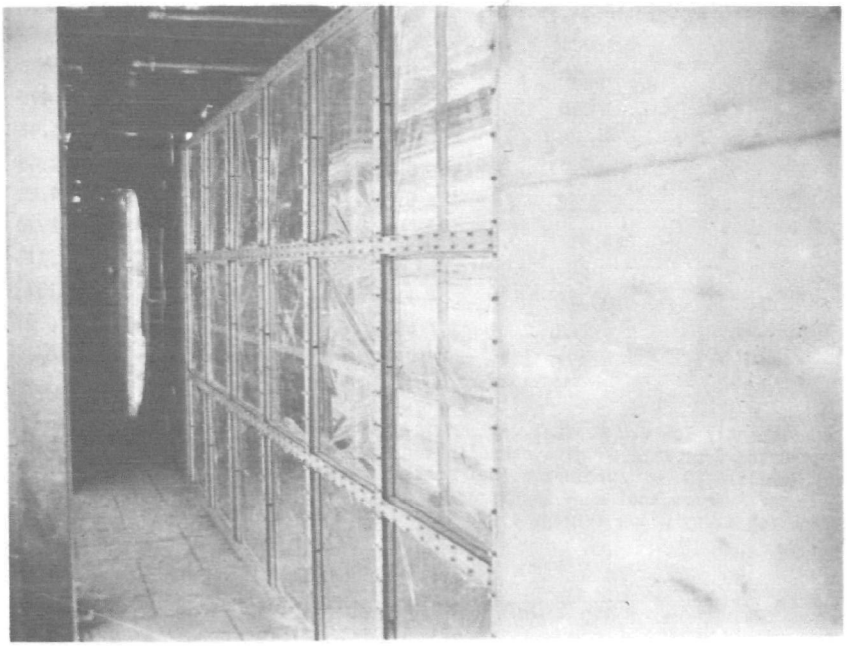


Figure 3. Typical irradiation chamber

The "TAME Average Generation Conditions Summary" (Table 1) provides relevant information regarding the studies, such as fuel, tuning and dilution ratios. A clean engine was operated for 150 hours (3000 miles) on a modified 7-mode California Cycle using the reference fuel (Indolene) to stabilize engine operation and pollutant emission factors. TAME A represents a 7-day run after the break-in period with the carburetor set as tuned by the factory. TAME B is also a 7-day continuous run with the carburetor tuned for maximum vacuum to assist in obtaining identical engine performance for future studies.

TABLE 1. TAME AVERAGE GENERATION CONDITIONS SUMMARY

CONDITION	TAME A	TAME B	TAME C	TAME D	TAME E	TAME F
FUEL	REF. ONLY	REF. ONLY	REF. ONLY	REF. + 3/8MMT	REF. ONLY	REF. + 1/4MMT
TUNING	FACTORY SET	MAX. VAC.	LEAN (CO)	TAME C (CO)	TAME D (CO)	TAME E (CO)
ENGINE	'B'	'B'	'B'	'B'	'B'	'B'
ENG. HRS.	153-254	279-450	521-696	779-1115	1354-1698	1792-2131
STUDY HRS.	101	171	175	336	344	339
FUEL CONSP. LB/HR	N.A.	7.2	6.9	6.6 *1	7.1	7.4
EXH. CO, PPM	1500	10000	1705	*3 2135/1925	1924	2294
EXH. THC, PPM	1580 *2	2000 *2	1965	2626/3429	2025	2236
EHX. NO_x, PPM	435 *2	600 *2	347	335/231	475	457
CO: THC RATIO	0.95	5.00	0.87	0.81/0.56	0.95	1.03
CO: NO_x RATIO	3.45	16.7	4.91	6.37/8.33	4.05	5.02
THC: NO_x RATIO	3.63	3.33	5.66	7.84/14.8	4.26	4.89
EXH. OXYGEN, %	NA	NA	NA	NA	2.30	2.64
AIR/FUEL RATIO	NA	NA	NA	NA	14.7/1 *5	14.8/1
ROTOR CAP	D-137R	D-137R	D-137R	D-137R	D-137	D-137
ADJUSTMENTS	NONE	NONE	NONE	(2SP, 9 CYCLE)	(3 CARB, 5 CYCLE)	(2 CARB.16 CYCLE)
DILUTION RATIOS *4	16/1	102/1	18.2/1	19.2/1	19.0/1	18.4/1

*1 Cycle speeds consistantly low due to fuel contamination problems.
*2 These values estimated from chamber data and dilution ratios.
*3 1st week AVGS./Overall AVGS. - Experienced fuel contamination 2nd week of study.
*4 Dilution by RATIO of Average engine CO to Average Chamber CO. (Data for Highest Chamber Concentrations)
*5 A/F ratio average for Study using average cycle CO & oxygen and correcting for unburned HC.

A bar graph (Figure 4) illustrates the increase in CO during study B with the engine tuned for maximum vacuum to provide reproducibility, rather than at the factory setting of lean CO. Study D is a replicate of Study C plus the additive MMT. Study F repeats Study E and includes 0.25 g MMT per gallon of gasoline.

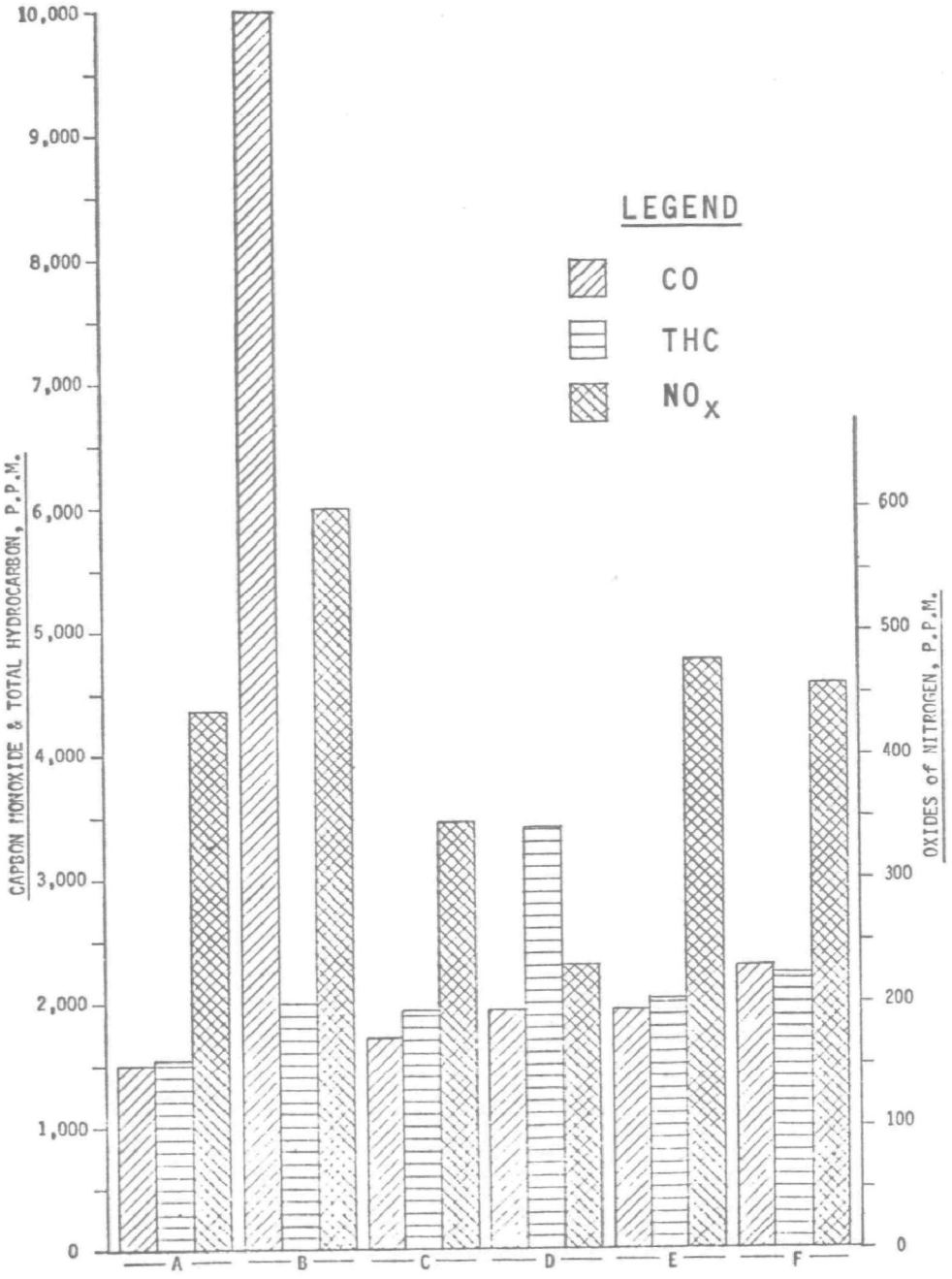


FIGURE 4. Engine "B" average gaseous emissions comparison for TAME studies A,B,C,D,E and F.

Curves of the average daily gaseous emissions for Study C (7 days-Indolene only) and Study D (14 days-Indolene and additive) are shown in Figure 5. The large increase in total HC and decrease in NO_x during the second week of Study D is attributed to fuel contamination by water rather than the MMT additive.

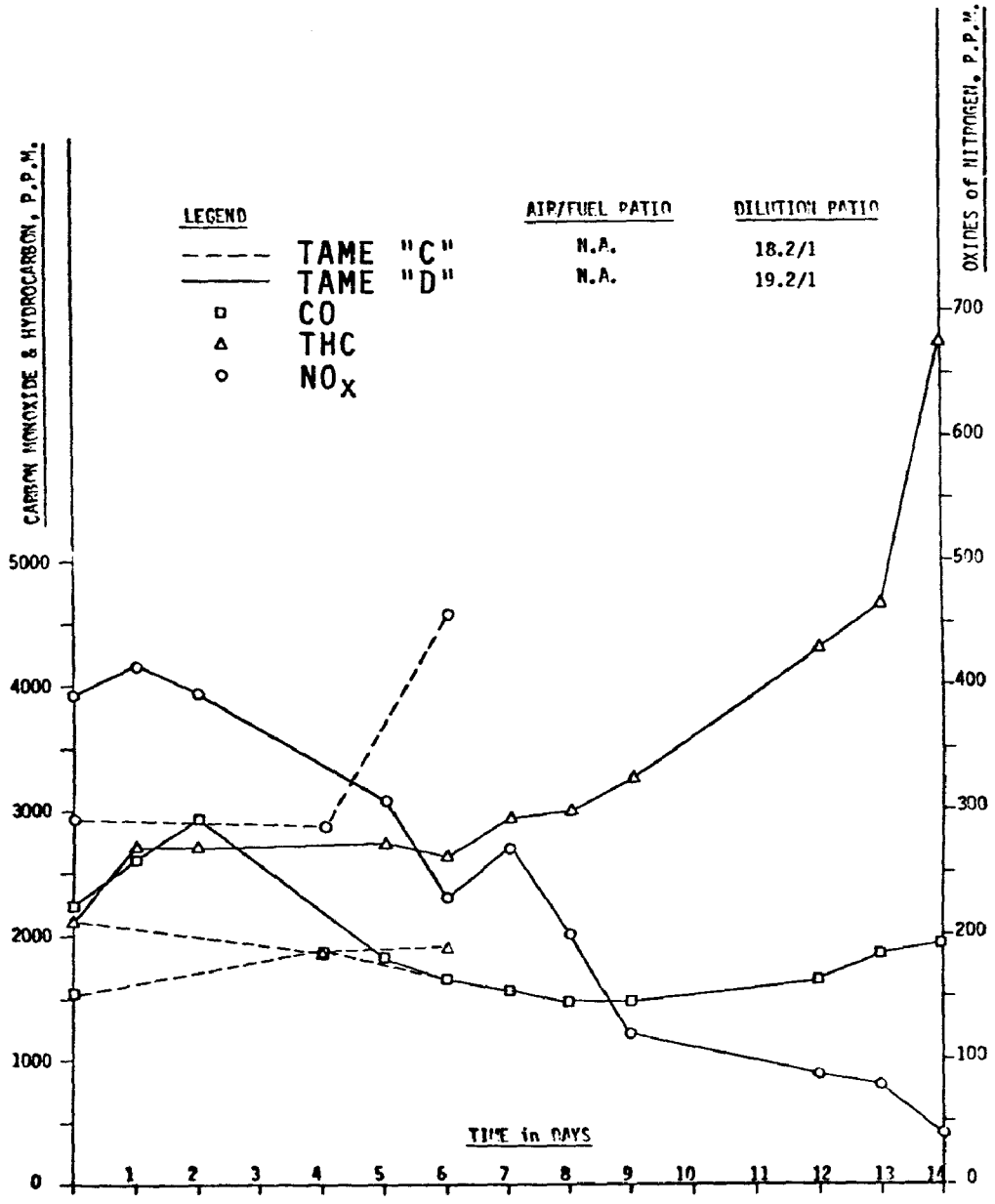


Figure 5. Engine "B" average daily gaseous emissions comparison for studies C and D.

The average gaseous emissions curves for TAME E (Indolene) and TAME F (Indolene and additive) for 14 days of continuous engine performance are shown (Figure 6). In these studies, the engine was tuned at the start to match the CO output from the preceeding study, and illustrates the degree of engine reproducibility obtained. As the engine hours increase, CO also increases and the emissions of studies E and F are as comparable as can reasonably be expected for a 2-wk continuous run.

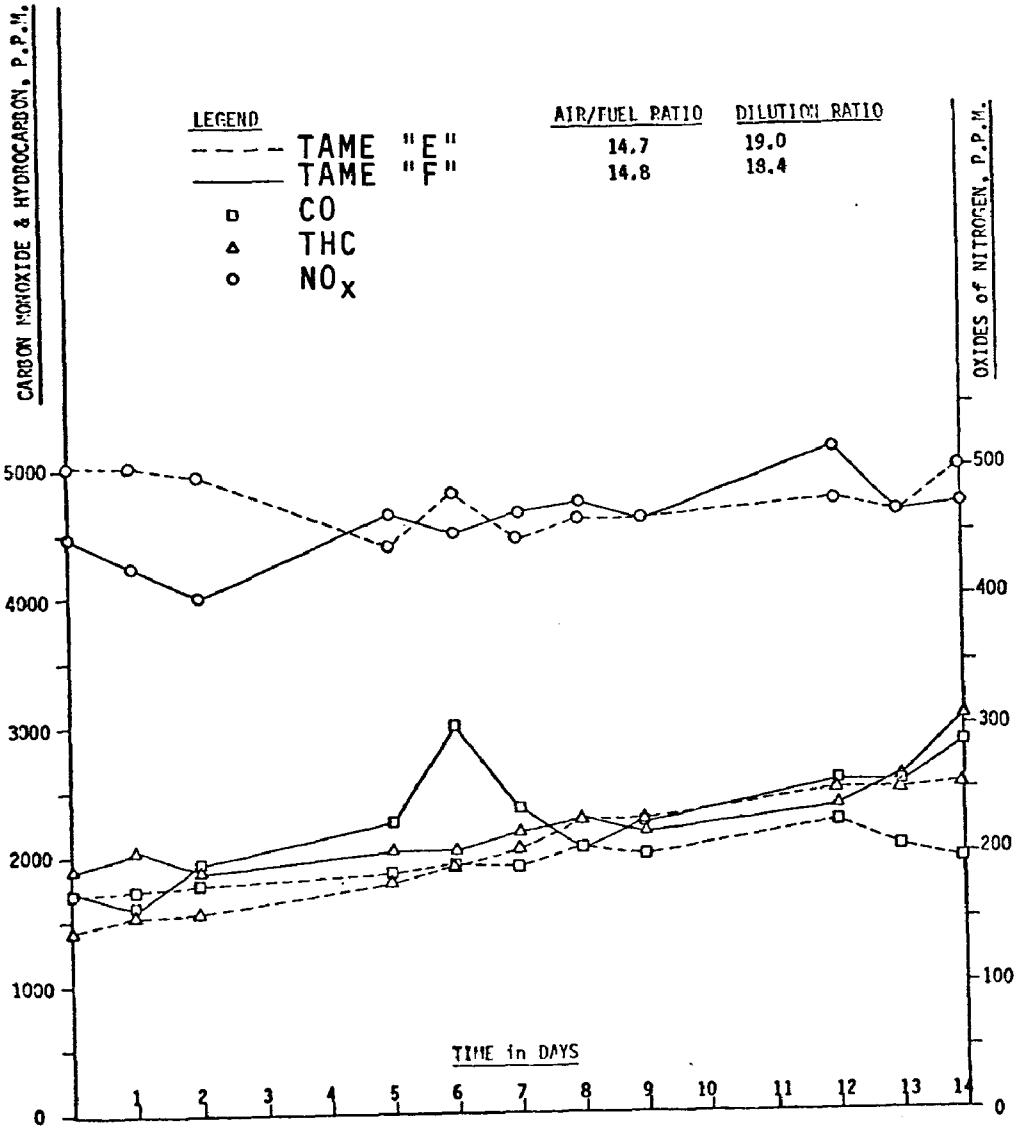


Figure 6. Engine "B" average daily gaseous emissions comparison for studies E and F.

A new air dilution system for mixing raw exhaust with CBR filtered and temperature controlled air has just been installed. This has eliminated the water cooled heat exchanger and surge tank, which is believed responsible for some particulate loss in the former system. As may be seen in the schematic drawing (Figure 7) the raw, hot exhaust is introduced into the cooling air dilution tube at an orifice plate and then flows into a large mixing chamber. The diluted exhaust is then directed back into the existing distribution piping that feeds the various irradiation and animal exposure chambers.

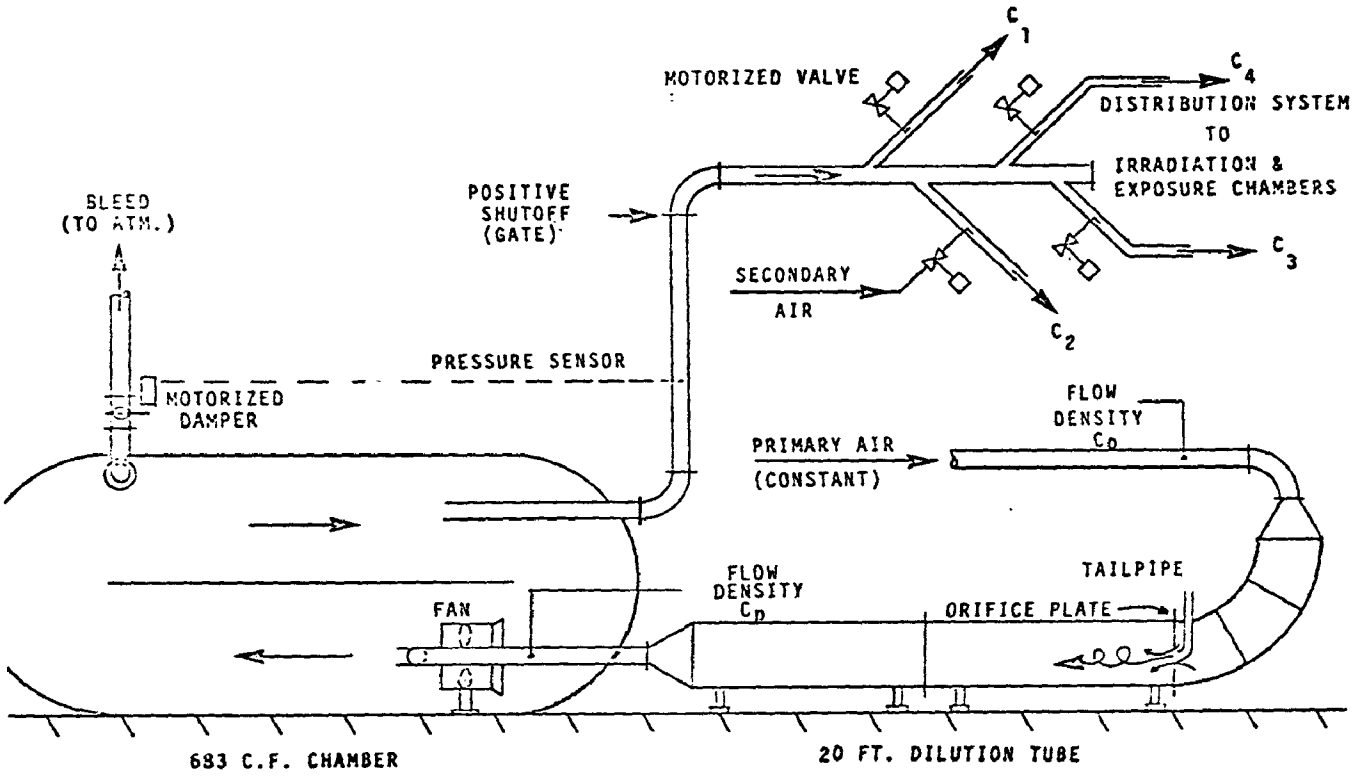


Figure 7. Schematic of air cooling and dilution utilizing constant dilution air flow

Future experiments will utilize the exhaust emissions from a Lister SR-2, 4-cycle, direct-injected, and naturally aspirated type diesel engine, in conjunction with the new air cooling dilution system. Future planning includes operating a 1973 Chevrolet 350 C.I.D. engine with added controls as now marketed, also a 1975 engine prototype with catalytic converter and possibly a Wankel rotary engine if the animal exposure time can be appropriately scheduled.

Table 1. AEROMETRIC CHARACTERIZATION OF EXHAUST EMISSIONS

Pollutant component**	Analytic method	Automatic	Manual	Where determined*
Carbon monoxide (CO)	Nondispersive infra-red spectroscopy	X		EPM, EC
Total hydrocarbons (THC), as CH_4	Flame ionization spectroscopy	X		EPM, EC
Nitrogen oxides (NO_x includes NO and NO_2)**	Colorimetry using Saltzman reagent	X	X	EPM, EC
C_1 to C_5 hydrocarbons (several compounds)	Gas chromatography		X	EC
C_6 to C_{10} aromatic hydrocarbons (several compounds)	Gas chromatography		X	EC
Aldehydes, total	MBTH according to Hauser		X	EC
Particulates, total mass	Filtration gravimetry; optical density (Sinclair-Phoenix)		X	EC
Particulate size distribution: Aerodynamic	Stage impaction (Anderson)		X	EC
Photonomeric	Photoelectronic (Royco)		X	EC
Particulate composition	Infra-red and ultraviolet spectrophotometry		X	EC
Ozone, "oxidant" **	Coulometry (mast)	X		EC
	Iodometric colorimetry		X	EC

*EPM - Exhaust or primary exhaust: air mixture; EC - exposure chamber
**NO_x and ozone methods to be replaced by automatic chemiluminescence instruments.

ATMOSPHERIC CHARACTERIZATION IN AUTO EXHAUST EMISSIONS

M. Malanchuk, G. Contner and R. Slater

In conducting biological effects studies of mobile emissions, it was necessary to evaluate the atmospheres at various points in the engine-to-exposure chamber system for gases, vapor$, and particulates.

The methods employed for measuring component concentrations are summarized in Table 1.

The average values over the entire period of the exposure run for the six different runs are given in Table 2. The measurements are listed for those chambers in the RH (nonirradiated, "high" concentration) group and in the IH (irradiated, "high" concentration) atmosphere chambers.

TABLE 2. SUMMARY OF EXPOSURE CHAMBER AEROMETRY TAME STUDIES

Pollutant	Treatment	A	B	C	D	E	F
CO	RH	93	99	92	101	101	125
	IH	96	99	92	98	101	118
THC	RH	98	19.6	109	182	99	106
	IH	102	19.4	95	154	81	87
NO	RH	23.7	5.8	17.4	10.8	24.3	21.4
	IH	18.9	5.1	10.8	4.5	18.7	13.0
NO_2	RH	3.6	0.3	3.2	1.8	3.4	2.9
	IH	8.6	2.0	9.2	5.8	8.8	7.6
Aldehydes	RH			5.2	14.1	2.8	3.3
	IH			17.2	36.4	6.5	7.3
GC-HC's: C_1-C_5	RH					6.8	9.6
	IH					5.8	6.9
C_6-C_{10}	RH					2.1	2.7
	IH					1.9	2.1
Particulates $\mu g/m^3$ (Total)	RH	311	38	164	556	164	387
	IH	433	75	1726	3293	980	1290
Particulate Ratio	IH/RH	1.4	2.0	10.5	5.9	6.0	3.3

*Control air levels for CO and HC range 3-6 ppm

Concentrations in ppm except as noted

Although various comparisons can be made among the several runs, TAME-A to TAME-F, with respect to atmospheric compositions, a most useful comparison can be made between TAME-E and TAME-F. During those two exposure studies, the system conditions were closely controlled so that the results could be reliably compared for the effect on exhaust emissions of introducing additives into the gasoline. A quick appraisal of the close control of engine operation can be seen in the consistent values for CO, THC, and NO_x concentrations over the length of the runs of TAME E and TAME F in Figures 4 and 6 of the previous report.

TAME E used the reference fuel, Indolene. TAME F used the reference fuel to which methylcyclopentadienyl manganese tricarbonyl (MMT) at the ratio of 0.25 g (as Mn) MMT per gallon of fuel was added.

The particulate concentrations produced in various parts of the system can be compared in Table 3.

TABLE 3. PARTICULATE CONCENTRATIONS IN TAME E AND TAME F STUDIES

	PARTICULATE, mg/M^3	
	TAME E	TAME F
Heat Exchanger, Effluent	2.90	-
Surge Tank	2.55	5.01
Exposure Chamber #6 (Nonirradiated)	0.17	0.39
Irradiation Chamber #3	0.15	0.31
Exposure Chamber #15 (Irradiated)	0.66	1.31

The TAME F/TAME E pairs of values show a consistent 2:1 ratio. It appears that the presence of the MMT in the fuel, which was the one major variable in the two studies, was primarily responsible for the considerable increase in particulate emissions in TAME F. Irradiation chamber #3 was the source of irradiation treatment of the atmosphere introduced into exposure chamber #15.

Key components analyzed in TAME E and F were calculated to a g/mile basis for comparison with other data presented as typical for a 1971 Ford-8 operated on a dynamometer, using Shell no-lead gasoline (John Sigsby, Jr. Conference on Health Intelligence for Fuels, RTP, 05-07 January 1973). For that purpose, the TAME values of ppm were converted to g/mile by assuming that the modified California cycle used with engine "B" of the TAME studies resulted in an average exhaust flow of 1 m^3/min and an average of 22 mpg equivalent road speed. The values are listed in Table 4.

TABLE 4. GASEOUS EMISSIONS OF AUTOMOBILE ENGINES

Component	TAME E	TAME F	'71 Ford Operated on Dynamometer using Shell No-Lead Gasoline, Hot Start
THC	3.46	3.84	1.81
CO	5.95	7.02	15.60
NO_x	1.57	1.51	4.06
Ethylene	0.083	0.166	0.212
Propylene	0.055	0.125	0.117
Butene	0.013	0.021	0.026
Acetylene	0.053	0.106	0.158
Benzene	0.041	0.105	0.142
Toluene	0.071	0.159	0.142
o-, p-Xylene	0.076	0.160	0.050
Butylbenzene	0.028	0.061	0.031
i-Butane	0.009	0.009	0.005
n-Butane	0.055	0.046	0.077
i-Pentane	0.072	0.071	0.085
n-Pentane	0.042	0.043	0.019

From analyses of the particulate collected at the surge tank, it was determined that 27.0% of the Mn burned in the engine (combustion) was recovered in the particulate from the surge tank. That may be most all the particulate from the exhaust that is of airborne size (<10μ) and, therefore, of potential biological significance.

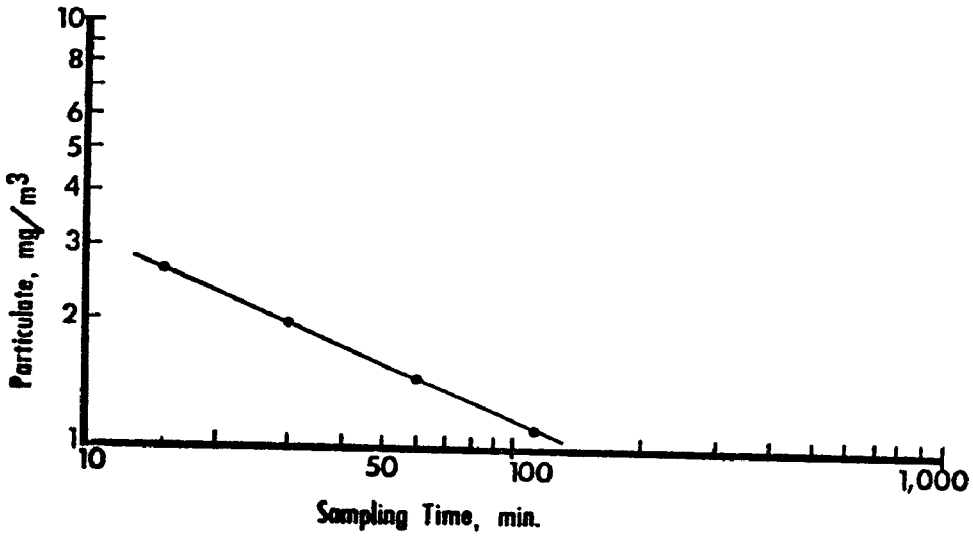
PARTICULATE SAMPLING PROCEDURES FOR AUTO EXHAUST EMISSIONS

M. Malanchuk and A. Cohen

Group samples of auto exhaust atmospheres were taken (during the TAME-F study) to explore the adequacy of currently widely-used procedures for particulate evaluation. One group of samples were collected on glass fiber filters for particulate mass measurements. Another group of samples were collected by an Andersen sampler for particle size distributions.

The filter sampling conditions consisted of sampling from an exposure chamber (#16) containing irradiated exhaust emissions that had been diluted with clean air by a factor of about 24-25 before the irradiation treatment. The identical sampling rate, 3 cfm, was used for 15-, 30-, 60-, and 120-minute collections on 142-mm glass fiber filters. The initial weighings (immediately after the sampling) established sample weights that indicated concentration values in a logarithmic relationship to the sampling times (Figure 1).

Figure 1. Particulate Concentrations Measured in the Irradiated Atmosphere of Exposure Chamber #16



The atmospheric particulate concentration calculated from the 15-min. sampling was more than twice the value for the 120-min. sampling although the concentration values should be the same, since the same source of atmosphere was sampled. Later (by several days) weights of the self-same filter samples showed a progressive tendency for the samples to lose weight (the shorter-term samples lost at a greater rate than the longer-term samples); the recalculated concentrations approached a more common value.

A different group of particulate samples were collected from the surge tank unit by an Andersen sampler. The flow rate was also 3 cfm. Collection times were 15, 30, 60, and 75 minutes. Glass fiber filters were used on each stage of the sampler to retain the impacted particles with maximum efficiency. Weighings were amde as soon as possible after the sampling was concluded. Calibration values determined for the increased flow rate were used to plot the weights on the log-probability scale (Figure 2).

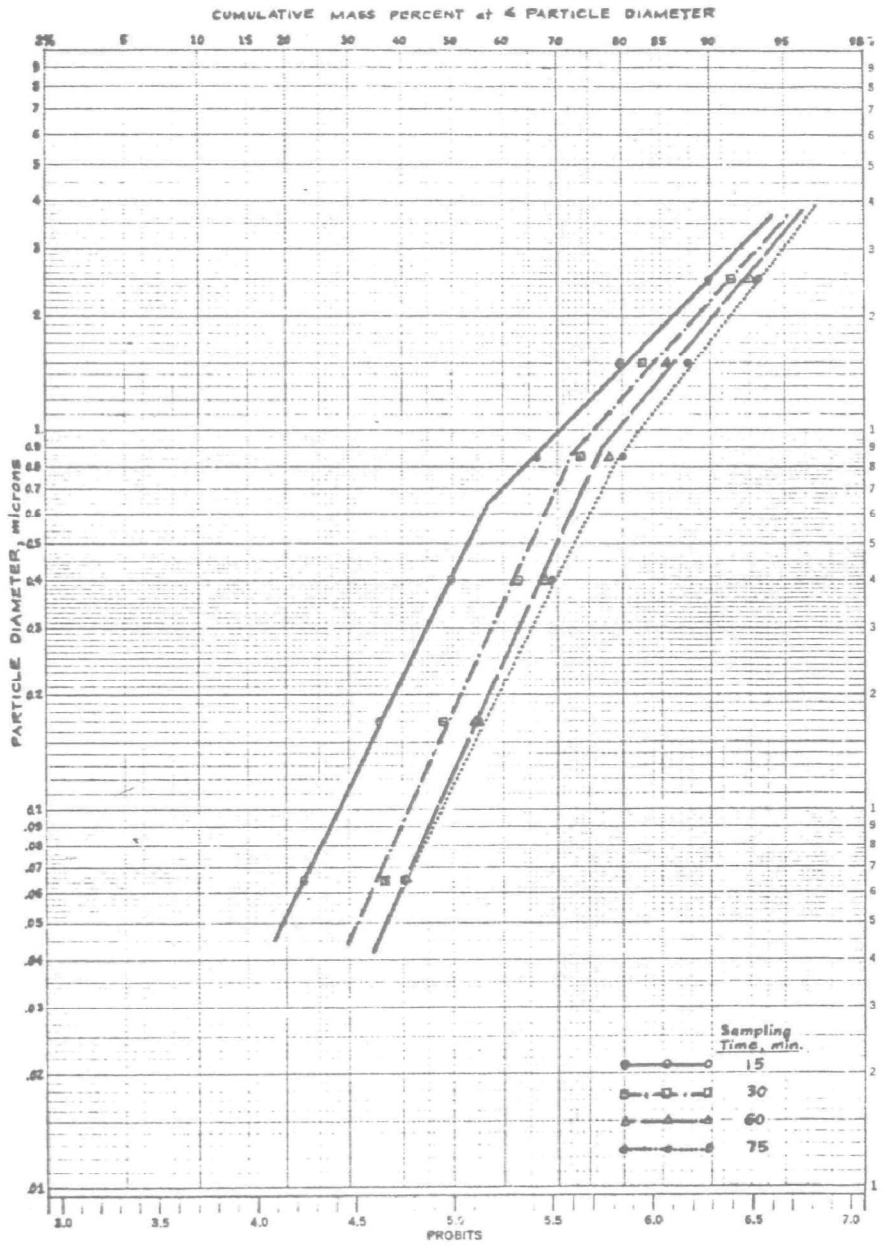


Figure 2. Effect of Sampling Time Upon Particle Size Distribution Measured in Auto Exhaust emissions

The parallel relationships of the plots emphasize the similarity of distributions. The broken line characteristic indicates a skewed effect upon the normal distribution picture. The equivalent mass median diameter (EMMD) for these (uncorrected for normal distribution appearance) samples show decreasing values of such diameters with longer sampling times - from 0.41 μ for the 15-min. sample to 0.11 μ for the 120-min. sample.

From the data obtained (pictures evident in the figures) the filter and the Andersen sampler measurements, it is obvious that modifications or alternatives in the procedures, or equipment, or both should be used to attain greater accuracy in such measurements (as made on systems like auto exhaust emissions).

DIFFERENCES IN FUEL EMISSION COMPONENTS IN TAME E AND F: TENTATIVE INFERENCES CONCERNING INFLUENCE OF A TEST GASOLINE ADDITIVE AND OF GENERATION VARIABLES

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R. Iltis, J. Burkart, R. Hinners, Y. Yang and J. Stara

Comparisons were made concerning the composition of irradiated (I) and nonirradiated (raw, R) automotive engine exhaust resulting from the use, in two sequential experiments, of reference gasoline* in one study (TAME-E) and the same reference gasoline plus a test additive+ (TAME-F). Generation, control and distribution systems, and atmospheric characterization are described elsewhere (Hinners, et al. and Malanchuk, et al.).

Prior to addition of test additive in TAME-F, systems were adjusted to match as well as possible initial performance obtained during TAME-E, according to several criteria. These criteria, and the ratio of initial performance in F (i.e. before additive put in the fuel) to initial performance in E, are shown in Table 1. Average performance throughout the study period are also compared for the additional criteria of fuel consumption and exhaust oxygen content.

TABLE 1. CONTROL CRITERIA AND CONDITIONS IN TAME E AND F

Criterion*		TAME-E	TAME-F	Ratio, F:E	Data Base
Air:Fuel ratio		15.0	15.3	1.02	Initial values
Exhaust dilution ratio		16.5	15.7	.95	"
Exhaust gases: (ppm)	CO	1701	1680	.99	"
	THC	1407	1747	1.24	"
	NO_x	502	439	.87	"
Fuel consumption, (gal/hr)		1.18	1.23	1.04	Overall mean
Exhaust oxygen, %		2.28	2.66	1.17	"

*Amoco unleaded test fuel ("indolene clear")

+An organic manganese compound, used at twice the normally recommended concentration in the fuel (0.25 gm Mn/gal).

Chamber atmospheric monitoring data for several components in TAME E and TAME F are compared in Tables 2 and 3. The direct ratio of F:E means are shown to express magnitude of differences; also shown are the results of simple student's t-tests of statistical significance. It can be seen that for some emission components differences between E and F are larger in proportion than might simply be expected in the light of relatively small differences in the control criteria, and that several of the larger proportional differences are supported by statistical significance.

TABLE 2. COMPARISON OF ENGINE EMISSION COMPONENTS IN TAME E AND F

Component	Atmosphere	TAME-E	TAME-F	RATIO F/E	t-Test[+]
CO, ppm	R	102.6	126.9	1.24	***
	I	108.1	123.6	1.14	**
Tot.hydro-carbons, ppm	R	101.4	107.6	1.06	NS
	I	88.8	99.6	1.12	*
NO, ppm	R	24.6	22.5	.92	NS
	I	20.7	15.9	.77	**
NO_2, ppm	R	3.3	2.8	.87	?
	I	8.6	7.5	.87	NS
Tot. alde-hydes, ppm	R	3.1	4.0	1.30	*
	I	5.4	5.8	1.07	NS
Aliphatics,[++] ppm	R	1.42	1.34	0.94	No test
	I	1.26	1.25	0.99	"
Olefinics,[++] ppm	R	6.03	8.28	1.37	"
	I	5.40	6.90	1.28	"
Aromatics,[++] ppm	R	2.49	2.67	1.07	"
	I	2.11	2.35	1.11	"
Tot.particu-late, $\mu g/m^3$	R	173	388	2.24	***
	I	658	1311	1.99	***
Particulate Mn, $\mu g/m^3$	R	0.10	99	-	Obvious signif.
	I	0.26	90	-	Obvious signif.

[+]NS = not significant, $p > 0.1$; ? = questionable significance, $0.1 > p > 0.05$; *, **, and ***, significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

++See Table 3 for components whose concentrations are summed herein.

TABLE 3. COMPARISON OF EXHAUST HYDROCARBON COMPONENTS IN TAME E AND F[+]

COMPONENT	TAME E		TAME F		RATIO, F/E		t-TEST	
	R	I	R	I	R	I	R	I
Aliphatics:								
n-Butane	0.50	0.44	0.42	0.41	0.84	0.92	NS	NS
i-Butane	.08	.09	.08	.07	.95	.77	NS	?
n-Pentane	.31	.29	.32	.30	1.04	1.06	NS	NS
i-Pentane	.52	.44	.52	.47	.98	1.06	NS	NS
Olefinics:								
Acetylene	1.54	1.55	2.15	2.03	1.39	1.31	**	*
Ethylene	2.37	2.27	3.14	2.84	1.32	1.25	*	?
Propylene	1.06	.88	1.58	1.15	1.48	1.30	**	?
Butene-1	.16	.13	.20	.14	1.22	1.10	NS	NS
Isobutylene	.59	.38	.77	.51	1.32	1.32	NS	*
Butadiene	.30	.18	.44	.23	1.46	1.29	*	*
Aromatics:								
Benzene	.56	.57	.72	.71	1.29	1.25	*	*
Toluene	.88	.76	.92	.83	1.04	1.07	NS	NS
o,m-Xylene	.57	.42	.58	.46	1.01	1.05	NS	NS
p-Xylene	.25	.18	.22	.17	.88	.93	NS	NS
Bu-Benzene	.24	.16	.24	.18	1.02	1.13	NS	NS

+ Concentrations are in ppm; statistical designations as in Table 2.

No attempt has been made to adjust TAME F data in proportion to control criteria differences, nor to attribute environmental significance to differences noted. In some cases relatively small differences in means were statistically significant because of small data variation, and may not be of chemical significance to the environment. On the other hand, true differences of some magnitude may fail statistically by virtue of great variability. Further experimentation and additional judgements are considered necessary for a conclusive assessment concerning the true effect of the additive and its environmental importance.

However, tentative inferences from the preliminary data include:

1. That of the components monitored here, the following are the most worthy of further attention as being affected by this additive and which might also be of biological importance:

   (a) Total and manganese particulate

   (b) Certain olefinic hydrocarbons

   (c) Benzene

   (d) Other exhaust species in low concentrations to be considered are:

      (1) Phenols
      (2) Polynuclear aromatics
      (3) Nitroorganics
      (4) Epoxides
      (5) Long chain aliphatics
      (6) Amines
      (7) Sulfonates
      (8) Azoarenes

2. That an additive should and may be tested in a system carefully engineered, controlled, and monitored, and that the impact of an additive may be through its influence on other exhaust components than merely its own products.

3. It is also quite evident that there are numerous engine-related and operational factors to be controlled, monitored and assessed in the conduct and interpretation of such engine emission studies.

The data of these studies also reflect the influence of irradiation-induced photochemical reactions on exhaust composition in this facility. Although because of quanity and relationships of hydrocarbons and nitrogen oxides there has been negligible, if any, oxidant formation in this test series, the following effects do appear significant.

1. Moderate reduction of NO and considerable increase of NO_2

2. Considerable increases in total aldehydes and total particulate.

3. Slight to moderate decreases in specific hydrocarbons

Determination of effects of fuel factors on emissions composition is important in suggesting areas of environmental impact and health concern, but the establishment and evaluation of standards and regulations concerning fuel products will likely also require definitive demonstration of associated effects (comparative toxicity) in billogical systems exposed to these emissions, such as increased body burden and functional and pathologic alterations.

CHANGES IN PATHOLOGY OF RATS AND HAMSTERS FOLLOWING INHALATION EXPOSURE TO MOBILE EMISSIONS

D. Hysell and W. Moore

In TAME C and D (the latter with MMT) sufficient animals were maintained in clean air, CO control, and high raw (RH) and high irradiated (IH) exhaust chambers so that five rats and five hamsters from each exposure group could be sacrificed on days 2, 4, and 6 for pathologic evaluation. In addition, in TAME D, sufficient rats were maintained in clean air and in RH and IH exhaust so that tissues from 18 rats in each group could be chemically analyzed for tissue Mn; of these, tissues were saved from six rats of each group for pathologic evaluation. In TAME E and F (with MMT), the numbers were increased so that animals could be examined on days 1-5 as well as 20 rats per each exposure group for the 2-wk exposure period. No gross abnormalities were noted in any animals except for chronic respiratory disease (CRD) in a significant number of rats. Tissues collected for histology were larynx and trachea, lung, liver, and kidneys. Tissues collected for chemical analysis from the rats exposed for 2 wk, were brain, heart, lung, liver, and kidney. One eyeball was saved from each 1-to 5-day-exposed hamster and rat in TAME E and F and processed for corneal mitotic rate determination.

Microscopic evaluation of tissues from TAME C and D has been completed. In those rats sacrificed on days 2, 4, and 6, no abnormalities were noted other than CRD. In TAME D, the rats maintained for 2 wk all showed rather severe CRD, but in all the IH exhaust animals there was a marked acute purulent bronchopneumonia superimposed. One third of the RH exhaust group showed these changes. In the hamsters from both TAME C and D, there were rather marked changes in pulmonary tissue compared with control animals (Figure 1).

As early as day 2, the exhaust animals manifested a rather prominent increase in leukocytes and macrophages in alveoli at the level of the terminal brochioles (Figure 2).

By day 6, there appeared to be thickened alveolar septae, a possible increase in Type II alveolar septal cells, and early epitheliazation of more peripheral portions of respiratory ducts (Figure 3). These changes occurred in both RH and IH exhaust groups but affected a higher percentage of the latter. No abnormalities relatable to the Mn additive were noted. If anything, pulmonary changes appeared more severe in TAME C.

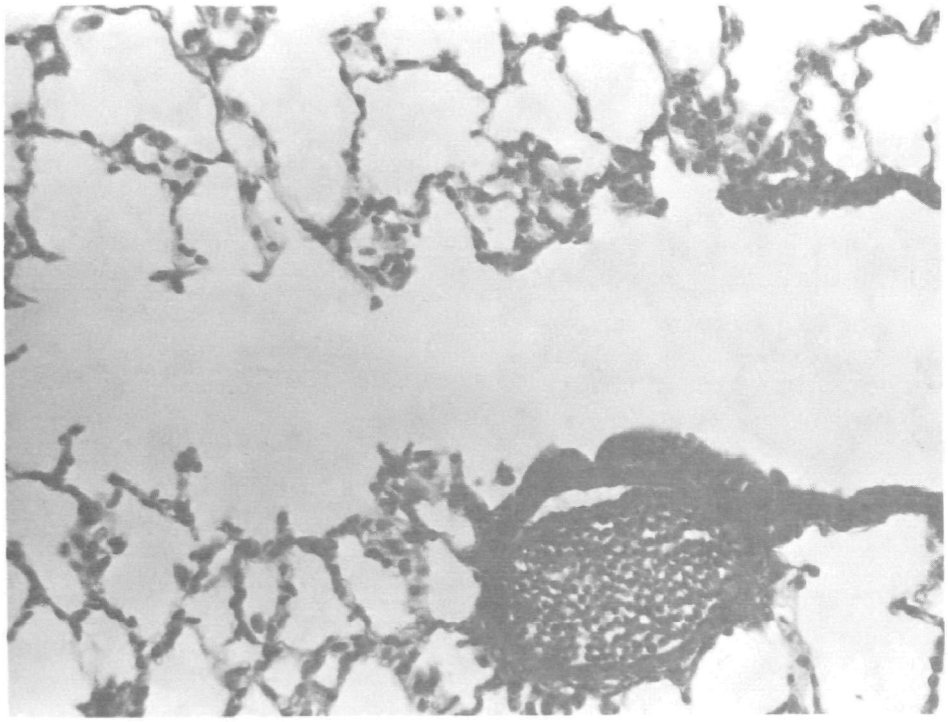

Figure 1. Normal hamster lung.

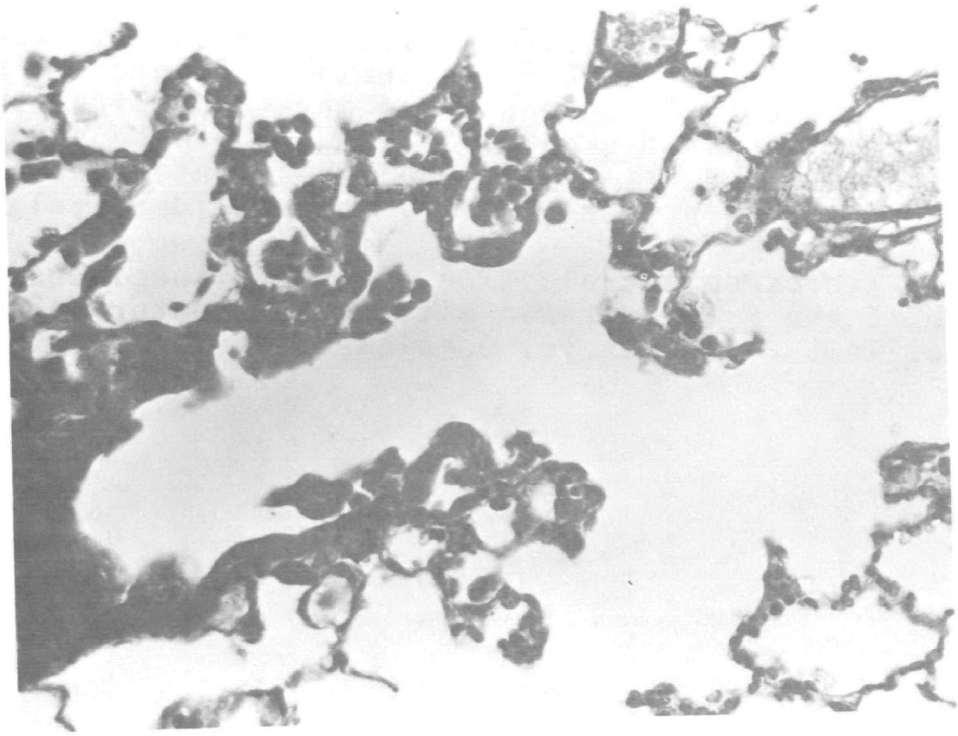

Figure 2. Hamster lung after 2 days exposure to exhaust in TAME study.

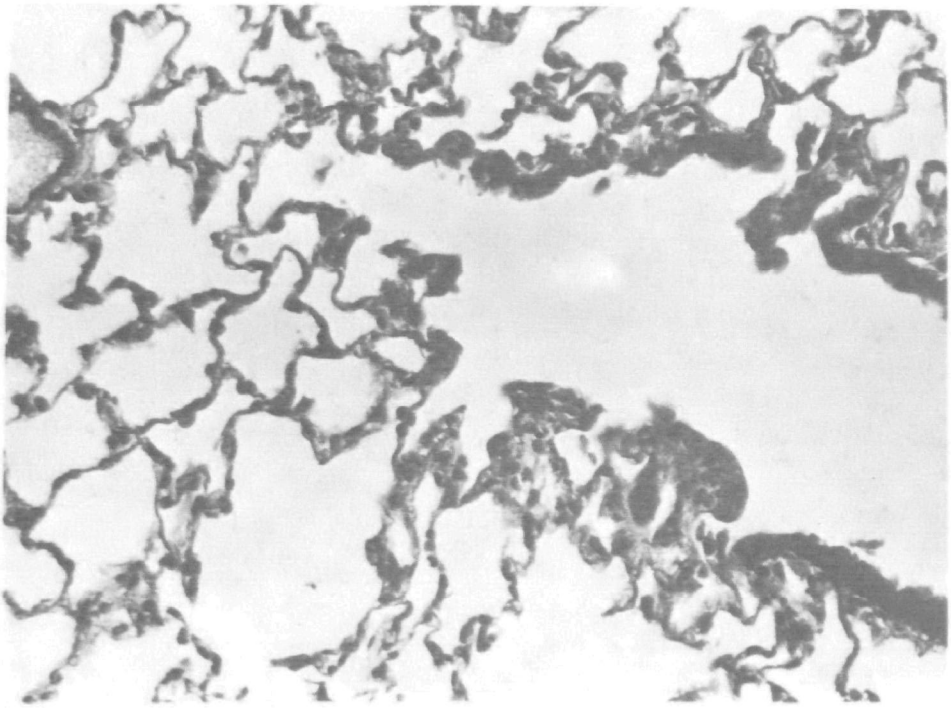

Figure 3. Hamster lung after 6 days exposure to exhaust in TAME study.

The results of corneal mitotic rate determination in hamsters from TAME E are shown in Figure 4. As may be noted there is an early and marked cyclical response in the IH exhaust group. The RH group also shows a response which is somewhat delayed. Determinations in the rats (not presented here) show an identical pattern.

Microscopic evaluation of tissue specimens from TAME E and F and corneal mitotic rate determination from TAME F are not yet completed.

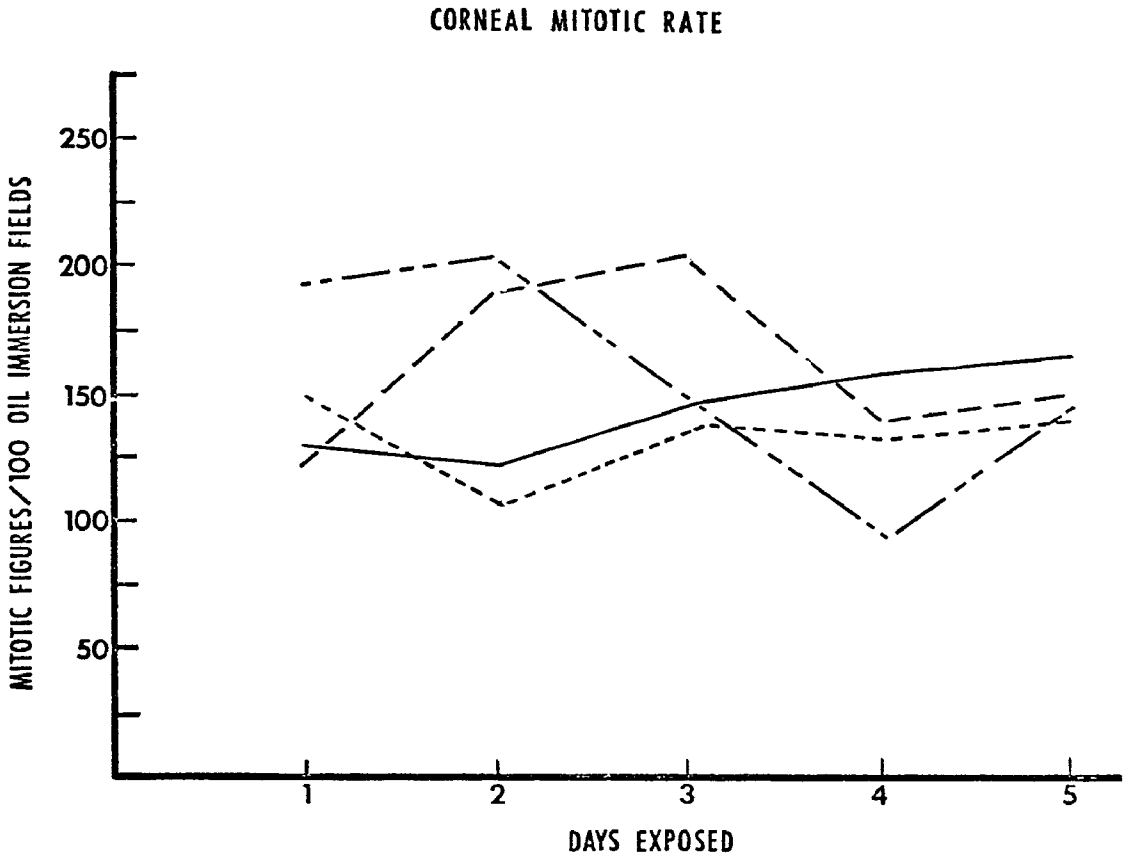

Figure 4. Corneal mitotic rate of control and exhaust exposed hamsters in TAME E.

EFFECTS OF MOBILE EMISSIONS ON BODY WEIGHT AND TISSUE LEVELS OF Mn IN RATS

R. Miller, W. Moore, and D. Hysell

Groups of 18-20 animals were exposed for 14 days to each of the atmospheres in TAME D, E, and F. During the exposure, the animals were weighed at intervals of time, and the data are presented in Figures 1, 2, and 3. Although some of the body-weight curves for different types of exposure were not significantly different, it should be noted that there is a consistent trend in the weight data. Exposure to the raw (RH) and irradiated (IH) exhause atmospheres consistently depressed the weight gains which indicates that these atmospheres, in some unknown way, influenced the growth rate of these animals. The irradiated atmosphere in each experiment had a greater effect than the nonirradiated exhaust atmosphere.

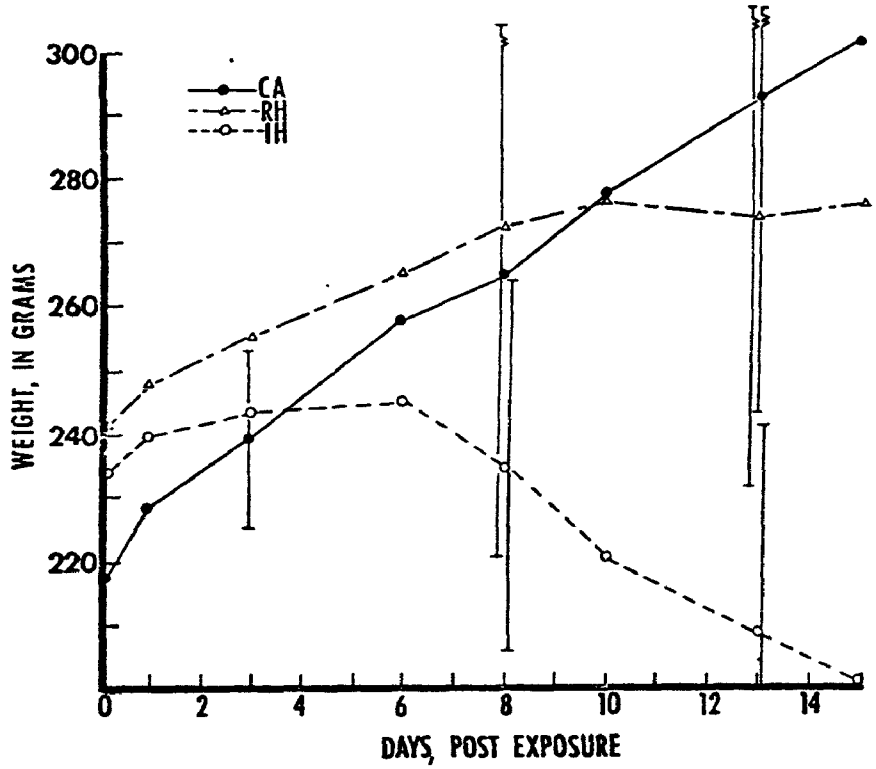

Figure 1. Weight of rats after exposure to auto emissions when 0.37 g MMT added to reference fuel, TAME D.

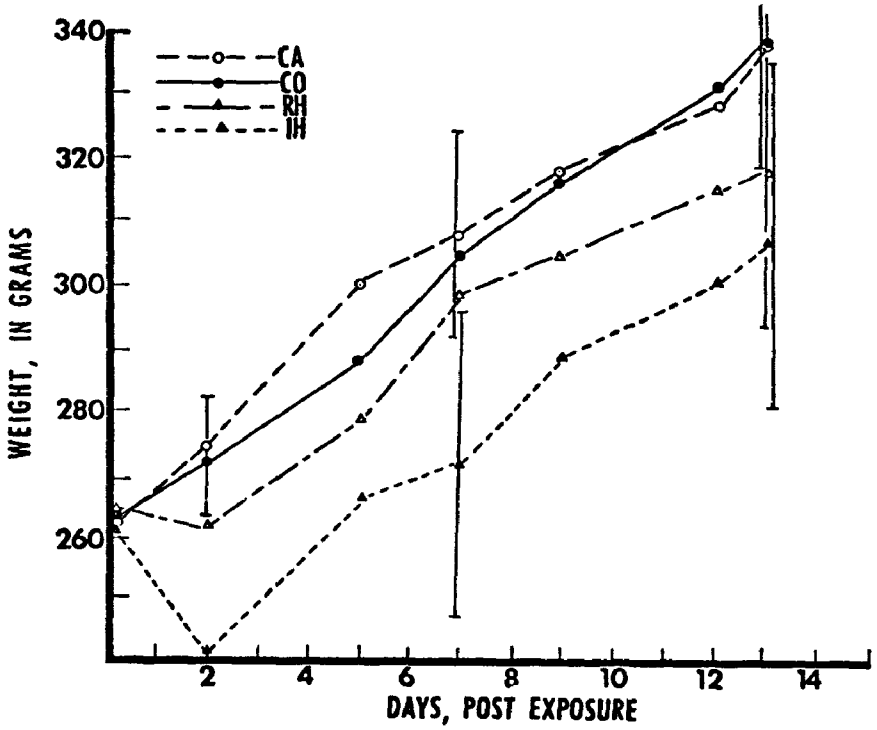

Figure 2. Weight of rats after exposure to auto emissions with reference fuel only, TAME E.

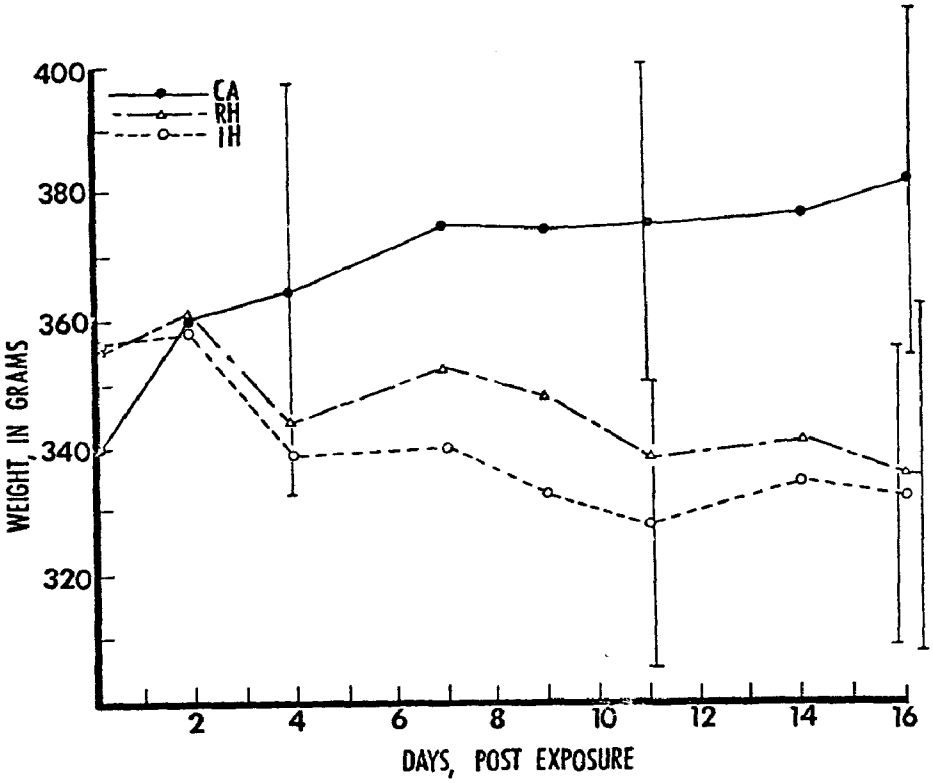

Figure 3. Weight of rats after exposure to auto emissions when 0.25 g MMT added to reference fuel, TAME F.

At the end of the experiment (14-15 days), the animals were sacrificed and selected tissues taken for Mn analysis by atomic absorption spectrophotometry. The Mn content for tissues taken in TAME D, E, and F are given in Table 1. There was some variation in the tissue

TABLE 1. MANGANESE TISSUE CONCENTRATIONS FOLLOWING INHALATION EXPOSURE TO AUTO EXHAUSTS FROM INDOLENE WITH AND WITHOUT MANGANESE

Experiment	Type of fuel	Mn µg/g Dry Sample		
		Clean Air	Nonirradiated exhaust (RH)	Irradiated exhaust (IH)
Lung				
TAME E	Indolene	2.86	1.80	2.16
F	Indolene 0.25 g Mn	3.19	5.47	3.98
D	Indolene 0.37g Mn	5.98	8.15	7.37
Kidney				
TAME E	Indolene	3.15	3.03	3.58
F	Indolene 0.25 g Mn	4.13	4.49	3.97
D	Indolene 0.37 g Mn	4.85	6.57	6.46
Liver				
TAME E	Indolene	6.99	6.65	8.41
TAME F	Indolene 0.25 g Mn	7.58	7.57	8.22
TAME D	Indolene 0.37 g Mn	8.19	7.93	7.72
Brain				
TAME E	Indolene	4.49	6.16	6.67
F	Indolene 0.25 g Mn	5.03	5.65	4.77
D	Indolene 0.37 g Mn	5.42	5.18	3.23

levels of Mn among the control groups when the different TAME studies were compared; for example, all the Mn levels for the control tissues in TAME E are lower than TAME F,

and the control values for TAME F are lower than TAME D. These differences may be attributed to either slight deviations in chemical analysis or to slight differences in the tissue Mn levels of these three different rat populations. The rats used for each TAME experiment were received approximately 10-14 days before onset of the study, and thus, the animals did not have sufficient time for their Mn tissue levels to equilibrate with Mn levels in our rat diet. It has been shown that the concentration of Mn in the tissues is directly related to the level of Mn in the diet. Some of the lung and kidney samples for TAME D and F showed increased Mn concentrations over the control values. These studies do not, however, resolve the question of whether or not increased atmospheric levels of Mn would significantly effect the total body burden.

DETECTION OF EARLY BIOCHEMICAL ALTERATION IN HAMSTERS EXPOSED TO AUTO EXHAUST GASES

S.D. Lee, R.M. Danner, L. McMillan,
K.C. Butler, W. Moore, and J. Stara

Recently, we have demonstrated oxidative destruction of polyunsaturated fatty acid films exposed to O_3 and NO_2 in terms of gravimetric change, spectrophotometric change, and malonaldehyde formation. Furthermore, these changes were shown to be reaction-time and concentration dependent. Thus, in this study, polyunsaturated fatty acid films were exposed to irradiated auto exhaust to investigate whether similar alteration would take place or not.

Figure 1 shows the gravimetric changes observed with methyl esters of linoleic and linolenic acid in TAME F. Approximately 7% and 13% increases were observed in linoleic and linolenic acid films, respectively, in a 4-hour period. A comparison of relative changes in the two polyunsaturated fatty acid esters in two Indolene and Indolene + Mn-carbonyl studies is made in Figures 2 and 3. These data indicate that a greater change occurred in the studies in which Mn-carbonyl was used as the fuel additive. However, a positive correlation between these changes and Mn-carbonyl could not be made, as yet.

There was an apparent increase in blood glutathione level in hamsters exposed to raw (RH) and irradiated (IH) exhausts and an increase in the group that was exposed to IH auto exhaust (Figure 4). A marked increase in GSH was observed in the experiments in which Mn-carbonyl was used as the fuel additives; however, a concommitant increase was observed in the CO control. For this reason, further studies are necessary to clarify the above findings.

Lactic dehydrogenase appeared to have altered with a decreasing trend in Mn-carbonyl studies and perhaps an increasing trend in a control study (Figure 5). No appreciable difference or changes were observed in leucineaminopeptidase and glucose-6-phosphate dehydrogenase.

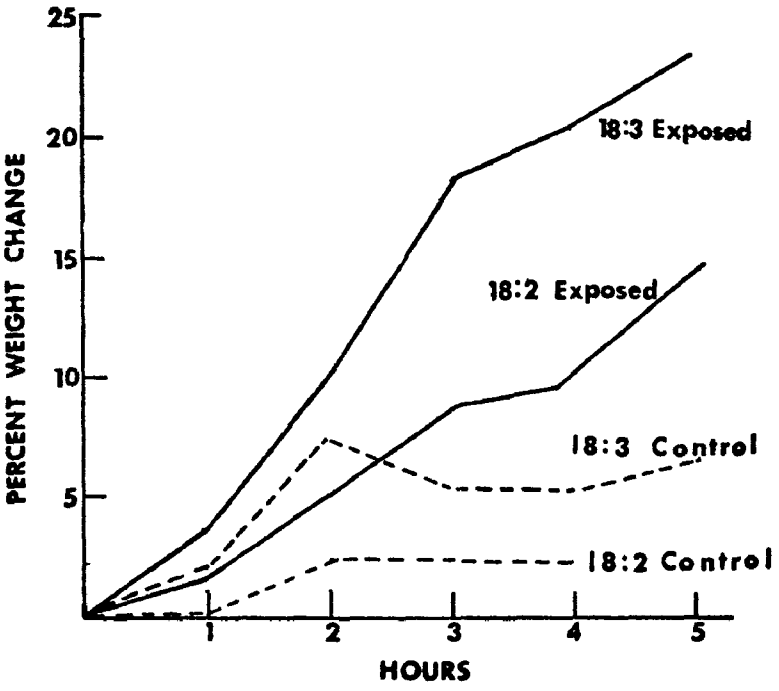

Figure 1. Relative changes in weight in Methyl esters to high irradiated exhaust and clean air in TAME F.

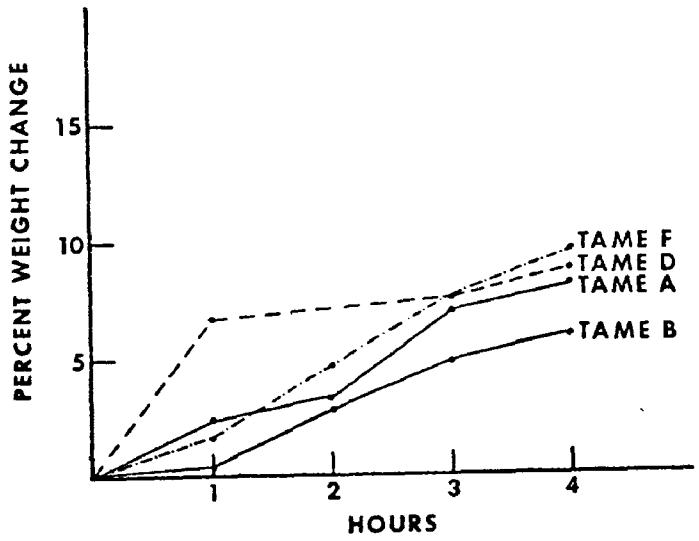

Figure 2. Gravimetric change in fatty acid (18:2) in vitro.

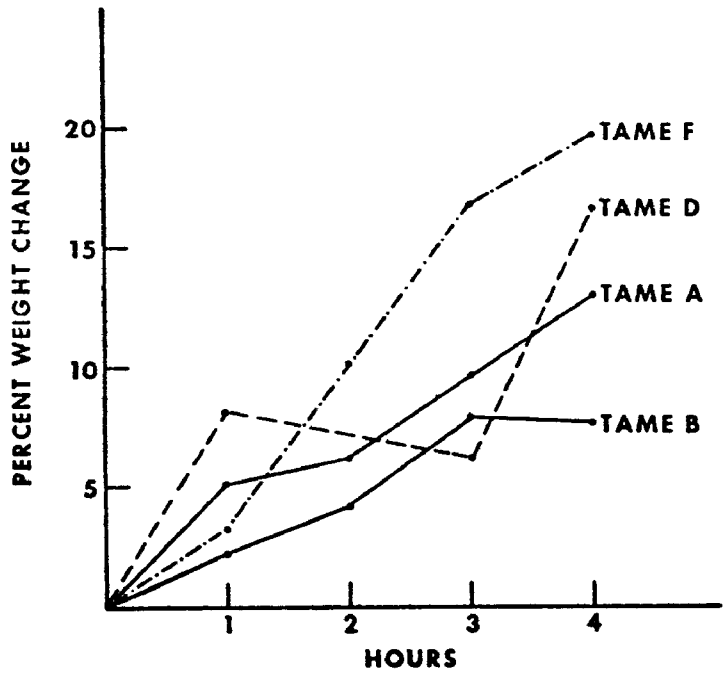

Figure 3. Gravimetric change in fatty acid (18:3) in vitro.

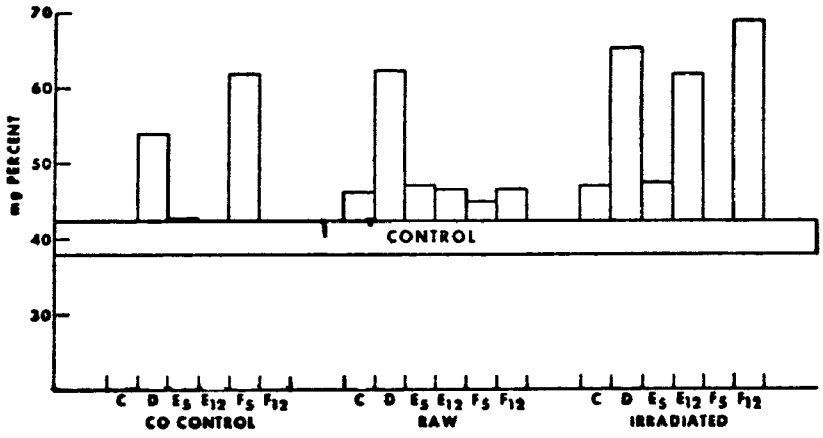

Figure 4. Comparison of blood glutathione levels of hamsters exposed in TAME.

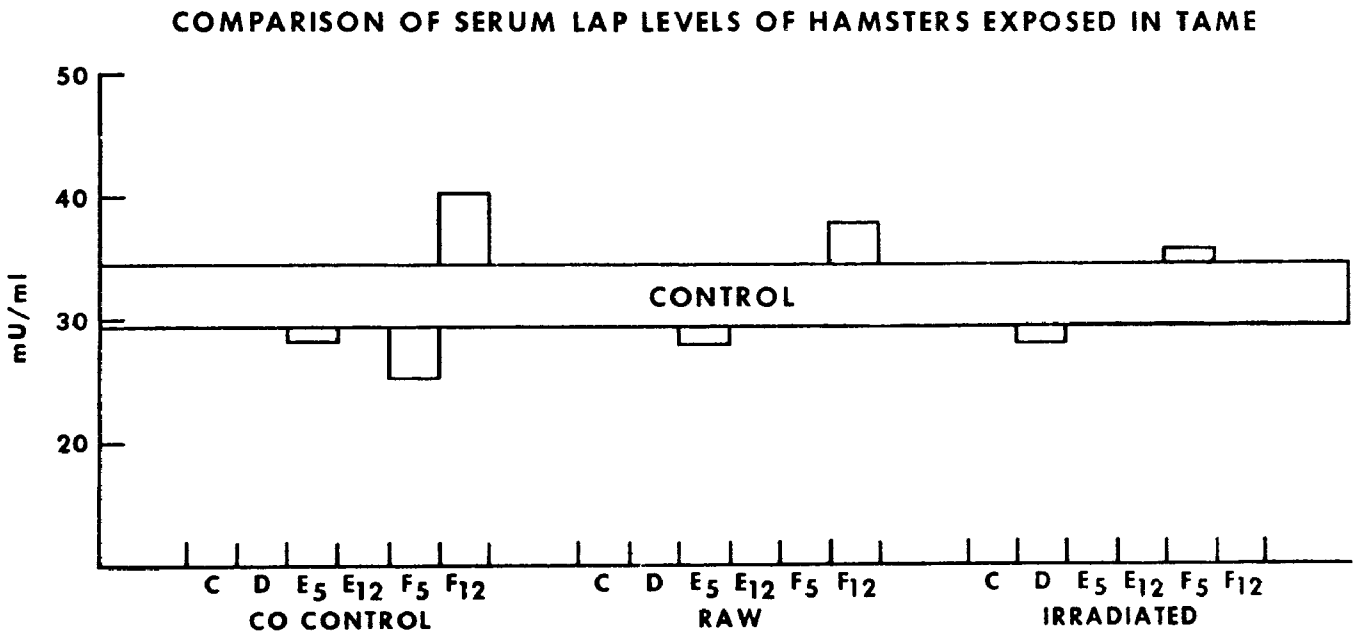

Figure 5. Comparison of serum LDH-P levels of hamsters in TAME.

DISTRIBUTION, EXCRETION, AND BIOTRANSFORMATION OF PARTICULATE POLYCYCLIC HYDROCARBONS

L. Hall, I. Washington, H. Ball, J. Adams and K. Campbell

Several compounds resulting from automobile and diesel motor fuel emissions are known to be carcinogenic in experimental animals. The determination of the biological fate of these compounds following inhalation of total exhaust emissions is of utmost importance since biotransformation of arenes like benzo(a)pyrene has been implicated mechanistically in the initiation of carcinogenesis through epoxide formation. Experiments in whole animals, however, show a decrease in tumor incidence with induction (Conney, A.H. and Burns, J.J., 1972, Science 178: 576-586). The question of whether this biotransformation is a true detoxification is unresolved at this time.

Preliminary investigations have begun for assessing the interaction of whole exhaust emission with the microsomal enzyme system that metabolizes the polycyclic hydrocarbon. Biotransformation by aryl hydrocarbon hydrolase (AHH), a cytochrome P-450 mixed function oxidase, results in metabolites, principally phenol and quinoids, with greater water solubility that are more readily excreted when compared with the lipid soluble parent compounds. Increased excretion results in a decrease in the biological residence time. Lung AHH activity has been assessed in old male hamsters (10-14 months) following exposure to diluted auto exhaust. The activity was assayed spectrofluorophotometrically (as described by Sunderman, F.W., Jr., 1967, Cancer Research 27: 950-955) on whole lung homogenates. Figure 1 shows the AHH activity after 5 days continuous exposure to CO (100 ppm), raw exhaust (100 ppm CO), and irradiated exhaust (100 ppm CO). No statistical difference was found between control (0.0145 $\pm$ 0.005) (mean $\pm$ S.D. relative fluorescence/minute/mg lung protein) and the CO animals (0.016 $\pm$ 0.002). However, raw (0.009 $\pm$ 0.003) and irradiated (0.006 $\pm$ 0.001) are different from control and from each other.

Figure 2 shows the temporal effects of exposure on AHH activity. A significant decrease (62%) occurred in 2 days. After 13 days of exposure, the activity is still depressed although variability has increased, which suggests perhaps that some animals may be recovering. This facet needs to be resolved.

The goal of our research is to discriminate between effects of different fuels and fuel additives. In Figure 3 the effects of a fuel additive (MMT) can be compared with effects of a reference fuel following 5 days continuous exposure. No statistical difference was observed between controls. The raw exhaust exposures, however, are different at the P = 0.06 level and the irradiated exposures are different at the P = 0.085 level. Since small numbers of animals are used in the comparison (five hamster/group), the true significance of this difference is not known although the data suggest a difference between exposures. Further research is intended to resolve this question.

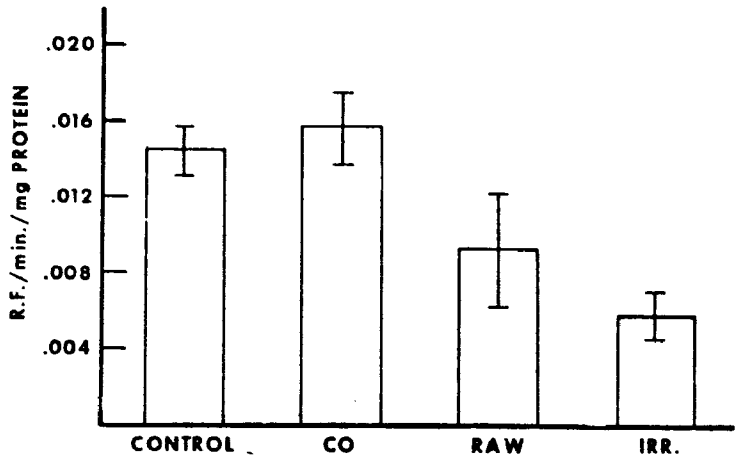

Figure 1. Effect of auto exhaust pollutants on hamster lung aryl hydrocarbon hydroxylase (AHH); comparison of different atmospheres.

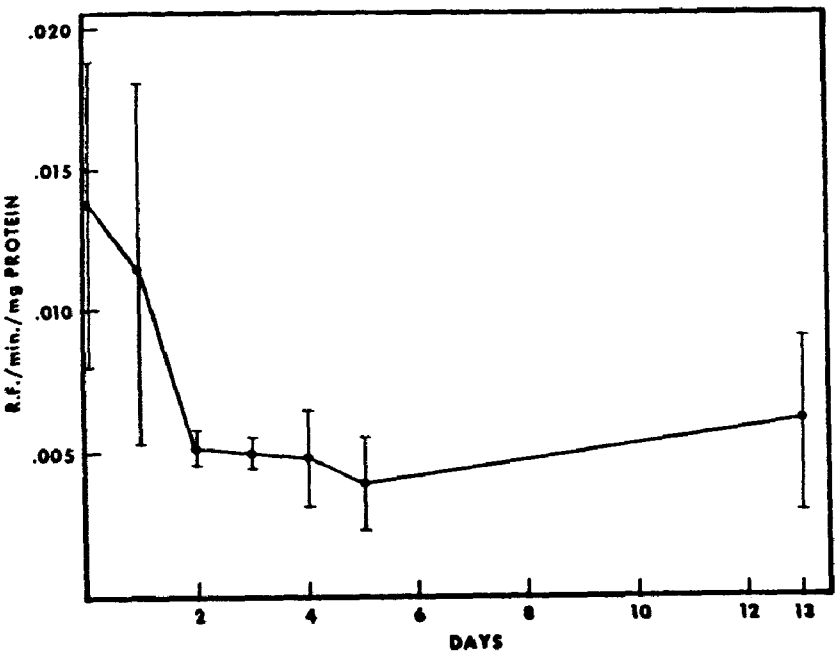

Figure 2. Temporal effect of exposure to auto exhausts on AHH activity in hamsters.

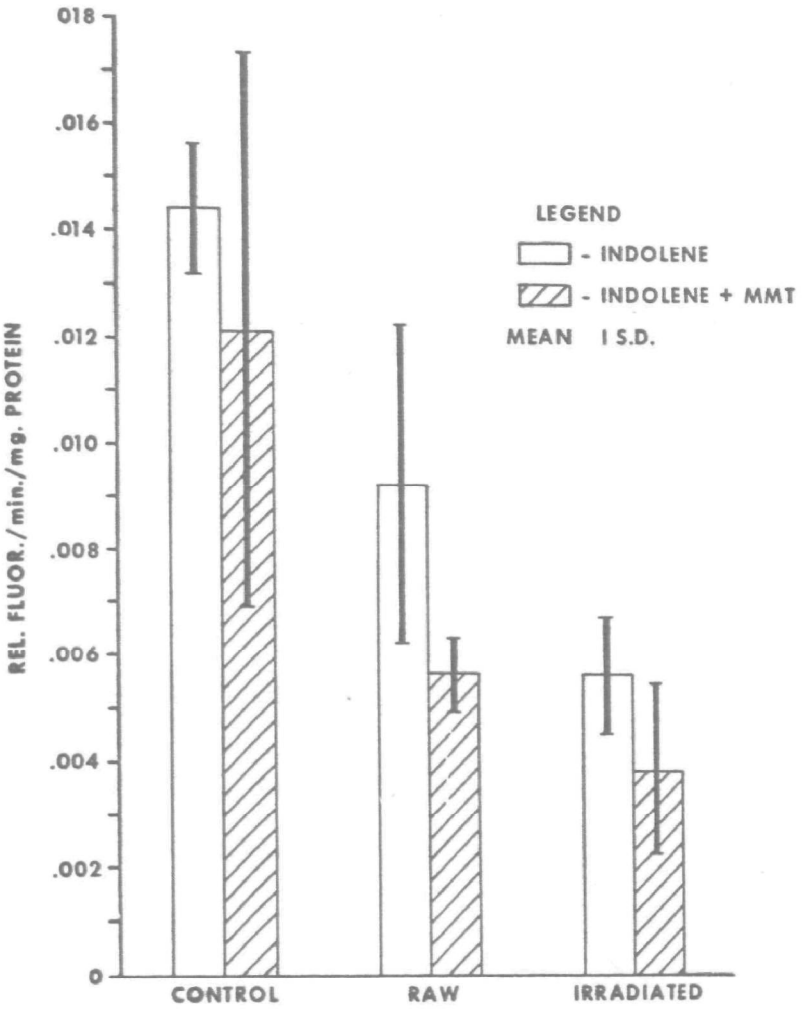

Figure 3. Comparison of hamster lung aryl hydrocarbon hydroxylase (AHH) activity following exposure to indolene and indolene + MMT emissions.

EFFECT OF ATMOSPHERIC POLLUTANTS ON PULMONARY DEPOSITION AND CLEARANCE

L. Hall, M. Malanchuk, A. Cohen, J. Adams, K. Campbell and J. Stara

An investigation is being conducted on pulmonary deposition and clearance as affected by exposure to fuel and fuel additive combustion products. With a surface area of approximately 70 m^2, the respiratory tract is a major route of entry for environmental pollutants and a major target organ for toxic effects. Effects on the self-cleaning processes (phagocytosis, mucociliary transport, and solubilization) of the lungs play a prominent role in the development of chronic lung disease and carcinogenesis.

In order to assess the integrity of this system, the method as described by Ferin, J. (1971) AIHA 32, 157-162) was used. After 13 days, continuous exposure to irradiated auto exhaust, the mice (10-14 wks) were exposed to the test challenge (TiO_2) at 10-15 mg/m^3 for seven hours, generated with a Wright Dust Feed. The mice are sacrificed at 0, 8 and 25 days post-treatment and the titanium lung burden determined spectrophotometrically with 4,4'diantipyrylmethane. Figure 1 shows the titanium lung burden in mice exposed to auto exhaust. Although no definite conclusions can be made because of limited sample size (2 animals/group), the data suggest a difference between control and exposed animals. The apparently increased deposition and lung clearance in exposed mice is being tested in larger groups of animals. The method appears to be a satisfactory tool for studies of effects on pulmonary physiology and lung defense mechanism.

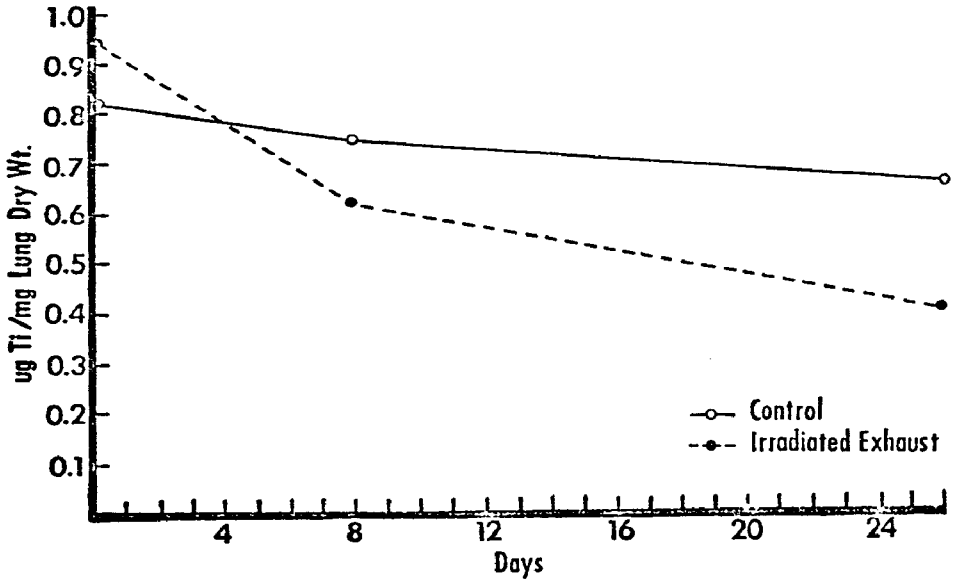

Figure 1. Retention of titanium dioxide test aerosol in mice exposed to irradiated auto exhaust.

SERUM ALPHA-1-ANTITRYPSIN IN ANIMALS EXPOSED TO FUEL COMBUSTION EMISSION

L. Hall, I. Washington, H. Ball,
J. Adams and K. Campbell

Alpha-1-antitrypsin (AT), a low molecular weight glycoprotein, inhibits the activity of a variety of proteolytic enzymes. It is believed to be synthesized at least by the liver (Sharp, H.L., 1971, Hosp. Practice 6:83-96). A deficiency, genetically transmitted, is associated with panacinar emphysema in adults and cirrhosis in children. Increased serum titers have been associated experimentally with NO_2 exposure, surgical procedures, and injections of turpentine (Ihrig, J. et al., 1971, Amer. Rev. Resp. Dis. 103, 377-388).

Because of the sequelae associated with decreased titers of this protein, investigations have begun to assess the effect of auto exhaust on this system. Serum from old, male hamsters (10-14 months) exposed to raw and irradiated exhaust for 0-13 days was collected and assayed for inhibitor activity as described (Eriksson, S., 1964, Acta Med. Scand., 175, 197). Although the sample size was limited and rather large control variation was seen, comparison of time-paired controls and exposed animals suggests a decrease in serum AT following exhaust exposure (Table 1). Additional studies are in progress.

TABLE 1. EFFECT OF IRRADIATED AUTO EXHAUST ON SERUM ALPHA-1-ANTITRYPSIN (AT) IN HAMSTERS

Exposure Time Days	Control* mg AT/ml	Exposed mg AT/ml	Percent Decrease
1	1.40 (2)	1.20 (1)	14.3
2	1.21 (3)	1.09 (3)	9.9
3	1.10 (2)	1.08 (2)	1.8

* Duplicate analysis reported as mg of Trypsin inhibitor/ml Serum. Brackets represent sample size.

CHANGES IN BLOOD AND WEIGHT PARAMETERS IN AGED RATS EXPOSED TO FUEL AND FUEL ADDITIVE EMISSIONS

M. J. Wiester

The purpose of this study was to gain information about the change in general health of a homogenous group of aged animals as a result of fuel emission exposure. Attempts were made to identify a dose response to the atmospheres as well as to estimate quantitatively if differences could be detected between the toxicity of emissions from pure gasoline with that from gasoline plus an additive, methylcyclopentadienyl manganese tricarbonyl (MMT).

Charles River retired male breeder rats ($\pm$ 600 g) were exposed to selected "TAME" atmospheres for continuous periods of 7 and 13 days. At the termination of the exposure, animals were tested for arterial blood pO_2, pCO_2, pH, COHb, WBC, Hbg, and HCT. Body weight change was also noted. On the day of bleeding, the animals were lightly anesthetized (30 mg/kg pentobarbital I.P.) and catheters (50 PE) were surgically inserted in femoral arteries. Rats were returned to their respective atmospheres and allowed to recover from the anesthesia under continued exposure for 3 hours. Arterial blood was collected under anaerobic conditions from quiet unanesthetized animals while equilibrated with their atmospheres. Plastic glove bags were utilized for this procedure. Blood gases and pH were read on a Radiometer Copenhagen, and WBC's on a Coulter Counter. The cyanmethemoglobin method was used for hemoglobin analysis and a standard NH_4OH spectrophotometric method was used for COHb.

Table 1 lists the primary pollutant concentrations for each atmosphere to which rats were subjected. There were four exposure periods, two in which Indolene was burned and two with Indolene + MMT. Animal data from similar exposures were combined and the significance of the differences were determined by mean of the student t test (Table 2).

Results indicate that there were harmful effects caused by exposure to dilute emission atmospheres when the additive was used. After 1 week of continuous exposure, weight loss, anoxia, and CO_2 retention in three animals was significantly different from control animals as well as from their Indolene exposed counter parts. After 13 days, even the blood pH was significantly decreased. Exposure to the more concentrated

atmospheres produced more pronounced harmful effects with respect to weight loss, anoxia, CO_2 retention, blood acidity, and dehydration. A comparison of weight loss for all animals is shown in Figure 1. Increased hemoglobin and hematocrit levels do not appear to be a response to increased carbosy hemoglobin levels. Dehydration would be a factor contributing to the increased weight loss seen in the High Irradiated + MMT atmosphere. No effects seen could be directly attributed to CO. Undefined interaction of components in the complex atmospheres, however, could be a modifying factor. None of the animals showed significant changes in their WBC levels that might indicate that bacterial infections were not responsible for effects seen.

In summary, rats exposed for 13 continuous days to concentrated atmospheres of fuel emissions show marked harmful effects when MMT is added to the fuel.

TABLE 1. POLLUTANT CONCENTRATIONS IN TREATMENT TEST ATMOSPHERES

Pollutants	Control	CO Control	Low Med Irr.	Low Med Irr. + MMT	High Irr.	High Irr. + MMT
CO, ppm	5.4	102.0	48.0	53.0	96.0	95.0
H_xC_7, ppm	6.0	6.0	44.0	63.0	95.0	121.0
NO, ppm	0.125	0.1	7.11	5.05	16.6	9.7
NO_2, ppm	0.075	0.01	4.78	3.8	8.8	6.1
Particulates $\mu g/m^3$	22.0	-	3.42	1432.0	1698.0	2135.0
Manganese, $\mu g/m^3$					0.3	51.0

TABLE 2. PHYSIOLOGICAL EFFECTS IN RATS AFTER EXPOSURE TO FUEL EMISSIONS

TREATMENT	CONTROL		CARBON MONOXIDE CONTROL		LOW MED IRRAD		LOW MED IRRAD MMT		HIGH IRRAD		HIGH IRRAD MMT	
days of exposure	7	13	7	13	7	13	7	13	7	13	7	13
Δ weight (g)	-9	-3	-8	-8	-6	-4	-43	-42	-41	-2	-60	-94
± S.D.	23	22	17	8	26	16	37	45	40	20·91	31	66
Significance							Δf,▼e	Δe,▼c	Δf		Δf,▼b	Δe,▼e
pO_2 mmHg	85.9	91.2	88.3	89.8	90.7	90.8	82.5	74.7	78.9	80	78.3	65.9
±S.D.	9.6	8.5	9.3	12	6.5	4	12.6	17.5	7.9	3.6	12	26
Significance							▼c	Δe,▼b	Δe,	Δe,	Δd,	Δe,▼a
pCO_2 mmHg	38.9	37.1	38.6	37.5	33.5	39.8	39	43.2	37	42	41	45
±S.D.	4.9	4.4	5.4	4.3	4.3	2.7	4.6	14	4	5	10	12
							▼d	Δa		Δc	Δc	Δc
pH	7.401	7.424	7.376	7.421	7.409	7.416	7.397	7.380	7.416	7.433	7.388	7.368
±S.D.	.028	.046	.046	.053	.028	.020	.03	.1	.026	.017	.0460	.082
Significance								Δa			Δd	Δb,▼a
Hemoglobin g%	16.0	15.6	15.9	16.2	16.4	16.8	16.4	16.3	16.9	16.9	17	17
±S.D.	.98	1	1.4	1.3	1.5	.04	1.65	1.2	.94	.72	.91	.9
Significance				Δa					Δe	Δc	Δf,•e	Δf,•c
Hematocrit %	43	44	44	45	45	46	45	45	45	47	47	50
±S.D.	3	3	4	3.	4	.6	3.8	4	2.3	2.5	2.3	1.9
Significance							Δa,				•d,▼c	•e,▼c
WBC $1000/mm^3$	10800	12300	10800	11600	16420	17400	10440	13250	11000	14800	10300	14000
±S.D.	4.6	5.2	4	4	4.8	1.2	3	5	5	4	3.8	3.2
Significance												
COHB g%	4.	2	18	15	12	8	8	5	26	20	10	19
±S.D.	5.0	5.0,	7	4	6	6	7	4	·5	6	6	4.
Significance												

Legend Significance
a. = t.90
b. = t.95
c. = t.975
d. = t.99
e. = t.995
f. = t.9995

Δ = diff. from controls
▼ = diff. from pure indolene atmosphere (no MMT)
• = diff. from CO control
No mark indicates differences not significant or comparisons were not made.

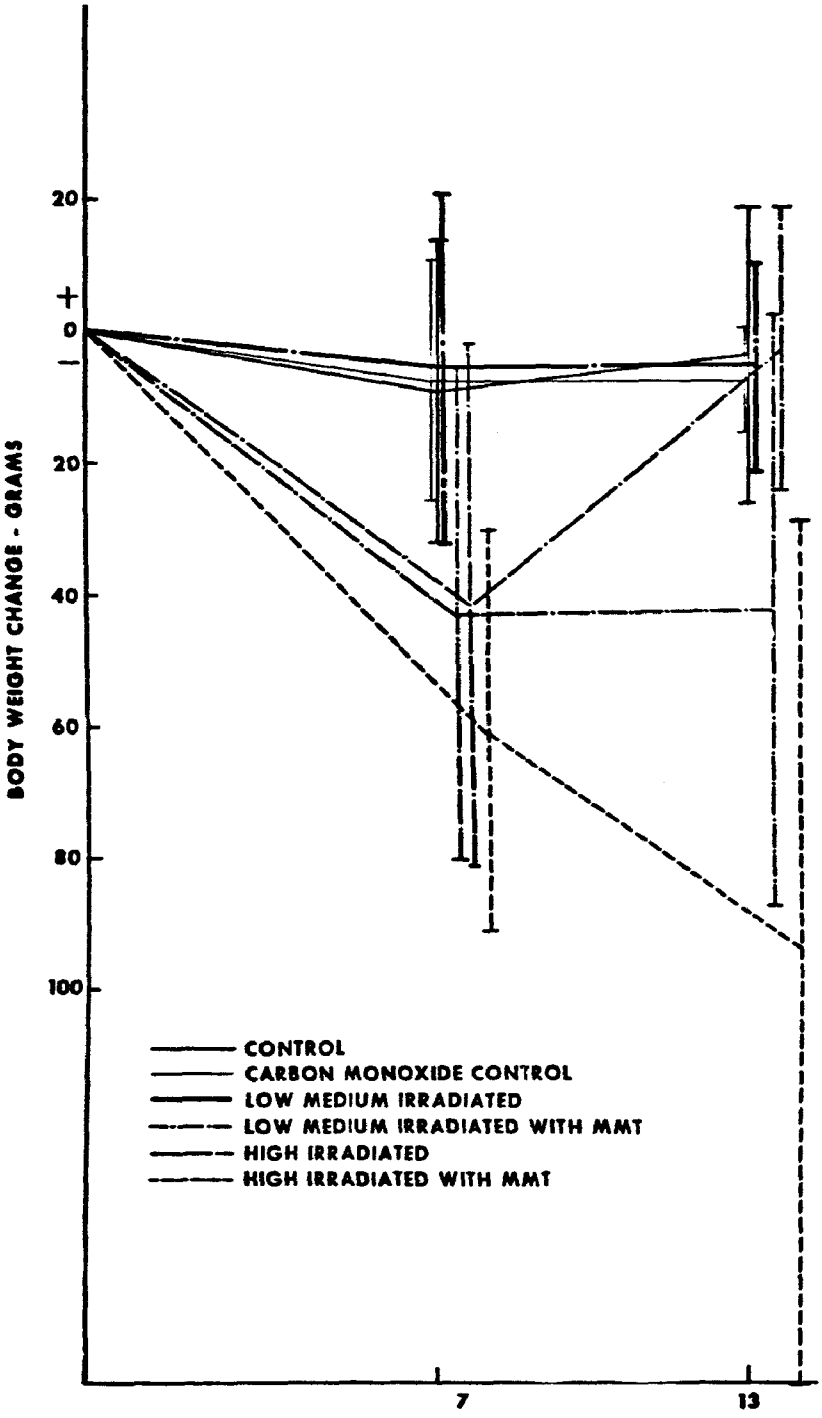

Figure 1. Effect of exposure to fuel emission on body weight of old male rats.

ACTIVITY WHEEL RUNNING OF MICE RELATED TO AUTOMOTIVE FUEL EXHAUST. I. EFFECTS OF EXHAUST LEVEL

M.I. Gage, Y.Y. Yang, A.L. Cohen and J.F. Stara

Evidence from a number of sources indicated that exposure to emissions from automotive and diesel fuels altered the functioning of the nervous system and behavior of animals. Most of the reported behavioral changes have been decreases in animal activity as a function of exposure to automobile engine exhaust, or constituents of exhaust in ambient air or laboratory created atmospheres. The present research was undertaken to evaluate the use of activity wheel running of mice in discriminating the relative severity of effects of exposure to exhaust emissions when the composition or gasoline is changed by using different brands or additives, or when emission control devices are added to the engines. This evaluation differed from prior studies in the use of 1972 engines tuned either lean or rich to alter relative pollutant levels and in the simultaneous exposure of different groups of subjects to multiple levels of gasoline exhaust containing no additives.

Albino mice (Charles River CD-1, COBS) from 10 to 12 wk of age were divided randomly into groups of three male and three female mice. Several days before the start of exposures, they were placed in individual stainless steel activity wheels 5-3/4 in. in diameter, with small attached compartments enabling continuous access to food and water. Groups of six mice in activity wheels were placed on the top shelves of appropriate inhalation exposure chambers so that one of the groups inhaled each level of nonirradiated and irradiated exhaust, clean air, and clean air with 100 ppm carbon monoxide (CO) during every exposure. Exposure periods lasted from 4 to 7 consecutive days. During exposures A and C, the engine was tuned to factory specifications. During exposure B, the engine was retuned for maximum manifold vacuum at idle, and total exhaust concentration in each exposure chamber was decreased so the amount of exhaust CO nominally matched the amount in each chamber during exposures A and C. A microswitch was activated by a flat portion on the shaft of each activity wheel causing a counter to increment once with every wheel revolution. Accumulated counter values for each wheel were recorded every 2 hours during the exposure period.

The mean daily counts of wheel revolutions for each group in exposures A, B, and C are shown in Figure 1. Separate bars represent counts for male and female mice as a function of exhaust level and atmosphere. The results of analysis of variance indicated a significant effect due to treatment in exposures A ($F = 4.43$, $df = .9/40$, $p < .001$) and C ($F = 28.0$, $df = 7/32$, $p < .001$), not in exposure B ($F = 0.95$, $df = 7/32$, $p = 0.48$). There were significant differences between the sexes in all three exposures ($F = 17.7$, $df = 1/40$, $p < .001$, in A; $F = 16.1$, $df = 1/32$, $p < .001$, in B; and $F = 35.1$, $df = 1/32$, $p < .001$ in C), with the males generally making fewer daily revolutions than the females. There were significant treatment by sex interactions in exposures A ($F = 2.3$, $df = 9/40$, $p < .05$) and C ($F = 7.8$, $df = 7/32$, $p < .001$). A greater decrease in wheel running was seen with increasing levels of exhaust and a slightly greater decrease was seen with exposure to irradiated than non-irradiated exhaust of the same concentration in exposures A and C. The decrease in activity levels appeared within the first day of exhaust exposure and remained fairly consistent throughout the duration of the exposure period. Within the first day after termination of exhaust inhalation the behavioral activity returned to or exceeded the pre-exposure levels.

Wheel running of mice served to measure behavioral consequences of exposure to automotive exhaust, confirming several previous studies. The results of the current study were particularly interesting considering that no attempt was made to preselect mice for high pre-exposure activity or low variability of the measured behavior. The observed decrease in behavior was not a consequence of the CO in the atmosphere because, in exposures A and C, mice exposed to 100 ppm CO had behavioral changes that were different from those of mice exposed to exhaust containing the same level of CO. In exposure B, no significant activity decreases were observed; this was when levels of CO were comparable with those in other exposures but levels of other measured exhaust constituents were lower in all chambers than in the lowest levels in other exposures.

Automotive exhaust can be said to have suppressed the wheel running activity of mice because the counts fell and rose with the onset and offset of exposure. The amount of suppression was a direct relation to the concentration of exhaust. The fact that a behavioral measure that did not require training and that responded rapidly to the onset of exposure was a toxicological indicator was important because it provided an approach to rapidly acquire data about the effects of some environmental pollutants on certain aspects of behavior.

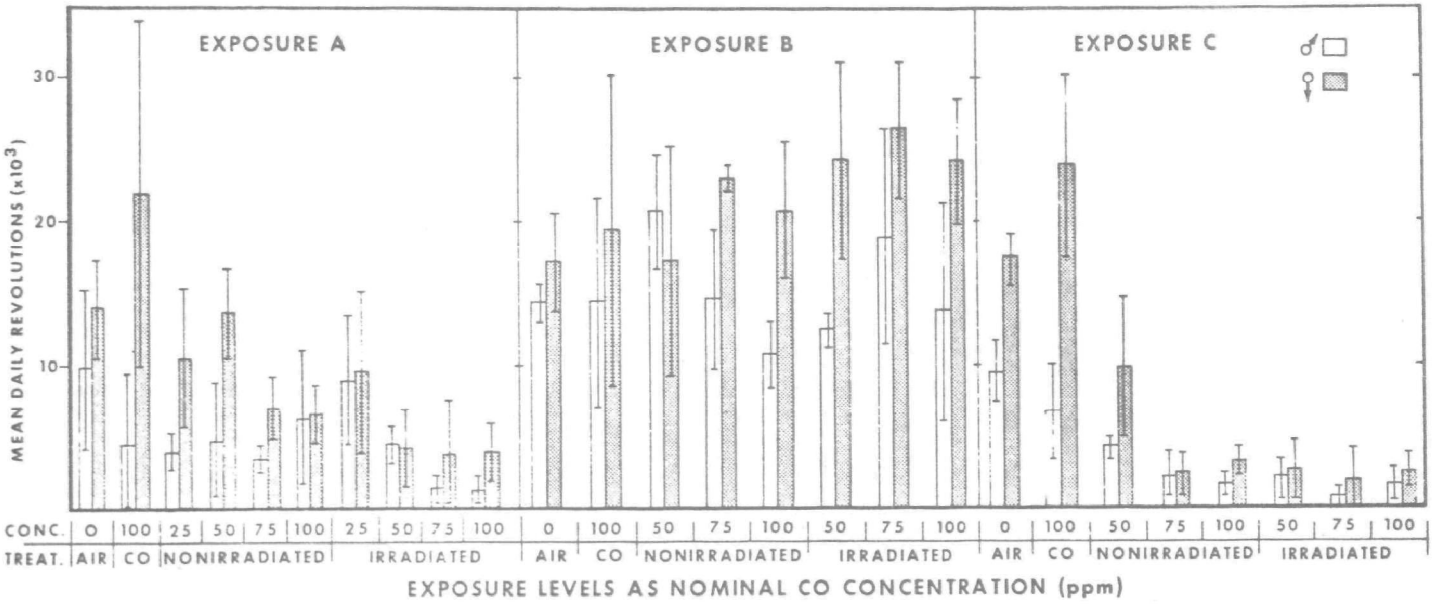

Figure 1. Daily wheel running of mice as a function of inhalation atmosphere in three exhaust exposures. Daily revolution counts for each mouse were averaged over the duration of exposure. Bars indicate the means and standard deviations of these individual averages for groups of three mice. Components of exhaust other than CO during exposure B were lower than in the lowest levels of exhaust during exposures A and C.

ACTIVITY WHEEL RUNNING OF MICE RELATED TO AUTOMOBILE FUEL EXHAUST. II. EFFECTS OF A MANGANESE ADDITIVE

M. I. Gage

The mean number of daily revolutions made by mice in an activity wheel decreased when these mice were exposed to emissions from automotive fuel exhaust. This decrease was directly related to the concentration of exhaust in the exposure atmosphere. The purpose of the present experiment was to see if this behavioral measure, which was sensitive to quantitative differences in exhaust concentrations, was able to show changes in performance as an effect of inserting a methyl cyclopentadienyl manganese tricarbonyl (MMT) additive.

Subjects and methods were similar to those used in earlier exposures. Groups of three male and three female albino mice were placed in activity wheels with continuous access to food and water five days before the start of exposures. The wheels were connected to counters that recorded revolutions of each wheel independently. Exposures E and F lasted 2 weeks. One week before the start of exposure F a 1-day exposure to exhaust of fuel containing MMT occurred. Activity of the mice was measured before, during and for 5 days after the 2-week exposure. During these periods, the room with inhalation chambers was kept in a constant state of illumination. In each exposure, one group was exposed to every level of irradiated and nonirradiated exhaust, clean air, and clean air with carbon monoxide (CO).

Exposure to automotive exhaust emissions decreased activity wheel running of the mice. Results of analysis of variance of revolution counts of male mice indicated a significant effect due to exhaust concentration ($F = 7.42$, $df = 2/24$, $p < .005$) but no effect of irradiation or additive. Results of a similar analysis of the data from female mice indicated a significant effect due to both concentration ($F = 9.93$, $df = 2/24$, $p < .001$) and additive irradiation. Separate analyses of variance showed there were no differences among groups of male or female mice exposed to clean air or just CO polluted air in both exposures. Figure 1 shows a decrease in revolutions as a function of atmospheric type and level, with each animal serving as its own control, for both exposures and sexes.

For each animal, the mean daily revolutions averaged over the entire exposure period was divided by its own mean daily revolutions obtained 3 days before and after exposure (when it inhaled clean air) and is expressed as percentage. The mean and standard deviation of the percentage change of all animals in a group were plotted as

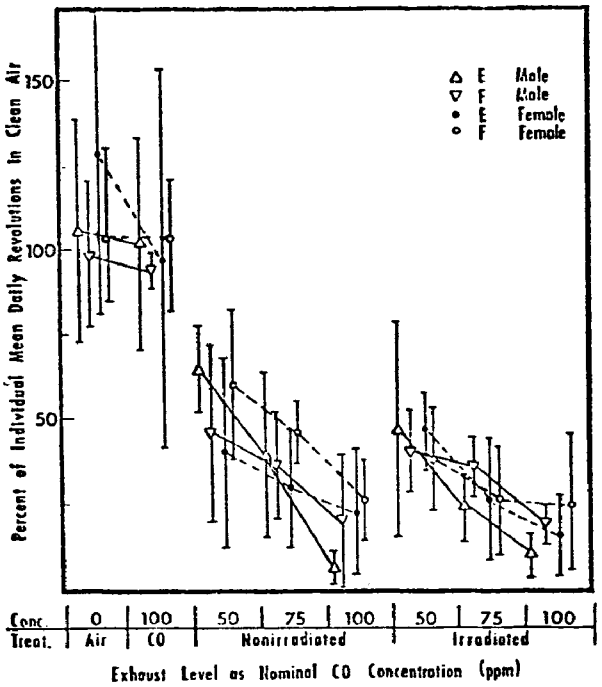

Figure 1. Percent change of wheel running as a function of inhalation atmospheres for one exposure without (E) and one exposure with (F) a manganese additive in the fuel. Points represent means and standard deviations for groups of 3 mice of the percent change from control values of each mouse

the ordinate values. Considering each animal as its own control, no differences appeared during the exposure period in mice inhaling clean air or CO. However, decreases in revolution counts appeared in all groups exposed to exhaust. The amount of decrease was a direct function of exhaust concentration. Little differences appeared between the changes in male and female performance and between changes during the different exposures. There seemed to be slightly less of a decrease in activity of females exposed to non-irradiated exhaust from fuel containing the MMT. Figure 2 shows the mean daily revolutions for four of the eight groups of each sex over the course of each exposure. As in earlier exposures, activity of mice exposed to exhaust decreased within the first day of exposure, remained low during the exposure period, and returned to the level of control groups soon after the termination of exposure. Data from the aborted 1-day exhaust exposure at the start of exposure F indicated that the behavioral results can be adequately predicted from only a very short period of exhaust inhalation.

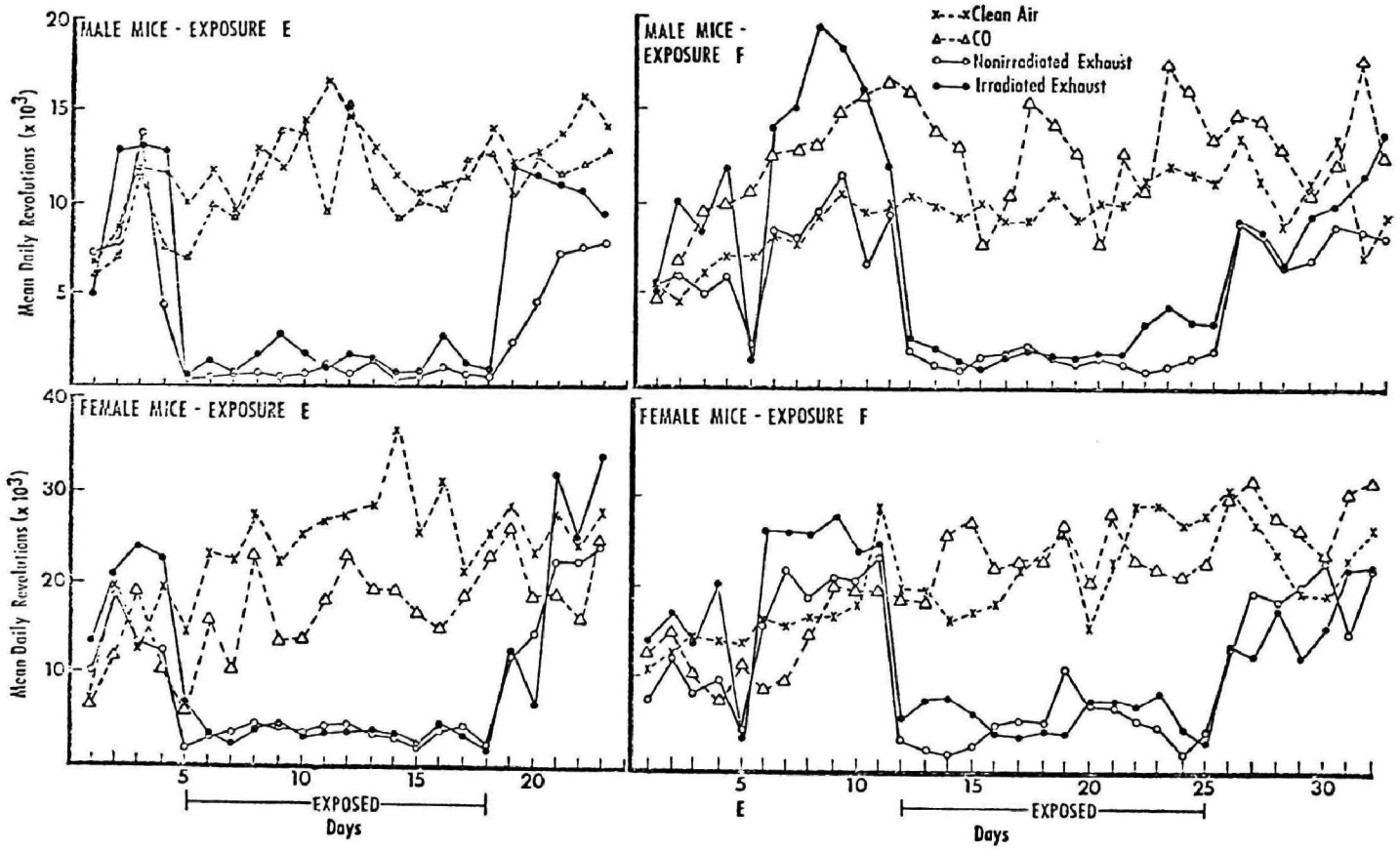

Figure 2. Mean daily wheel running of different groups of 3 mice over the course of two exposures. Data are shown only for the mice exposed to the highest levels of irradiated and nonirradiated exhaust. Fuel in exposure E had no additives, fuel in F had a manganese additive.

Wheel running activity of mice was suppressed by automotive fuel exhaust as it had been in earlier exposures. The amount of behavioral suppression was only minimally altered by introducing MMT into the fuel. This change in behavioral suppression reached significance only in the female mice. It was not surprising that the Mn additive exerted so little an effect in comparison to the effect of exhaust exposure itself. The Mn did little to alter the composition and quantity of measured gaseous constituents of the exhaust even though it increased the particulates measured. Results of this experiment suggest, therefore, that measured behavioral suppression was in some way related to the level of gaseous components of exhaust but not the level of CO or particulates.

GASOLINE EXHAUST EFFECTS ON WATER LICKING OF RATS

M.I. Gage and D. Schneider

Intake of adequate amounts of food and water is essential to the general health and well being of living organisms. If an environmental pollutant alters food and water intake, it will produce changes in the physiological and biochemical measurements of an organism leading ultimately to death. A study was undertaken to see if changes in a stereotyped motor act related to water ingestion, that of licking at a spout, was altered in response to exposure to automotive fuel exhaust emissions when food intake was in no way restricted.

Two groups of four or six male albino rats (Charles River, CO, COBS) were tested in each of the exposures C through F. From the time of arrival (1 to 3 weeks before the start of exposures), until 5 days after the exposure ended, the rats had daily access to water only during a 15-minute period when they were placed in a small operant conditioning chamber which contained clean, filtered air. Food was available at all other times in their home or exposure cages. The rats could drink water by licking at a spout, the tip of which was flush with one end panel, 1.5 in. above the floor and 2.0 in. from the right side wall of the testing chamber. The number of licks, and times of onsets to offsets and of offsets to onsets of licks (interevent times), were measured by an electronic contact switch passing a small alternating current through the animal as he licked. DUring the exposures, one group was kept in an exposure chamber containing clean air and the second was kept in an exposure chamber containing irradiated exhaust with nominally 100 ppm CO. They were removed from these chambers only for the daily testing.

Without special training, the rats began licking at the water spout and within a few days emitted from 2000 to 4000 licks during a session. Typical control performance of a rat is presented in Figure 1. The left graph is a cumulative record in which licks are represented cumulatively along the ordinate and session time along the abcissa. The graph restarts from the baseline after every 400 licks. The licking rate is therefore read directly as the slope of the line. Rats licked at the rate of about 7/sec., which is similar to rates reported by others. As a session progressed, periods of long pauses ensued. Most rats ceased licking some time before the 15-minute session ended. The right graph in Figure 1 is an interevent time histogram in which time, in milliseconds, between onset and offset and time between offset and onset of each lick is the abcissa and number of onsets

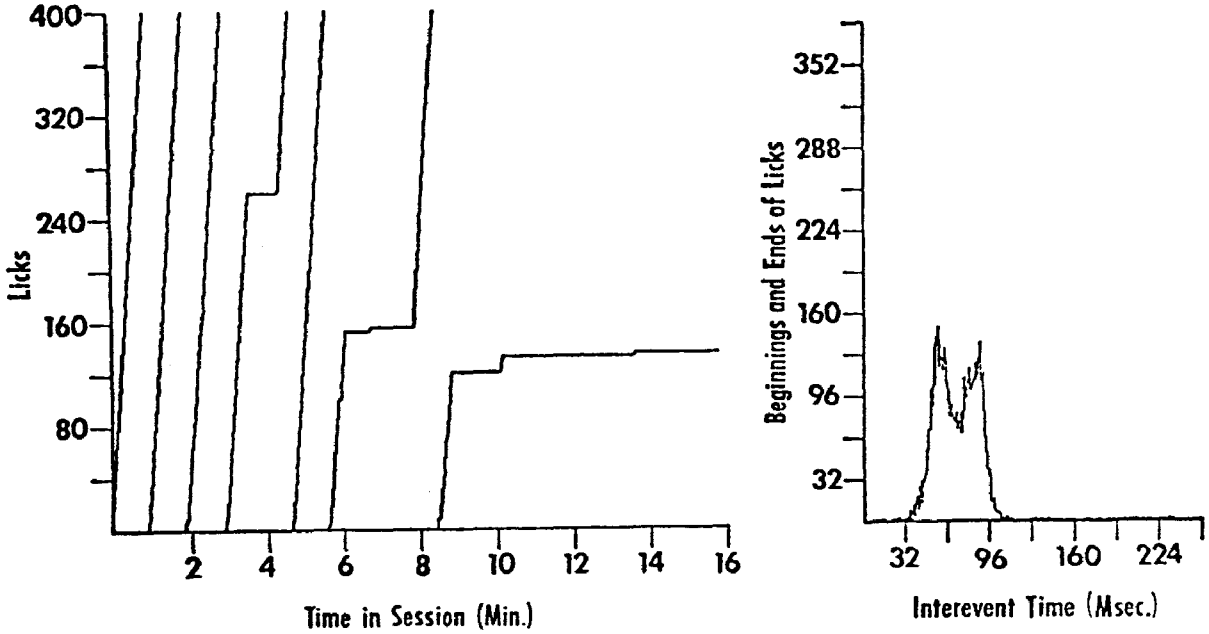

Figure 1. Computerized plots of typical performance of a control rat during one fifteen minute session of licking for water. Left graph is a cumulative record of the licks. Right graph is an interevent time histogram of the same licks.

or offsets occurring at that time is the ordinate. These graphs showed that most rats had a highly stereotyped pattern of licking which, although different from rat to rat, was similar from day to day in the same rat. Only in the data from some rats were two peaks in the interevent time histogram discernable. Oscilloscope tracings of the lick sensor output indicated that the time from the ordinate to the first peak was the modal offset to onset time and whereas the time from the ordinate to the second peak was the modal onset to offset time of the licks.

The daily performance of the rats before, during, and after exposure C (Figure 2) showed that licking was suppressed in the animals inhaling automotive exhaust throughout the course of exposure. The mean number of daily licks quickly returned to or exceeded the level of the control group when the experimental group again inhaled clean air. During the exposure period, the mean number of daily licks for rats in the exposed group was 2406.9, an amount significantly different ($t = 5.26$, $df = 6$, $p < .01$) from the 3304.7 mean number of daily licks for rats in the control group. During this period, rats inhaling exhaust gained less weight than rats breathing clean air (4.75 gm versus 34.5 gm, $t = 4.65$, $df = 6$, $p < .01$). The pattern of licking and the interevent times did not change in a consistent way related to the treatment during exposure.

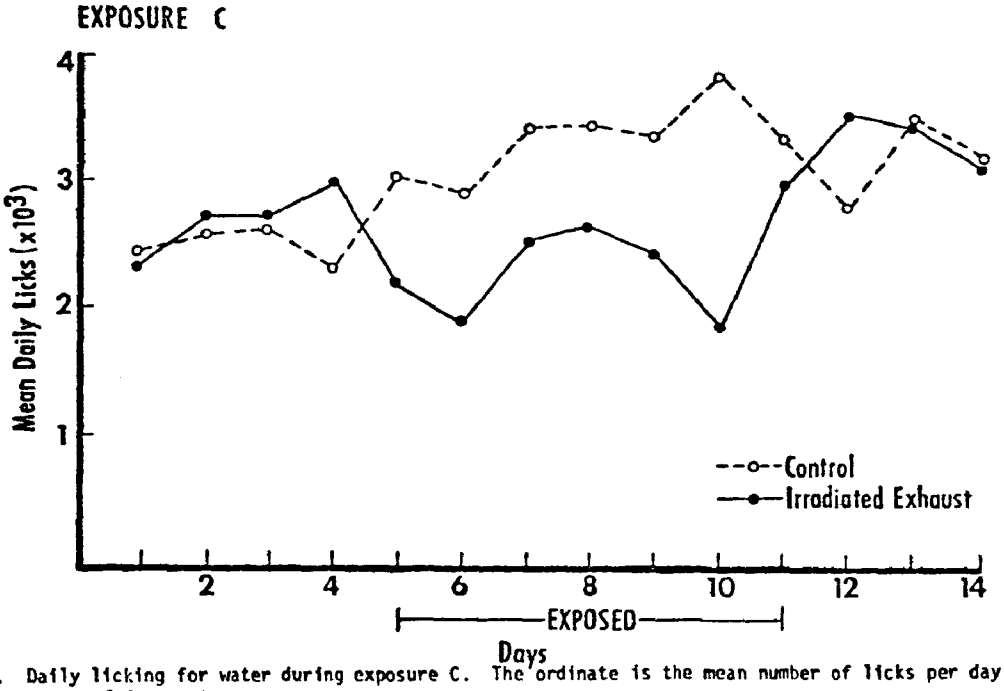

Figure 2. Daily licking for water during exposure C. The ordinate is the mean number of licks per day of each group of four rats.

Similar results occurred for other exposures. An analysis of the mean number of daily licks for the last 2 exposure days during exposures E and F showed a significant effect of exhaust exposure ($F = 5.89$, $df = 1/20$, $p < .05$) but no significant effect of the Mn additive or additive by exhaust interaction. Data were obtained for only 2 days during the exposure because of failure of the lick sensing apparatus.

The suppression of water licking by inhalation of automotive fuel exhaust emissions was complicated by the failure of the exposed rats to show a weight gain equal to that of the control rats. Water intake may have been lower in the exposed rats because they weighed less. However, cursory examination revealed no clear relationship between weight and number of licks. Rats in the exposed group may have gained less weight because they drank less water. Cause versus effect could not be elucidated. Water intake may have been decreased by a failure of the exposed rats to eat as much food as the control rats. Rough estimates of the amount of food eaten each day showed no difference between the groups. In a related experiment, hamsters deprived of both food and water were trained to press a bar for food and lick for their water during daily 15-min. sessions. There were no discernible effects of exhaust exposure on hamster bar presses or licks. It is clear, however, that automotive fuel exhaust emissions decreased the number of daily licks of rats for water. This decrease was not altered by the addition of MMT additive to the fuel, presumably because the additive did not greatly alter the gaseous constituents of the exposure atmospheres.

COMPARATIVE ACUTE TOXICITY OF EMISSIONS FROM AN INTERNAL COMBUSTION ENGINE USING TWO DIFFERENT NO-LEAD GASOLINES

K.I. Campbell and L.H. Hall

Preliminary to a program of studies to assess the comparative toxic hazard of fuel and fuel additive emissions, a pilot study was conducted to determine comparative susceptibility of laboratory animal species, strains, ages and sexes to inhalation of exhaust emissions from a small 4-cycle internal combustion engine. A further objective was to determine the ability of a multi-species acute-lethality test system to discriminate possible differences in the character of emissions as a consequence of using two different marketed gasoline fuels.

In both experiments the same engine was used, operated as identically as possible as to engine speed (average about 1270 rpm) and "tuning" (smooth performance). Also, an attempt was made to maintain a constant and comparable ratio of purified air:exhaust in the test atmosphere delivered to the exposure chamber (average 65:1 in experiment A, 74:1 in B). Two marketed gasolines were used in this experiment; both were unleaded and low-octane ("regular"). Males and females of the following species, strains, and ages were exposed continuously for 5 days: "old" albino rats, inbred and random-bred; "mature" and "infantile" random-bred albino rats; "old", "mature" and "infantile" Syrian hamsters. Corresponding controls were exposed to purified air. Timed mortality during exposure and pre- and post-exposure body weights were recorded. Samples of undiluted exhaust and of exposure chamber atmospheres were periodically analyzed for carbon monoxide (CO), total hydrocarbons (THC), and oxides of nitrogen (NO_2 and NO). Mean concentrations (ppm) of principal pollutants for experiments A and B, respectively, were as follows: CO, 1526 and 1498; TCH, 381 and 387; NO_2, 0.16 and 0.15; and NO, 1.46 and 1.11.

On the basis of acute lethality data, the following comparative susceptibilities were generally apparent: among aged rats, the random bred strain was more susceptible than the inbred strain; aged and mature male and female rats were more susceptible than hamsters of comparable age and sex, whereas the reverse was true for infantiles (hamsters more susceptible than rats); males more susceptible than females, with the exceptions that in aged rats and hamsters the sexes were about equally susceptible; aged rats were the

most susceptible, followed by mature and infantile, but among hamsters the infants were the most susceptible. Substantial loss in body weight occurred in most groups and in both experiments, but the loss did not permit differentiation of biologic or exposure factors. Ranked susceptibilities among exposure subject types were generally consistent in both experiments. Table 1 summarizes these acute toxicity data for both experiments.

Finally, there was an apparently greater degree of toxic response in experiment A than in B. However, despite the appearance of the data on the surface, we cannot conclude at this time that gasoline A is more hazardous than B. Reasons for this include incomplete quantitative characterization of the emissions, and the possibility of the exposure pattern influencing the severity of response; neither of these factors is specifically related to fuel itself.

TABLE 1. ACUTE TOXICITY IN RATS AND HAMSTERS EXPOSED TO EMISSIONS OF SINGLE-CYLINDER, 4-CYCLE ENGINE USING TWO DIFFERENT NO-LEAD GASOLINES

Test Subject	Experiment A			Experiment B		
	Total mortality %	Median lethal time* hr	Relative weight change+ %	Total mortality %	Median lethal time* hr	Relative weight change+ %
Rat, inbred, old, male	100	38	ND	ND	ND	ND
Rat, inbred, old, female	50	108	-22	ND	ND	ND
Rat, randombred, old, male	100	4	ND	80	9	-27
Rat, randombred, old female	80	10	-32	50	50	-24
Rat, randombred, mature, male	100	7	ND	60	60	-20
Rat, randombred, mature, female	90	21	-16	40	228	-14
Rat, randombred, infantile, both male and female	100	53	ND	100	69	ND
Hamster, old, male	70	84	-23	0	NA	-20
Hamster, old, female	80	80	-28	0	NA	-27
Hamster, mature, male	100	85	NA	0	NA	-31
Hamster, mature, female	20	193	-25	0	NA	- 3
Hamster, infantile, both male and female	100	3	ND	100	10	ND

* Calculated estimate

\+ In relation to controls

ND Not determined; NA Not applicable

NOTE: Among controls, no deaths occurred and body weight changed by only -6 to +13% from pre-exposure values.

CHRONIC EFFECTS OF AUTO EXHAUST AND OTHER ATMOSPHERIC POLLUTANTS IN FEMALE BEAGLES

J. Stara, G. Hueter, T. Lewis, K. Campbell, D. Coffin, R. Hinners, M. Malanchuk, K. Busch, W. Bloch, J. Orthoefer, M. Wiester, D. Hysell and W. Moore

This rather large and complicated study is aimed at assessing a population of female beagles to determine the long-term biological effects of inhaling auto exhaust emissions and other major air pollutants. The selection of beagles as an experimental animal has value chiefly because of their large size and long life span. This makes them better suited for various physiological studies, and the resulting degenerative changes more closely mimic man than is the case of other animals, such as rodents.

The study was initiated in September 1965. Female beagles were randomly distributed at 4 mo of age, into 26 exposure chambers, four dogs per chamber. The distribution and exposure pattern was as follows: Clean air controls (20), raw exhaust (12), irradiated exhaust (12), SO_X (12), R + SO_X (12), NO_L + NO_{2H} (12), and NO_H + NO_{2L} (12) for a total of 104 animals. They were exposed daily for 16 hr.

Exposure chamber design (Hinners, R.G., et al., Arch. Environ. Health 13: 609-615, 1966), atmospheric measurements (Crider, W.L., Anal. Chem. 37: 1770-1773, 1965, and Amer. Lab., Nov. 1969), and statistical design (Busch, K.A. and Ludmann, W.F. Presented at the 60th Annual Meeting APCA, June 11-16, 1967, Cleveland, Ohio) of the study have been reported in detail. Figures 1 and 2 summarize these systems. The average concentrations of individual pollutants are reported in Table 1. A 5-yr summary of the exposure levels is presented in Figure 3.

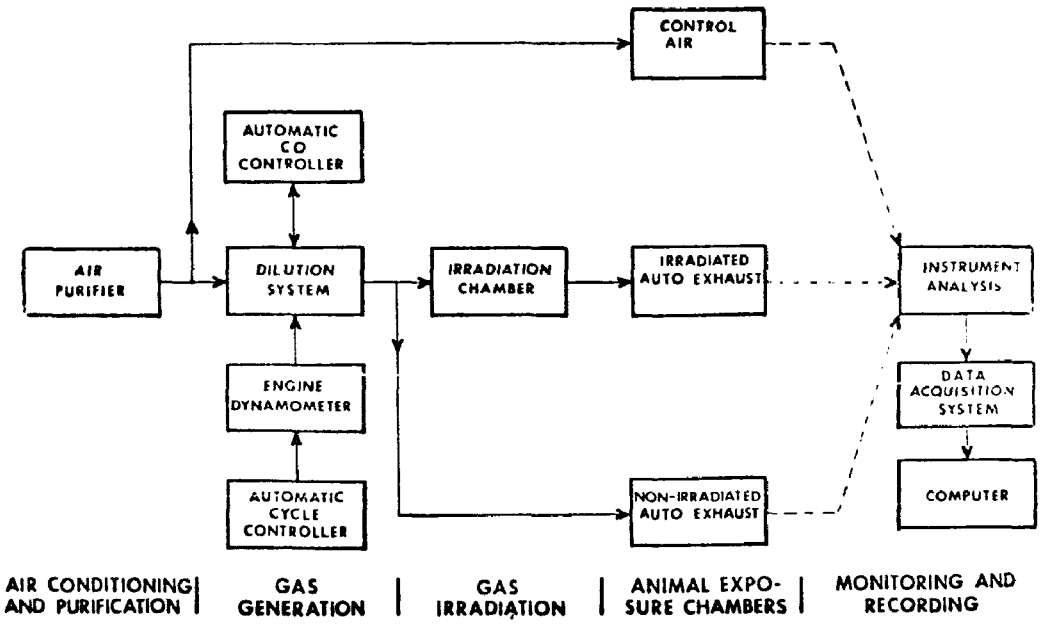

Figure 1. Schematic of auto exhaust study.

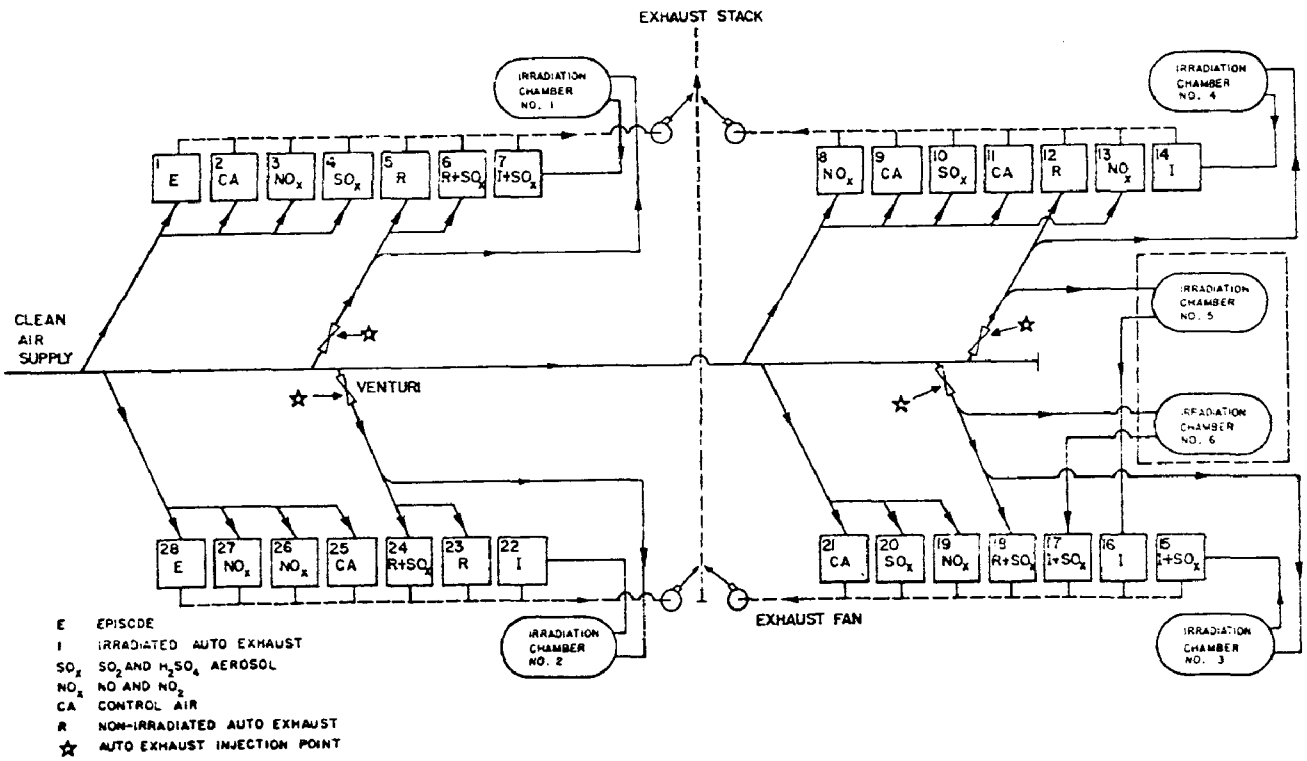

Figure 2. Schematic of exposure chamber supply and exhaust flow

Based on administrative decision, all animals were removed from the exposure atmospheres in August 1970; at this time they had experienced 70 months of uninterrupted exposure. Immediately thereafter they were shipped under a contract arrangement to the Pathology Department, University of California, Davis, for maintenance and eventual terminal studies. At the present time, the study is in its seventh year. It is hoped that following a thorough review of the data and a final set of physiological measurement, the animals will be sacrificed during FY'74 and the final report published.

During the animals exposure regime, until 1969, the major biological parameters studied were hematology and pulmonary function. Commencing with the fifth year of exposure, additional parameters and measurement of standard values were added, particularly in cardiovascular physiology, neurophysiology, blood chemistry, patterns in body weight changes and clinical medicine. During the exposure period, animals that died due to fighting or anesthesia were examined for pathological lesions. Just before their removal from the exposure atmospheres, lobectomies were performed on 15 dogs (5 from irradiated exhaust, 5 from NO_x and 5 from clean air atmospheres) to determine, using electron microscopy, possible lung tissue effects before the animals were removed permanently from the chambers. Even though the final set of physiological measurements is not completed and all such observations must be confirmed by pathological lesions, there are indications of progressive cardiovascular and pulmonary effects.

Table 1. COMPARISON OF SUPPLEMENTAL GAS CONCENTRATION LEVELS (ppm) IN THE CHRONIC DOG STUDY CHAMBERS WITH AMBIENT ATMOSPHERIC CONCENTRATIONS IN THREE CITIES*

Gas	Chicago		Cincinnati		Washington, D.C.		
	Yearly avg.	Max.daily avg.	Yearly avg.	Max.daily avg.	Yearly avg.	Max.daily avg.	Control level
CO	9.1	22.9	5.6	10.6	4.9	14.6	100
NO	0.072	0.189	0.032	0.358	0.047	0.359	1.5-2.0
NO_2	0.050	0.114	0.028	0.069	0.043	0.088	0.5-1.0
SO_2	0.125	0.654	0.024	0.067	0.043	0.132	0.50
HC	3.0	5.18	2.5	5.37	-	-	24-30
Oxidants	0.029	0.080	0.031	0.089	0.025	0.109	0.2-0.4

*NASN air quality data, 1967

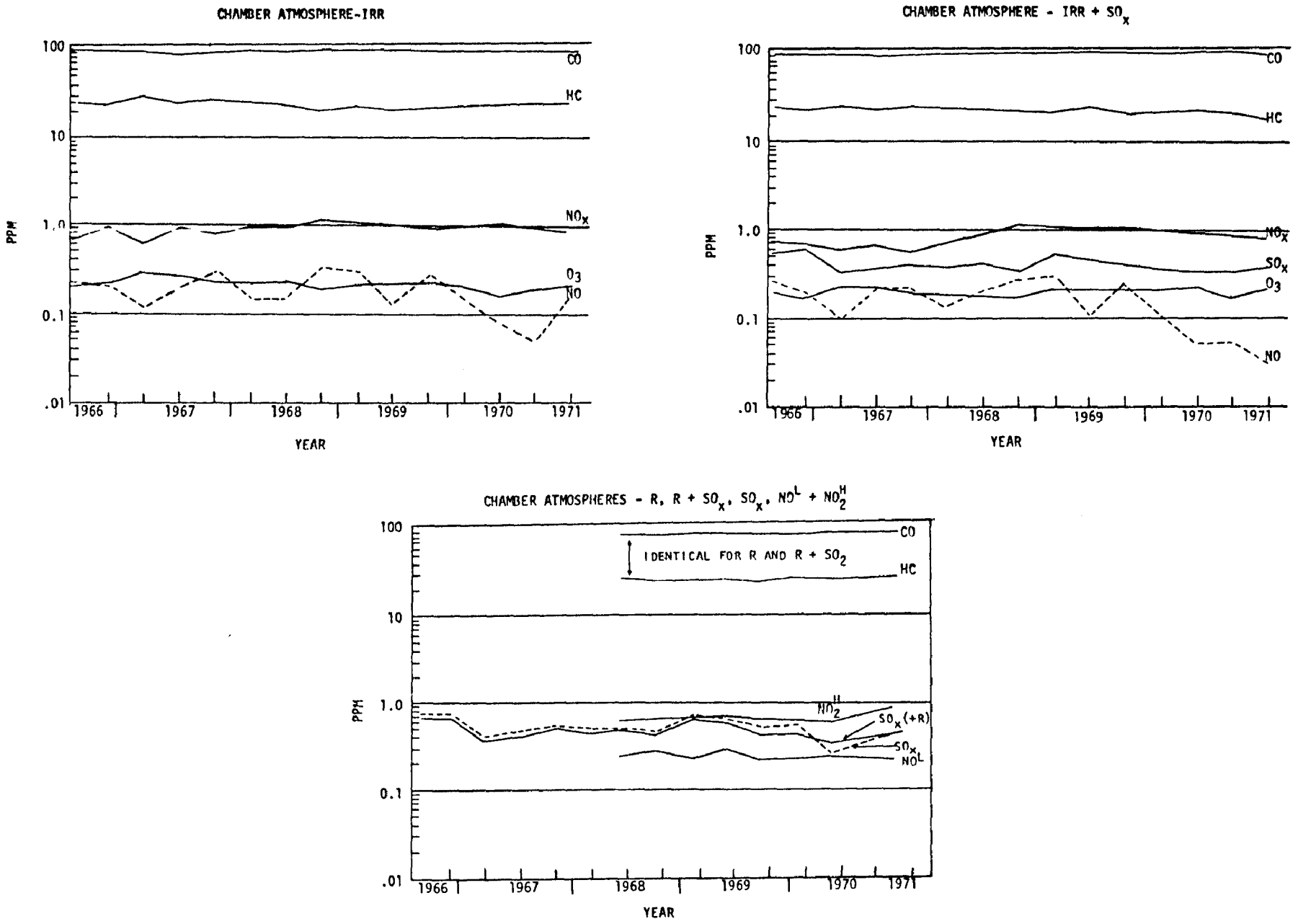

Figure 3. Five-Year Summary of Experimental Atmospheric Levels.

The cardiovascular results (Bloch, W.N., et al. Arch Environ. Health 24: 342-353, 1972) indicated that the pollutant-exposed dogs had a higher incidence of ECG and VCG abnormalities as well as documented or suspected myocardial infarctions than did the dogs exposed to clean air. As a result, these data strongly implicate air pollutants as a factor in causing cardiac electrophysiological damage. An example of the type of cardiovascular data obtained is seen in Table 2.

TABLE 2. EFFECTS OF CHRONIC AUTO EXHAUST EXPOSURE ON CARDIAC RHYTHEM IN DOGS

Type of Atmosphere	No. of Animals	W-QRS-C Index*
Clean air	19	Normal+
Clean air + SO_X	11	Normal
Clean air + NO_H+NO_{2L}	11	Normal
Clean air + NO_L+NO_{2H}	11	Normal
Raw exhaust	11	Normal
Raw exhaust + SO_X	10	Normal
Irradiated exhaust	11	30.8% > Normal
Irradiated exhaust + SO_X	10	42.0% > Normal

*Widened QRS complex.

+Normal index variation was 8.6%

The preliminary pulmonary function results indicate that after 60 months of exposure, the R + SO_X group had a significantly greater RV/TLC (ratio of residual volume to total lung capacity) than did the control group (CA). Furthermore, the R + SO_X, R, and $NO_{(H)}$ + $NO_{(L)}$ groups also had a higher RV/TLC than did the SO_X group.

After 63 months of exposure, the nitrogen washout in the I + SO_X and $NO_{(L)}$ + $NO_{(H)}$ groups was significantly lower than in the CA and R + SO_X groups. The vital capacity of the I + SO_X and $NO_{(H)}$ groups was significantly

higher than that of the control (CA) group. In addition, the vital capacity in the I + SO_x group was also higher than that found in the SO_x group. The data indicate that chronic exposure to air pollutants at realistic levels elicits harmful effects on pulmonary function. Results are not as yet completely conclusive, however, the data are being reanalyzed using various statistical methods to ascertain their biological significance. This review will be completed after the collection of the final set of data.

Neurophysiological studies and pathology studies, of course, are not complete at this time. Hematologic values throughout the study have demonstrated changes; however, these are contributable to the continuous 100 ppm carbon monoxide level. Soon after their removal from the contaminated atmospheres, all blood parameters have returned - on the whole - to normal values.

Clinically, the animals exposed to irradiated exhaust showed a higher incidence of epiphora (Figure 4). As may be seen, the condition underwent remission when the animals were removed from the exposure chambers. The animals exposed to exhaust products also showed a higher incidence of chronic dermatitis and weight changes.

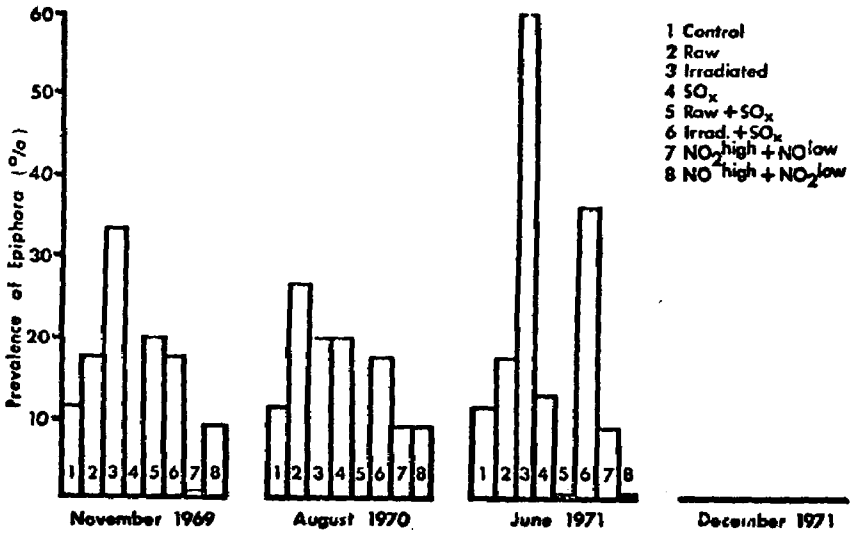

Figure 4. Incidence of epiphora among female beagles in chronic auto exhaust study.

Arrangements are being made to review thoroughly all available data in March 1973, at which time a decision will be made as to the termination of the experiment. It is most important to perform detailed tissue pathology, including light and electron-microscopy, since this is the sole long-term study of auto exhaust and other air pollutants in large mammals at the present time. The data, therefore, will have an impact on future revisions of air pollution standards. In addition, it is expected that the study will be widely quoted; for these reasons, a precise evaluation of the pathological effects is of paramount importance.

GROSS PHYTOTOXICITY OF AUTOMOTIVE FUEL COMBUSTION EMISSIONS TO EPISCIA AND AFRICAN VIOLET

K. I. Campbell

It has been well documented that many plant species of ornamental, agricultural, or otherwise economic importance are adversely affected by exposure to atmospheres containing internal combustion exhaust and its components. Requirements of recent legislation include research concerning relative hazards to public health or welfare of emissions from motor vehicle fuels and additives.

In partial implementation, a project entitled "Toxicologic Assessment of Mobile Emissions ("TAME") has been in progress at NERC-Cincinnati for the purpose of determining comparative toxicologic potential of emissions resulting from and as they may be affected by the use of various fuels and additives. To evaluate their utility as test subjects for comparative fuel-emissions testing, two species of ornamental plants have been included along with the primary battery of laboratory animal bioeffect systems. Such groupings of biological systems for toxicologic studies is increasingly being used. In serial tests, these subjects are experimentally exposed in environmental chambers to emissions produced by appropriate engine systems in which reference fuel or test fuels and additives are used. Of the six experiments conducted to date, information regarding plant effects in the last two, TAME E and F, are reported herein.

The generation and exposure facilities (engines; dynamometers; exhaust dilution, distribution, and irradiation facilities; controls; fuels and fuel storage; and exposure chambers) are described elsewhere, as are the atmospheric analytic methods and data. Study E was a test of emissions resulting from use of reference fuel (essentially no additives) only, and F was a comparative test in which the test additive was added to the reference fuel. The additive compound in this test was an organic manganese antiknock compound proposed for use in gasoline with or in lieu of alkyl lead. It was added at the rate of 0.25 gm Mn/gallon, twice the recommended concentration.

Two plant species, Episcia cupreata (Silver Sheen) and African Violet (Ultra Blue in E, Bloomin' Fool in F), comprised the experimental test unit. Since it was desirec to determine the plants' abilities to discriminate influence of exhaust treatment (irradiated compared with non-irradiated), exposure level (concentration), and pattern of exposure (continuous compared with interrupted) on response magnitude, as well as to estimate their possible utility in discriminating between atmospheres differing due to the test variable (e.g., use of fuel additive), single units were exposed to: both irradiated and raw exhaust types at two levels (low and high, characterized by CO concentrations of about 50 and 100 ppm, respectively), and in three exposure patterns (intermittent, 4 hr/day x 12 days, total pollutant exposure of 48 hr; interrupted, two 24-hr periods with an intervening 24-hr clean-air exposure, total 48 hours; and continuous exposure, total 48 hrs). Units were also exposed to clean air (CA) and to carbon monoxide (CO) atmospheres as controls.

Toxic response was assessed in terms of grossly visible foliar damage (e.g., spotting, discoloration, droop, curl, bronzing, wilt, death, and dehydration), and degree of severity was graded on an arbitrary subjective scale ranging from zero (no apparent effect) up to +12 (maximal effect for the species concerned). In addition, specimens of representative leaves were weighed, dried, reweighed, and frozen. For Episcia, per cent of original post-exposure weight retained during dehydration was used as an additional quantitative index of injury. The exposure conditions and results are summarized in Table 1.

Generally, in both species, but more demonstrable in Episcia (which was the more sensitive), there were differentiable exhaust-exposure effects with respect to atmosphere type (irradiated more effective than raw), exposure level or total dose (magnitude of effect reasonably proportional to concentration), and exposure pattern (increasingly severe effect associated with intermittent, alternate, and continuous exposure at equivalent total dose). CA and CO exposures produced no effects.

TABLE 1. CONDITIONS AND PLANT EFFECTS OF EXPOSURE TO GASOLINE AND GASOLINE ADDITIVE COMBUSTION EMISSIONS*

Exposure type Pattern, level+	Actual Total dose, ppm-days	TAME E Study Effect: Visible damage, Episcia	TAME E Study Effect: % Weight retention, Episcia	Actual total dose, ppm-days	TAME F Study Effect: Visible Episcia	TAME F Study Effect: Damage	TAME F Study Effect: % Weight retention Episcia
Intermittent							
CA	11	0	7.6	12	0	0	4.4
CO	202	0	5.9	210	0	0	3.3
RL	106	+	15.6	128	8(?)≠	0	36.8≠
RH	206	T	15.9	256	3(?)≠	0	10.0≠
IL	92	2	20.9	101	2	0	4.2
IH	214	8		216	4	0	55.3
Interrupted							
CA	11	0	10.6	12	N.D.	N.D.	N.D.
RL	106	4	41.7	128	3	2	5.6
RH	206	N.D.	N.D.	256	10	3	38.4
IL	92	5	29.7	101	8	±	29.4
IH	214	N.D.	N.D.	216	11	3	62.3
Continuous							
CA	11	N.D.	N.D.	12	0	0	2.3
RL	106	N.D.	N.D.	128	12	5	53.4
RH	206	N.D.	N.D.	256	12	7	71.7
IL	92	N.D.	N.D.	101	12	4	77.6
IH	214	12	≈90	216	12	8	81.2

*TAME E, reference fuel only; F, reference fuel plus additive; engine, 1972 Chevrolet V-8, 350 CID; engine dynamometer and California-cycle operation; exhaust dilution ratios: E, 19.0:1, and F, 18:4:1.

+See text for explanation of pattern. CA = clean air, CO = carbon monoxide, RL and RH = nonirradiated low and high, and IL and IH = irradiated low and high.

≠ Atypical. Suspect laboratory error.

Foliar damage resulting from exposure to these automotive emissions appears due primarily to olefinic hydrocarbons and nitrogen oxides constituents (e.g., ethylene at 2-3 ppm, NO_2 at 3-9 ppm), particularly since ozone and other reactive oxidants were absent or negligible. Plant tissue specimens have not yet been assayed for determination of manganese uptake.

In these experiments this particular plant-effects system, although more sensitive in terms of effect magnitude than several animal response criteria used, appeared unable to clearly discriminate the test variable (additive) effect through its influence on total emissions. It seems fair to suppose, however, that the system would discriminate larger alterations in emissions (say 40% or more, particularly if phytopathic constitutents are involved) and that with refinements (e.g., multiple selected species and more sophisticated quantitative effect parameters) a more useful and sensitive model may result for application in future studies.

METHODS DEVELOPMENT STUDIES

THE USE OF CORNEAL MITOTIC RATE AS A MEASURE OF OCULAR IRRITATION

D. Hysell, W. Moore and L. Garner

Ocular irritation is an often reported discomfort in human populations exposed to smog and other atmospheric pollutants. Since this effect is often subjective, the development of an animal model for assessing relative irritability of various pollutants was considered important. Clinical symptoms like epihora, conjunctivitis, and photophobia were considered to be not sensitive enough to permit meaningful comparisons. After a review of the pertinent scientific literature, it was believed that determination of corneal mitotic rate might be an appropriate technique. The methodology required that an animal be killed and the eyeballs be extirpated as soon after death as possible. The enucleated eye was immedately placed in an acetic acid-alcohol fixative, stained in orcein, decolorized, and then prepared for mounting. The latter involved separating the dome-shaped cornea from the rest of the eye, making sure that no pieces of conjuctiva or iris adhered to the cornea. Four radial incisions to facilitate flattening and mounting were made in the cornea. The cornea was mounted in glycerin jelly with the epithelial surface uppermost. The coverslip was ringed with finger nail polish to preclude dehydration of the preparation. When examined under oil immersion microscopy, the mitotic figures were readily recognized by their staining characteristics and morphology. One hundred oil immersion fields were examined, and the total number of cells in mitosis were tabulated.

Corneal epithelium exhibits a marked diurnal variation in numbers of mitotic figures. For the technique to be useful, there should be no extreme day-to-day variations in mitotic rates if the animals were sacrificed at essentially the same time each day. To check this, groups of five male hamsters, each weighing 75-100 g and maintained in an animal room, were sacrificed at 10:00 a.m. on 5 consecutive days. Similar groups of hamsters, maintained in stainless-steel exposure chambers and receiving only clean air were also examined. In contrast to the rather stable mitotic counts from the colony room animals, the chamber-maintained animals showed marked day-to-day variation (Figure 1).

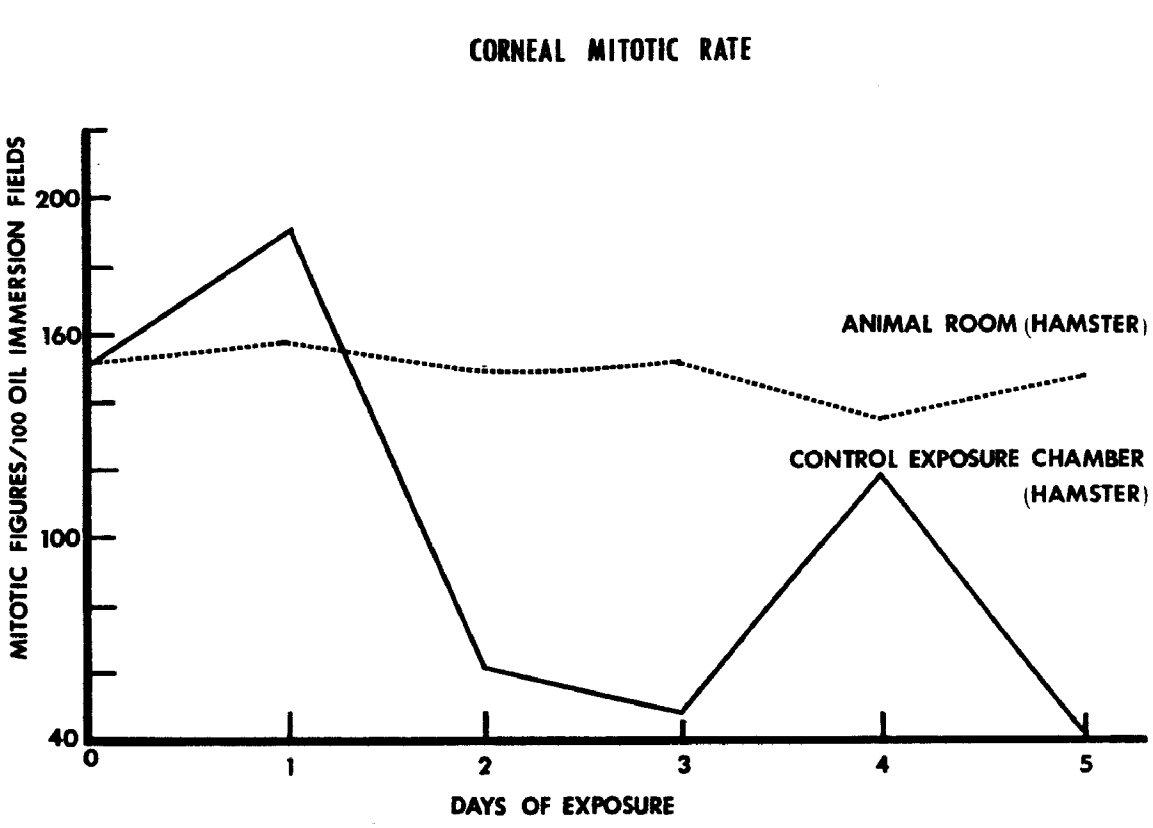

Figure 1. Corneal mitotic rate in hamsters maintained in exposure chambers and animal room.

The chamber conditions (identical to those used in TAME studies) that might result in these variations were 24 hr of daylight (12 hr light, 12 hr dark in animal room) and an air flow of 15 changes/hour (about 7 changes/hour in animal room).

A second study was performed in the exposure chambers in which the animals were maintained on a 12-hr-light, 12-hr-dark cycle and sacrificed at 10:00 a.m., but received exposure to either 5, 10 or 15 chamber air changes/hr. As may be seen by the results (Figure 2), maintenance of between 5-10 air changes/hr is critical in reducing day-to-day variation; however, the light cycle is also important as the 15 air changes/hr did not show the extreme variation seen in the first study where there was 24 hr of light/day plus 15 air changes.

The cyclical rather than sustained response is what might be expected of an irritant. It has been found that every 3-4 days a cell in the corneal germinal layers undergoes mitosis. Therefore, if an irritant produces a peak mitotic response, it would be followed in several days by a low mitotic rate because of the synchronization of mito tic cycles in a large proportion of the cells. From these data, the technique appears to be reproducible if certain parameters are met and yet is sensitive enough to detect a response from an irritant as innocuous as changes in air flow. Because of the results of this study, TAME exposure conditions have been changed so that all animals maintained for pathology receive 12 hr light, 12 hr dark, and no more than 7-8 air changes/hr. This system is being used currently in the bioeffect studies of mobile emissions.

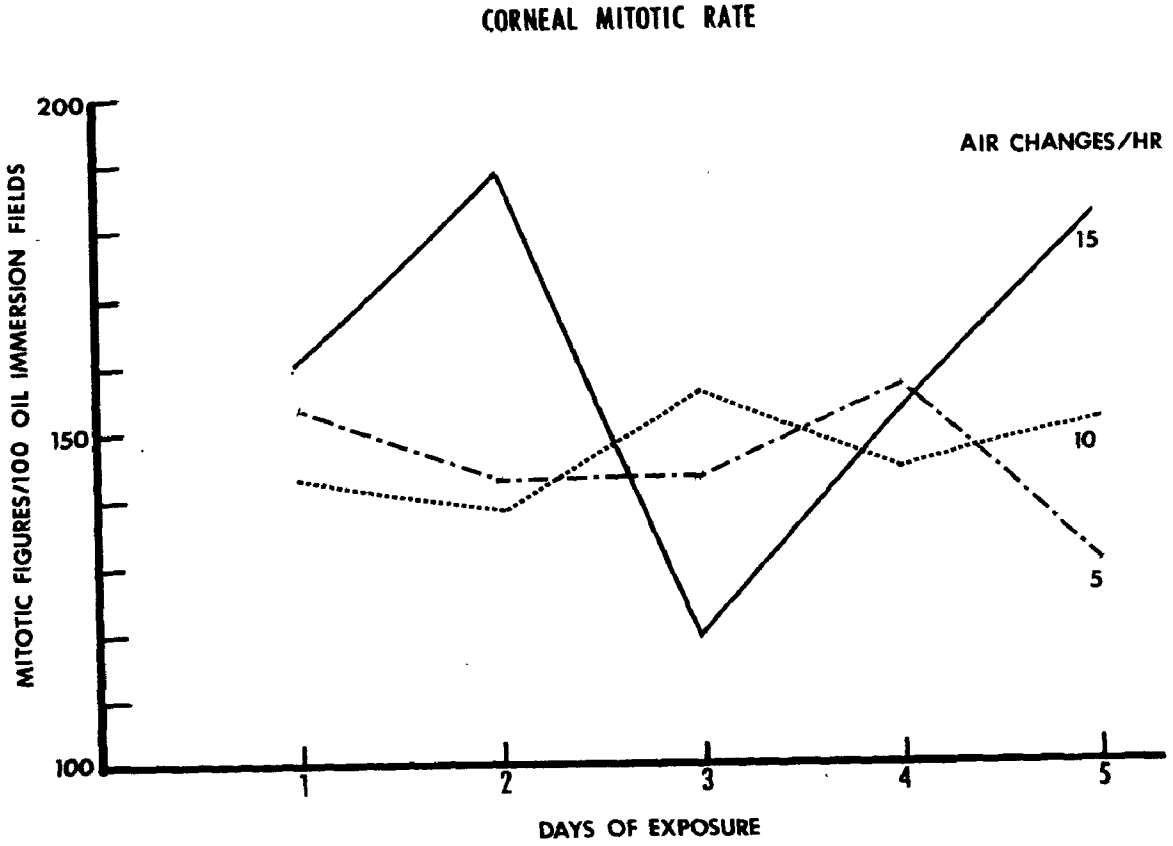

Figure 2. Variations in mitotic counts between hamsters maintained under differing numbers of air flow changes/hr.

BLOOD PRESSURE OF MONKEYS: NON INVASIVE

M.J. Wiester and R. Iltis

Studies in our laboratory require a simple but reliable method to measure blood pressure in monkeys over an extended period of time. Since indwelling catheters are time consuming and difficult to maintain, a tail cuff method was adpoted. In applying the method, we found that systolic pressure, as well as diastolic pressure could be determined and, thus, mean pressure calculated. To evaluate the accuracy of tail cuff pressure readings, a series of experiments was carried out in which monkey tail cuff pressures were compared with simultaneously recorded abdominal aortic pressures, measured directly. The blood pressure range was changed by means of a hypertensive drug and hemorrhage.

Rhesus monkeys (5 kg) were anesthetized with pentabarbital, and a 5 French pressure transducer (Millar Instruments) was inserted via the femoral artery. The output of this direct pressure measurement was amplified and recorded (Sanborn 350). Mean pressure was measured by means of a planimeter and at least three pressure pulses were averaged. The system for indirect pressure measurement includes two sensors: a pulse transducer (Narco) fed into an AC amplifier to monitor tail artery pulses, and a pressure transducer (Statham p23Db) fed into a carrier amplifier (Sanborn) to measure tail artery pressure. The outputs of the two amplifiers are fed to a recorder. If a chart recorder is used, pressure is represented on the Y axis and pulses are superimposed (Figure 1).

If an X-Y recorder is used, pressure is fed to the X axis and pulses to the Y axis (Figure 2). In both recordings, the initial point where a pulse is observed designates systolic pressure and the "less-definite" area where pressure pulses reach a uniform amplitude indicates diastolic pressure. In Figure 1, the pressure pulse is diamond shaped, and in Figure 2, it is spade shaped. Mean pressure is calculated by an emperical formula:

$$\bar{P} = \frac{P\ (\text{systolic}) + 2\ P\ (\text{diastolic})}{3}$$

A block diagram for the indirect blood pressure system is shown in Figure 3.

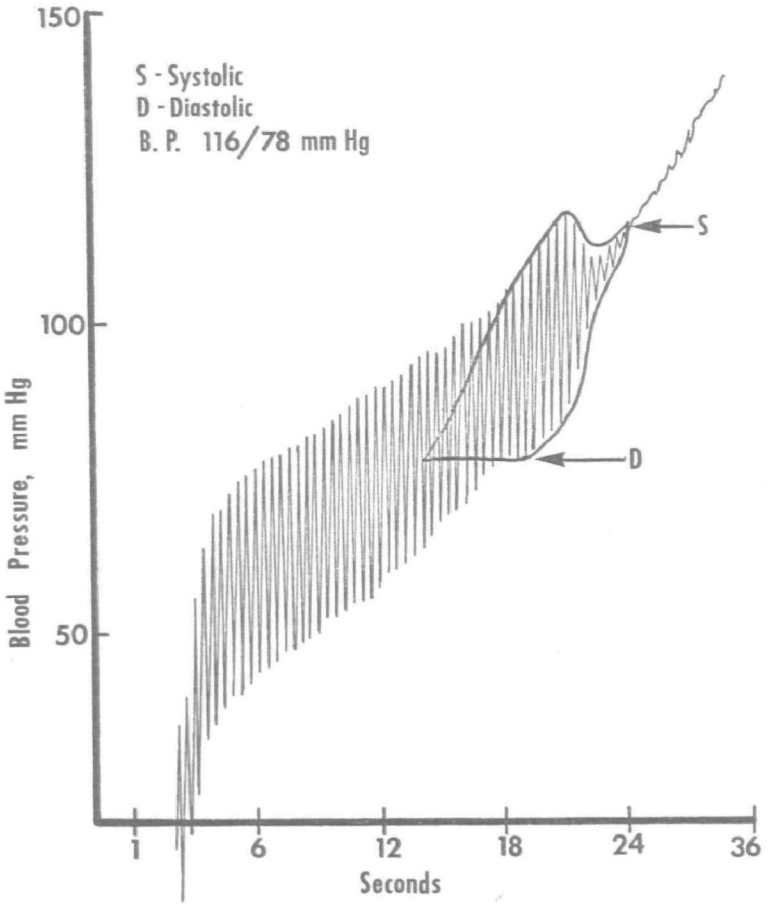

Figure 1. Typical tail-cuff blood pressure (chart) recording from an unanesthetized rhesus monkey.

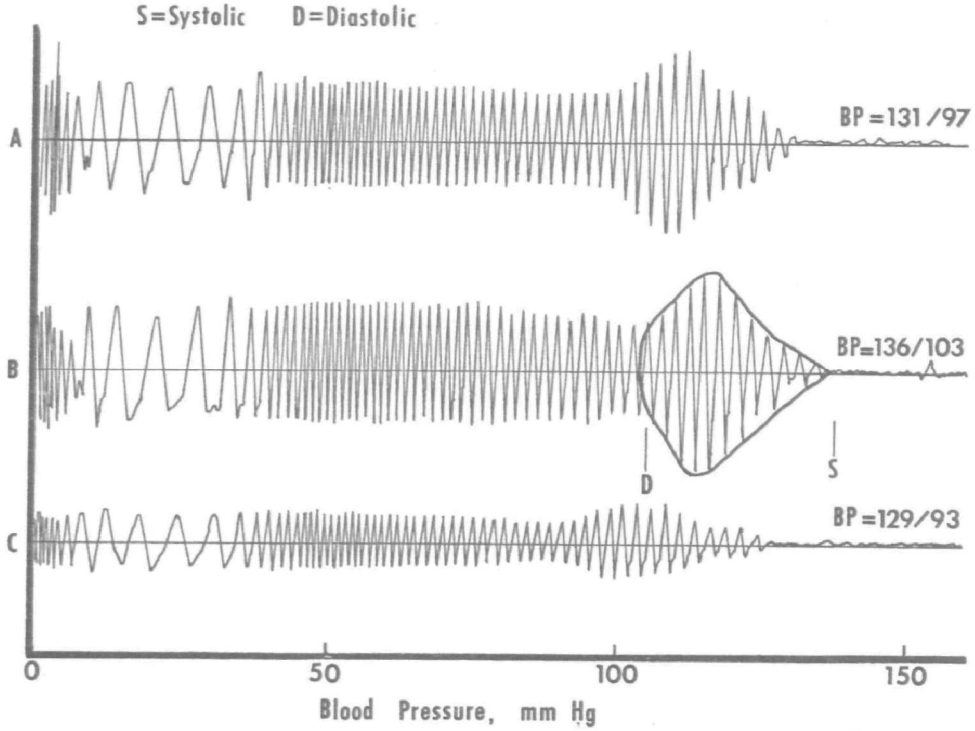

Figure 2. Typical tail-cuff blood pressure (X-Y) recordings from an unanesthetized rhesus monkey.

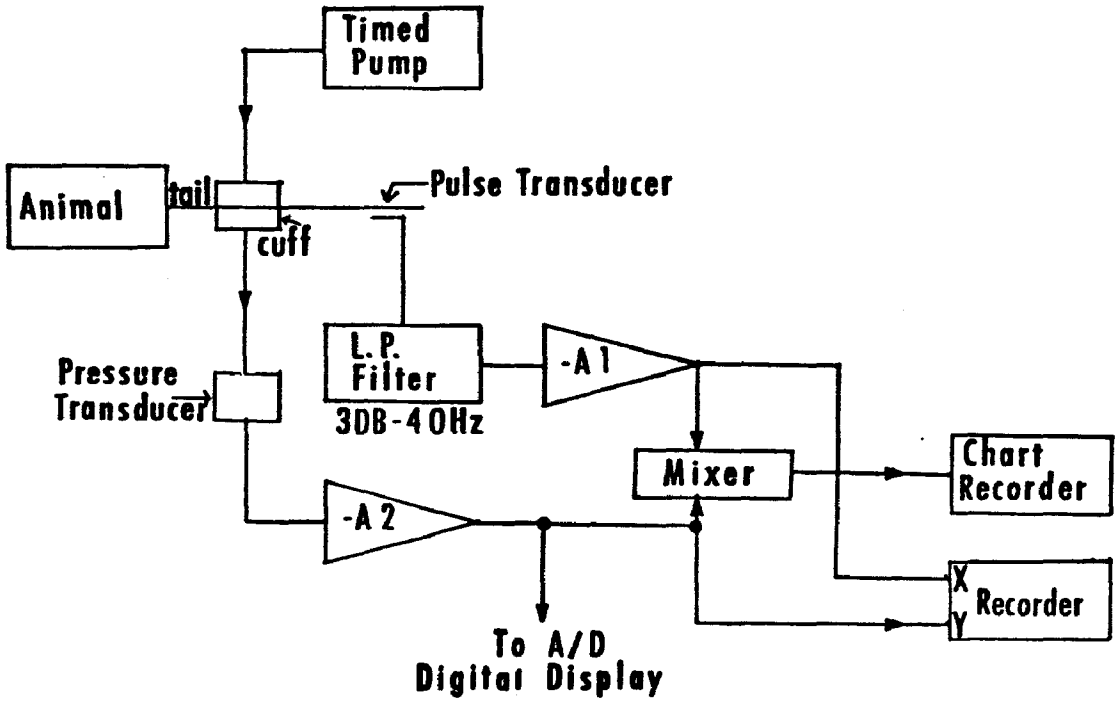

Figure 3. Block diagram for tail cuff blood pressure measurement.

Of the five monkeys used in this experiment, three were given methoxamine hydrochloride I.V. (Vasoxyl from Burroughs Wellcome & Co., N.Y.) in doses necessary to raise blood pressure 30-50 mmHg. All monkeys were hemorrhaged to the point of shock. Results from a typical experiment are found in Figures 4, 5 and 6. They show direct and indirect pressure readings for systolic, diastolic, and calculated mean pressures. As can be seen in the diagrams, the two types of pressure readings follow each other throughout the pressure range.

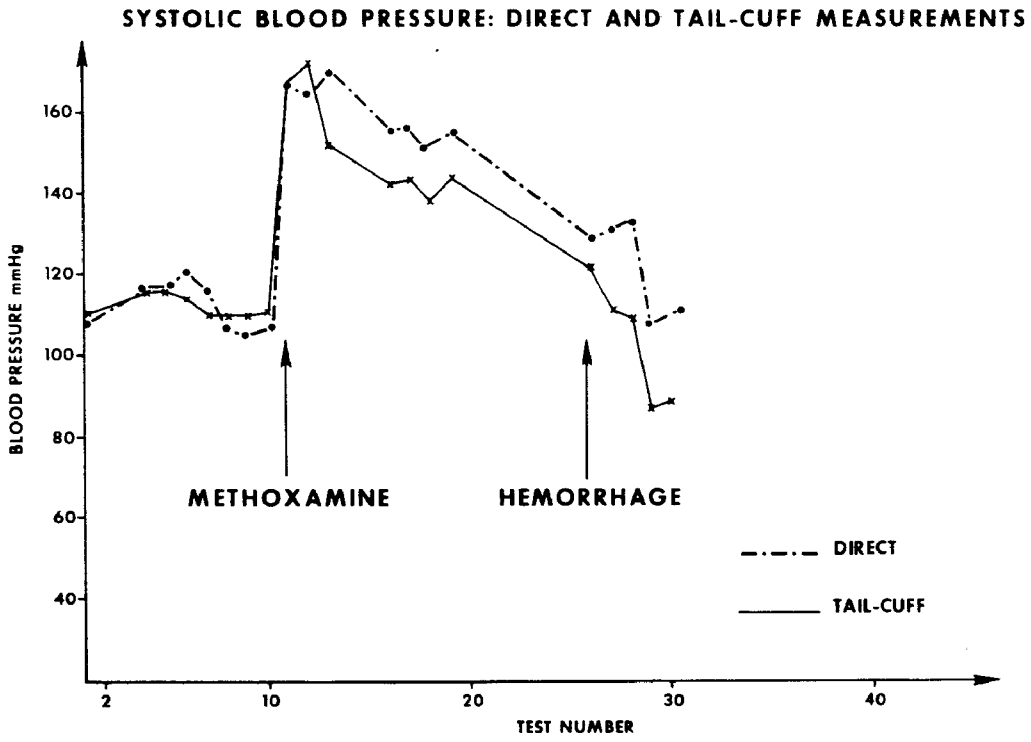

Figure 4. Systolic blood pressure of monkeys: direct and tail-cuff measurements.

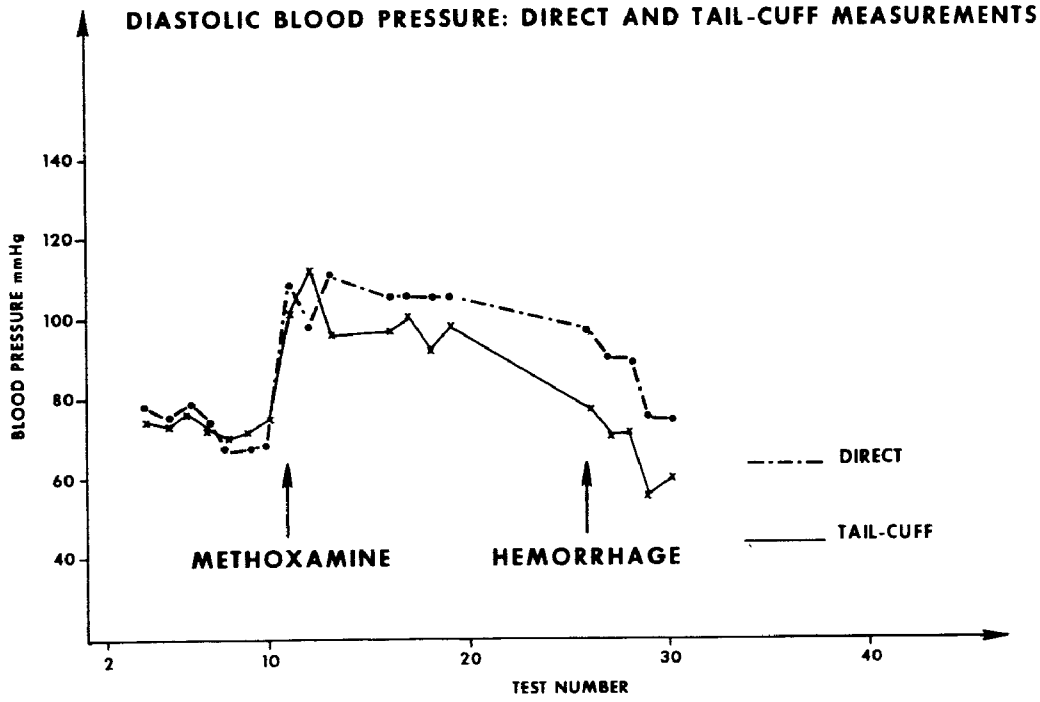

Figure 5. Diastolic blood pressure of monkeys: direct and tail-cuff measurements.

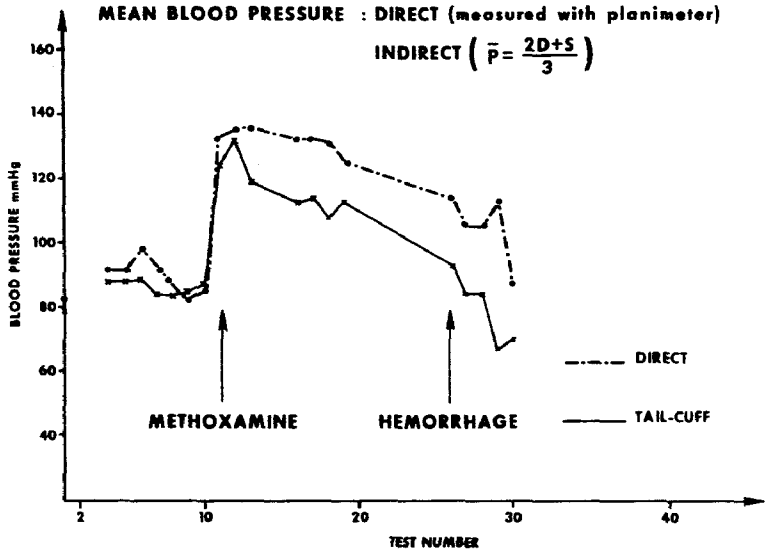

Figure 6. Mean blood pressure of monkey: direct (area under curve) and tail-cuff ($\frac{2D + S}{3}$)

The experimental findings are summarized in Table 1. Under conditions of light pentabarbital anesthesia or massive hemorrhage, tail cuff pressure readings closely reflect actual blood pressure of the animal. Tail cuff pressure usually is the lesser of the two. If methoxamine is administered, the differences and the standard deviation both increase, with the tail cuff pressure being the lesser of the two. Hemorrhage plus the hypertensive drug results in the greatest discrepancy in tail cuff pressure recordings.

TABLE 1. DIFFERENCES OF BLOOD PRESSURE OF MONKEYS OBSERVED IN mmHg (DIRECT PRESSURE - INDIRECT PRESSURE) UNDER SEVERAL PHYSIOLOGICAL CONDITIONS.

TREATMENT	NO. OF MONKEYS	NO. OF MEASURES	BLOOD PRESSURE DIFFERENCES OBSERVED IN mmHg (Direct-Indirect)								
			SYSTOLIC			DIASTOLIC			MEAN		
			Ave.	S.D.	r	Ave.	S.D.	r	Ave.	S.D.	r
Light Pentabarbital Anesthesia	5	44	6.1	6.6	0.94	-2.4	4.5	0.96	4.8	4.0	0.97
Anesthesia + Hemorrhage	2	14	8.3	6.7	0.98	1.5	4.4	0.98	2.8	5.0	0.98
Anesthesia + Methoxamine	3	33	16.9	11.6	0.87	7.2	8.8	0.80	14.6	8.1	0.87
Anesthesia + Methoxamine + Hemorrhage	3	12	17.7	16.0	0.77	12.3	10.3	0.83	17.3	14.0	0.79

S.D. = Standard Deviation of Difference in Readings
r = Correlation Coefficient
A negative sign indicates lower readings for the direct measurement.

The study demonstrates the reliability of monkey tail cuff blood pressure recordings. It is concluded that under conditions where blood vessels are under normal or intense neural influence, tail cuff pressure measurements are comparable with abdominal aortic pressure. The difference is increased, however, when a vasoactive drug is administered.

The tail cuff measurements are quickly and easily done on unanesthetized chaired monkeys as shown in the photograph (Figure 6).

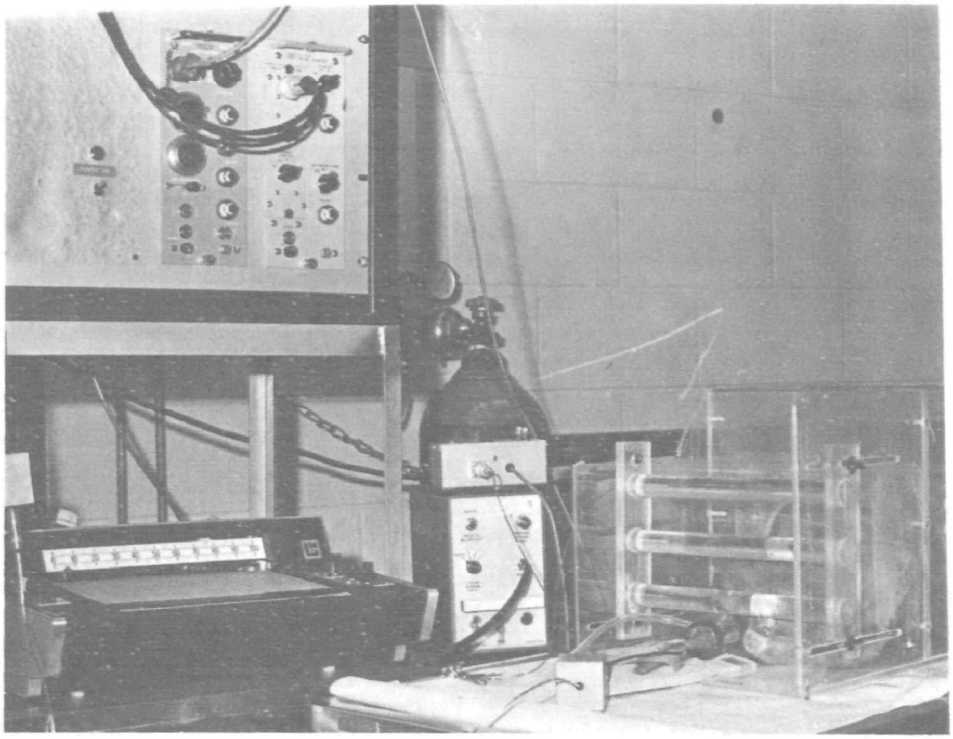

Figure 6. Equipment for tail cuff blood pressure measurements of monkeys. (A method prepared for the cadmium-cardiovascular effect study in primates.)

CORRELATION OF EVOKED POTENTIAL AND SPINAL CORD RESPONSES AS A METHOD FOR EVALUATING BIOLOGICAL EFFECTS OF ENVIRONMENTAL POLLUTANTS

J. P. Lewkowski

Unlike the visual evoked potential, which may be used as an indication of general brain function, the spinal cord is unique in that different types of reflex arcs may be studied in relative isolation from the influence of other reflexes. These reflex arcs consist of several types of cells that elicit facilitory or inhibitory effects by releasing different transmitter agents. These transmitter agents then either depolarize or hyperpolarize specific motoneurons. For a given population of motoneurons, the net effect is to inhibit or to facilitate a given reflex arc. For example, afferent Group I fiber stimulation of one head of a muscle has been shown to facilitate its synergists and inhibit its antagonists. Facilitation is maximal when conditioning and test stimuli are synchronous. Inhibitory effects are increased up to a conditioning-test stimulus interval of 0.5/sec.

As a result, facilitation and inhibition of spinal reflexes may be studied in isolation by stimulating and recording from the appropriate nerves. One may then determine the effects of toxicological agents by measuring the change in amplitude or waveform of the control test reflex. Furthermore, since many of the transmitters in these reflex arcs are now known, then this technique may be used to determine the effects of toxicological agents on the release of a single particular transmitter. Because the reflex arc is isolated, the effects will not be masked by complimentary inhibitory and facilitory influences. Thus, any changes may become evident a lower doses.

Furthermore, the visual evoked potential work and spinal work can be easily correlated. If the visual evoked potential is affected by a pollutant such as lead, then the spinal work may indicate that the release of a particular transmitter agent such as acetycholine is affected by this pollutant. As a result, one may conclude that the cholinergic component of the visual evoked potential may have been affected. The various transmitters responsible for the complex waveform of the evoked potential may thus be more easily elucidated.

These methods present potentially extremely useful tools for *in vivo* screening of central nervous system effects of a wide range of environmental pollutants.

ESTIMATION OF RELATIVE TOXICITY: A PROPOSED TREATMENT OF BIOEFFECTS DATA

Y. Y. Yang

In every dose-response situation, two components must be considered: the stimulus (for example, a vitamin, a drug, a metal test, or a physical force) and the subject (for example, an animal, a plant, a human volunteer, or a metal sheet). If the characteristic response is quantal, occurrence or nonoccurrence will depend upon the intensity of the stimulus. The resultant response is usually expressed in terms of the median lethal dose or the median effective dose.

If two series of quantal response data are compared, their behavior of response will be expressed by the relative potency. The estimation of the relative potency is possible only when the parallelism of two probit regression lines is true. In many types of investigations, however, the parallelism may not hold true and the estimation of relative potency is of no practical use. Instead of estimating relative potency, estimating relative toxicity is proposed when such a difficulty arises, i.e., to examine relative toxicity at different probit units. There are two important applications of this method: (a) to provide background information to investigators for further studies, and (b) to establish the optimal dose of the stimulus applied to the subject.

In Figure 1, for example, the two probit regression lines are parallel; hence, the relative potency can easily be estimated. In Figure 2, however, the probit regression lines are not parallel and the relative potency cannot be estimated. In this case, the investigator has to investigate what caused this phenomenon. If he is convinced that the phenomenon is true bioeffect, then he can obtain the optimal dose from the results.

The relative toxicity and its 95% confidence limits are calculated at different probit points corresponding to their expected response rates. Observe the relative toxicity (R) and its confidence limits in Table 1. In cases where confidence limits include unity, the young rats and infants had the same degree of relative toxicity to the treatment. If the relative toxicity is less (or more) than one, and its confidence limits do not contain unity, then the young rats responded to the treatment with less (or more) toxicity than the infant. Based on these results, the investigator can choose the optimal dose.

From Table 1, one can choose a value of relative toxicity with a satisfactory response rate; with this rate one can establish the optimal dose from Figure 2.

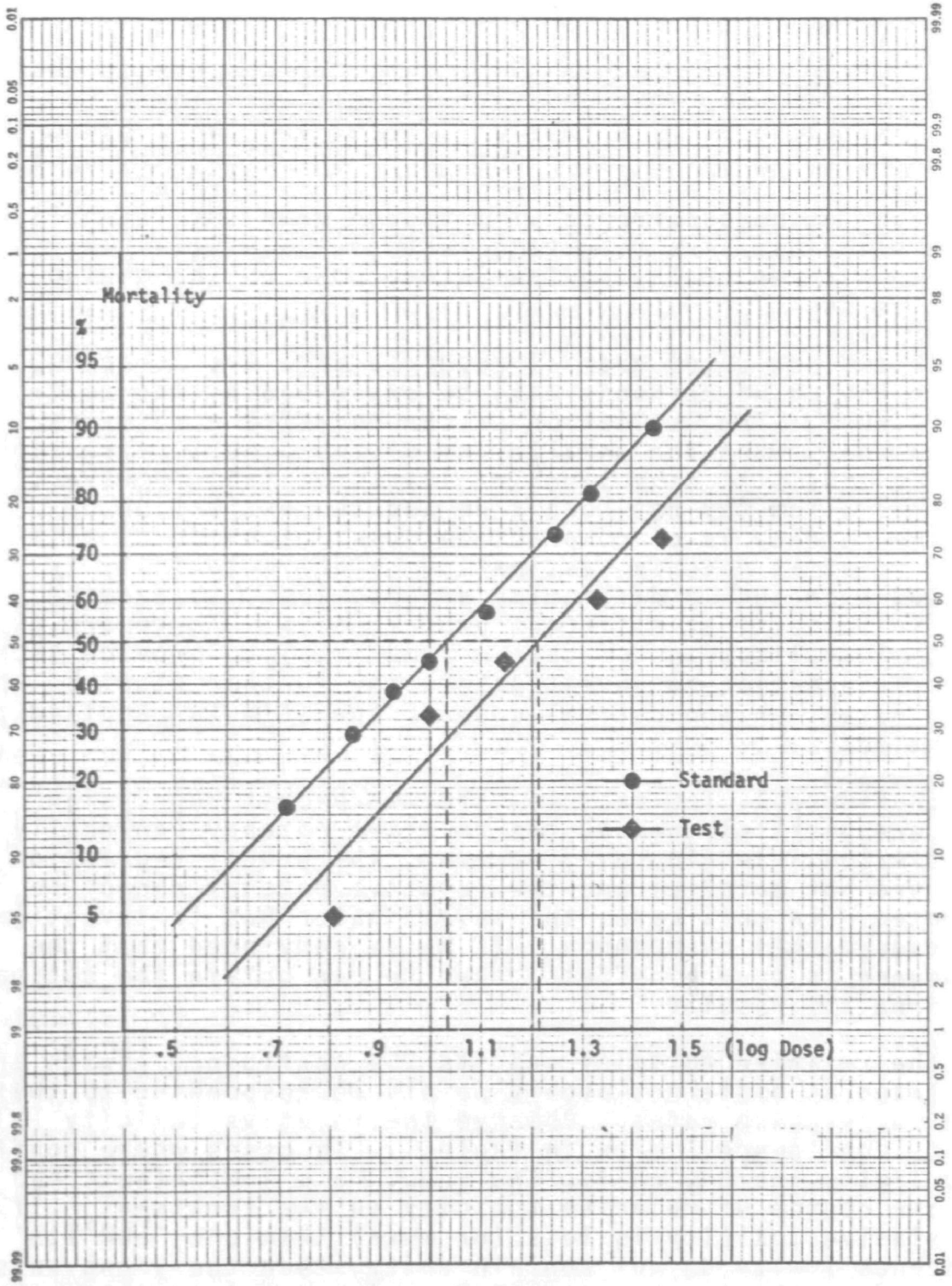

Figure 1. Probit Regression Lines for Insulin Assay (Hemingsen & Krogh, 1926)

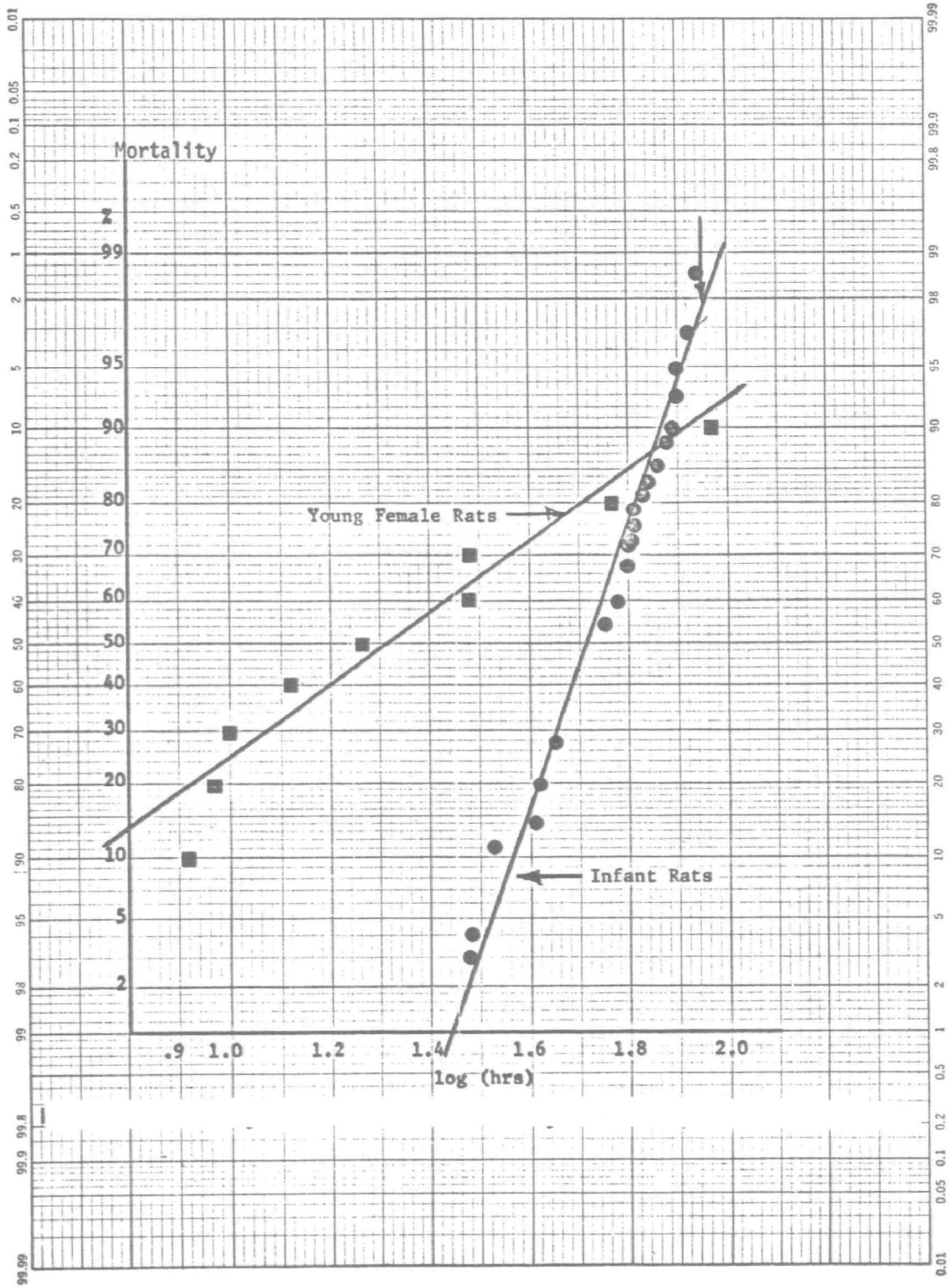

Figure 2. Probit regression lines for mortality of rats exposed to auto exhaust. (Kampbell, 1972, unpublished results).

TABLE 1. RELATIVE TOXICITY (R), COMPARING YOUNG RATS WITH INFANT RATS, AND ITS .95 CONFIDENCE LIMITS (R (L) AND R (U)) AT DIFFERENT EXPECTED MORTALITY PERCENTILES

%	R(L)	R	R(U)
5	8.25	9.41	10.90
10	6.25	7.05	8.03
15	5.18	5.81	6.57
20	4.43	4.95	5.56
25	3.87	4.31	4.83
50	2.24	2.50	2.78
75	1.29	1.47	1.65
80	1.12	1.28	1.44
85	.95	1.09	1.24
90	.77	.90	1.03
95	.57	.67	.78

THE APPROACH TO DATA ANALYSIS IN ETRL

R. Iltis

A. Analysis of Biological Responses

The Environmental Toxicology Research Laboratory is analyzing the biological data obtained through its experiments with mathematical modelling.

In this approach the biological systems are looked upon as functional compartments, each one having an input and output.

B. Objective

The fundamental objective is to relate mathematically the characteristic effect of a pollutnat (input) to the biological effect (output).

In case of experiments that require a long period of measurement, the objective is limited to establishment of a trend.

C. Purpose and Method

The purpose of mathematical modelling is the prediction of effects and the development of a suitable experiment for meaningful and logical answer.

Methods used are linear programming, "ranking" of data and by relating biological processes to analogus electrical systems that are then simulated on an analog computer.

As an example in the usage of the analog computer is simulation of a dose-response curve. The method used is that of R.G. Bickel*. Collected data is plotted on a graph paper. A fourth order system is used to describe the model. By adjusting respective coefficient potentiometer on the computer, the shape of the generated curve is changed until the error between the data and the generated curve is minimized.

*USAF School of Aerospace Medicine, "Simulation" Nov. 69

The settings of the coefficients represent the rate constant of each biological compartment. This technique has been used for simulation and curve fitting of data of the Cd toxicity.

D. Additional Work

1. Additional work done at ETRL is the use of "ranking" method to evaluate TAME data as a function of pollutants due to fuel emission.

2. A study on applicability of mathematical modelling to research program of the ETRL has been completed in cooperation of Dr. Carl Evert of the University of Cincinnati.

3. A computer program and method of analysis has been developed to analyze neurophysiological response pulses through FAST-FOURIER TRANSFORM and pattern recognition method. Data analysis and computer program was completed for studies of renal functions in pigs and monkeys.

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