ENVIRONMENTAL TOXICOLOGY RESEARCH LABORATORY

NERC - CINCINNATI

INTERIM REPORT

1 Studies on toxicology of catalytic trace metal components

2 Toxicology of automotive emissions with and without catalytic converters

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March, 1974

J. F. Stara

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Summary

INHALATION TOXICOLOGY OF AUTOMOBILE EXHAUST EMISSIONS AND THEIR TRACE METAL COMPONENTS ASSOCIATED WITH USE OF CATALYTIC CONVERTERS

J. F. Stara

Introduction

The Division of Health Effects, EPA, instructed the ETRL in July 1973, to reprogram ROAP 21 AFK and develop a new program for toxicologic assessment of emission products from oxidizing catalysts.

Members of ETRL staff had compiled early in FY 73 a monograph entitled, "Toxicology of Atmospheric Sulfur Dioxide Decay Products." In July 73, immediate emphasis was placed on the metabolism, kinetics, and biological effects of noble metals, palladium and platinum, because of the extremely limited data found in the literature on this subject.

The definitive toxicological investigations conducted in this laboratory utilize animal exposures, placing emphasis on those routes of exposure that are of major environmental significance. Other routes, such as intravenous, are used to obtain comparative toxicological data. For this purpose, ETRL developed a toxicologic matrix which was geared to investigate the biological effects resulting from inhalation of the individual catalytic components and the whole exhaust emissions after passage through the catalytic converter. The protocol used for the toxicologic investigations is outlined in Figure 1. Whether this matrix is followed in part or in full depends on the theoretical prediction of toxicity and the amount of knowledge presently available on the pollutant in question.

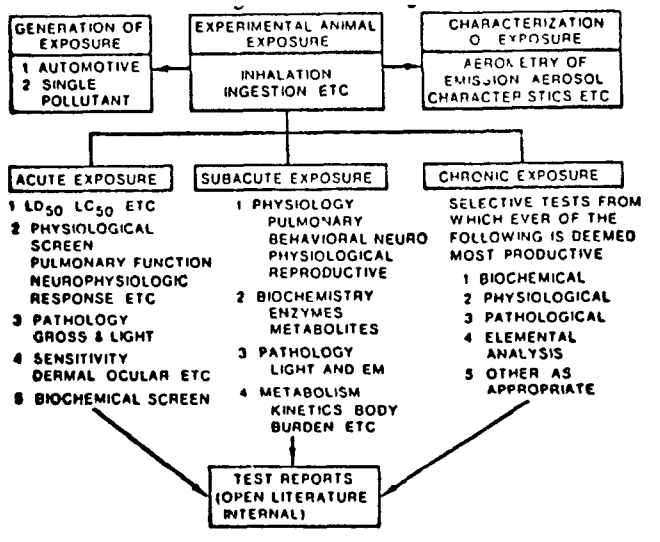

Figure 1

Toxicological Studies of Catalyst Metal Components

These studies were undertaken to ascertain the relative toxicity of catalyst metal components such as platinum and palladium. The acute and subacute studies are required in any comprehensive evaluation of the noble metals since data of this type is largely unavilable in the literature.

Intratrachael administration of platinum or palladium resulted in greater body retention than oral dosage (A.1). Furthermore, the differences among the intravenous, intraperitoneal, and oral toxic dosages of palladium indicated a difference in distribution of palladium depending on the route of administration (A 2). Biochemical studies have shown that the relative toxicity of platinum or palladium on glutamic oxalaoacetate transaminase and lactate dehydrogenase was dependent upon the chemical form of the metal (A.3) In addition, palladium elicited an increase in ^{14}C-leucine incorporated into heart and blood serum Platinum produced a dose-response change in ^{14}C-leucine incorporation in the lung (A.4). Dermal irritancy, dermal absorption, and ocular irritation tests are being utilized as screening techniques to determine the relative toxicity of various types of platinum and palladium compounds (A 5, A.6, A.7). Preliminary neurophysiological screening tests have shown that palladium elicited short-term changes at higher dosages; these changes may represent indirect effects produced by changes in other tissues such as the cardiovascular system (A.8). Preliminary cardiovascular studies have indicated that palladium acted as a non-specific cardiac irritant as well as a peripheral vasoconstrictor (A.9).

In summary, the preliminary results have indicated that platinum and palladium salts are toxic in high concentrations. However, the levels tested in studies reported here will probably not be found in the ambient atmosphere. Further work is underway to determine the atmospheric concentrations of these noble metals that may pose an environmental hazard.

Auto Exhaust Emission Studies

EPA has available at ETRL an auto exhaust generating system for the production of irradiated and nonirradiated gasoline engine exhaust-air mixtures. This system has been utilized in a series of acute and subacute studies which determined the biological effects of exposure of various experimental animal species to whole automotive exhaust emissions with fuel additives and/or with or without a catalytic converter (B.1).

Furthermore, the system was utilized to determine whether new pollutants may be emitted through the use of these converters. These new emissions may occur under three conditions· (1) conversion of organic sulfur compounds present in gasoline into sulfuric acid mist and sulfates, (2) platinum and palladium emission due to catalyst degradation, and (3) alteration of the emissions with the production of different quantities and/or new species (B.2). A report on the specific design and system performance is included (B.3).

Emission studies have indicated that the oxidation-type catalyst in the exhaust system has resulted in the following changes in exhaust

emissions. (1) large reduction of carbon monoxide, total hydrocarbons, and various organic compounds such as acetylene, (2) a nearly total elimination of aldehydes, (3) less photochemical reactions of hydrocarbons, (4) the presence of sulfuric acid as a major component in the particulate (B.4, B 5, B.6), and (5) trace emission of Pt and Pd particulates most of which are in the respirable range.

The bio-effect studies were undertaken to determine the general toxicologic effects of an acute exposure to exhaust from engines equipped with catalytic converters (catalytic exhaust) vs exposure to exhaust from engines without catalytic converters (non-catalytic exhaust) The results indicate that animals exposed to non-catalytic exhaust exhibited profound changes in weight of lactating female and infant rats, and in the survival rate of infant rats. There were no apparent effects on the rats exposed to catalytic exhaust with the possible exception of minute weight loss in lactating female rats (B.7).

The clinical data indicates that the only statistical exposure effect in the catalytic exhaust was an increase in total serum proteins. In the non-catalytic exhaust exposure, there were significant effects on total protein, platelet count, red blood cell and white blood cell count, white cell differential, alkaline phosphatase, hemoglobin, hematocrit, partial thrombo plastin tissue, serum glutamic oxalaoacetate transaminase, and serum glutamic pyruvate transaminase levels Lung pathology studies showed a greater incidence of pathological conditions after non-catalytic exposure than after catalytic exposure (B.7). In general, these data indicate a far greater potential hazard to several organ systems with prolonged exposure to non-catalyst exhaust.

The effect of catalytic and non-catalytic exhaust was tested in several biochemical systems. Lung aryl hydrocarbon hydroxylase (an enzyme which is responsible for the biotransformation of various carcinogens) activity is depressed with exposure to non-catalytic exhaust However, the catalytic converter significantly reduced the lung aryl hydrocarbon hydroxylase depression (B.8). Furthermore, serum lactate dehydrogenase was greatly elevated with non-catalyst exposure but was not significantly effected with exposure to catalyst exhaust (B.9).

Plant damage occurred in both catalytic and non-catalytic exhaust; it appeared to be somewhat less in the catalytic exposure. Since plants were damaged in both types of exposure, it appears that plants are inherently more susceptible to exhaust damage than animals and that the effect threshold was exceeded by emissions (B.11).

In summary, the acute whole emission studies have demonstrated that acute exposures to automotive exhaust without a catalytic converter elicited a profound effects on physiological and biochemical function and produced histo-pathological lesions. In contrast, exposure to exhaust from a system with a catalytic converter did not result in such demonstrable physiological or biochemical dysfunction. One may conclude that the introduction of the catalytic converter has reduced the levels of certain exhaust constituents. This has resulted in a decreased biological effect of exhaust emissions. However, it should be noted that catalyst-modified emissions produced changes in plants In addition, it is now known that catalyst-modified emissions are highly acidic. As a result, longer term studies are needed and are planned for the future (April, May) These studies will test the effect of long-term exposure to the more acidic catalyst exhaust.

PALLADIUM AND PLATINUM STUDIES

A.1. METABOLIC AND KINETIC ASPECTS OF PALLADIUM AND PLATINUM

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

Automotive manufacturers have indicated that palladium will be used in conjunction with platinum in automotive catalytic converters. These converters are designed to reduce the concentrations of carbon monoxide (CO) and hydrocarbons (HC) in the exhaust stream by oxidizing them into carbon dioxide and water The control of the concentrations of CO and HC in automotive emissions is necessary for light duty vehicles to comply with the CO and HC emission standards set forth in the Clean Air Act - 1970. With the use of palladium and platinum in automotive catalytic converters, there is the possibility that some of the material will be emitted to the atmosphere or enter into other segments of the environment following degradation or disposal of worn-out converters.

1. Palladium

Information on the biological effects of palladium is extremely limited. Meek et al. (1943) have summarized the toxicological findings and presented additional data on the toxicology following subcutaneous and intravenous injections in rabbits. Intravenous injections of $PdCl_2$ were extremely toxic, whereas subcutaneous injections were nontoxic due to the formation of an in-absorbable complex. When given in small amounts intravenously, the median lethal dose in rabbits was 18.6 mg/kg, with a survival period of 12 days (Orestano, 1933).

The effects of palladium, 5 ppm in drinking water, on the growth and life span of mice were observed from weaning until natural death (Schroeder et al. 1971). No significant differences were found in the body weights of the females, however, body weights of the exposed male mice were significantly less than those of the controls The life span of the exposed male mice was signifi-

cantly longer than that of the control male mice. The incidence of malignancy and amyloidosis was higher in the exposed animals

Information regarding human exposure to palladium is also extremely sparse. In older medicine, palladium has been given orally for the treatment of tuberculosis without success, and it has been injected into abdominal fat for the purpose of reducing obesity (Meek et al. 1943). Exposure of forearm skin to a 1 mg/ml buffered $PdCl_2$ solution for 24 hr. did not result in skin irritation (Meek et al. 1943). Men working with platinum salts occasionally developed an asthmatic response to platinum exposure These same men had no recurrence when transferred to jobs involving the handling of palladium (Hunter et al. 1945)

Palladium has been shown to be highly active in some enzyme systems, and it is possible that traces of palladium may influence, but not necessarily unfavorably, enzyme systems in plants and animals including man (Christensen, 1971-72).

It is apparent from very limited information that palladium is toxic when it is absorbed and that more information is sorely needed in view of the possibility of environmental exposure due to catalytic converters The purpose of this study was to provide information on the retention, tissue distribution, excretion and placental transfer of Pd following different routes of administration.

Animals and Treatments

The outbred albino rats (Charles River CD-1 strain) used in this study were maintained on a commercial diet (Purina Lab Chow) and tap water ad libitum except where otherwise noted The various treatment groups consisted of

1. Intratracheal Administration

Ten fasted male rats, 180-200 g, were anesthetized with pentobarbital sodium and placed in dorsal recumbency. The trachea was isolated through a

ventral midline cervical incision and blunt dissection of the overlying masculature. $^{103}PdCl_2$ (25 μCi in 0.1 ml saline) was injected intratracheally with a 1 cc tuberculin syringe and 5/8 in., 25 ga needle. Following closure of the incision, the animals were maintained in hanging wire cages for 104 days to determine whole body retention of the $^{103}PdCl_2$.

2. Oral Administration

Twenty fasted male rats, 180-200 g, were lightly anesthetized with ether and given 25 μCi of $^{103}PdCl_2$ in 0.2 ml saline by stomach tube. Ten rats were placed in metabolism cages for collection of 24 hr urine and fecal samples to determine routes of excretion They were maintained 104 days for determination of whole body retention of the $PdCl_2$. The other ten rats were sacrificed 24 hrs. following dosage to establish organ distribution of the $PdCl_2$. Fifteen non-fasted suckling rats, 30 g, were given a single dose of $^{103}PdCl_2$ (25 μCi in 0.2 ml saline) by stomach tube These animals were maintained for comparison with retention of Pd in the adult rats.

3. Intravenous Administration

Twenty male rats, 180-200 g were given 25 μCi $PdCl_2$ in 0.1 ml saline intravenously (iv) in a tail vein with a 1 cc tuberculin syringe and 5/8 in., 25 g needle. Ten rats were sacrificed 24 hrs. later for organ distribution, ten rats were placed in metabolism cages for collection of 24 hr samples of urine and feces and for whole body counting to determine retention. Thirteen female rats (16 days pregnant) were given 25 μCi $^{103}PdCl_2$ iv and maintained in metabolism cages for collection of feces and urine. They were sacrificed 24 hrs after dosage for determination of organ distribution and placental transfer of the $^{103}PdCl_2$. An additional group of 8 female rats were given

25 μCi $^{103}PdCl_2$ iv within 24 hrs post-parturition. The mothers and litters were maintained 25 days to determine if the ^{103}Pd was transferred to the young via the mother's milk.

Sacrifice and Tissue Sampling

All rats were euthanatized with an overdose of chloroform anesthesia. Samples collected routinely were blood, heart, lung, liver, kidney, adrenal, pancreas, abdominal fat, spleen, skeletal muscle, bone, brain and testicle from males, ovary from females. In the pregnant females, 4 placentas, 4 fetuses and a pooled sample of fetal livers were also saved. In the young rats from the milk transfer study, lung, liver, kidney, bone and spleen were saved. Tissue samples were placed in pre-weighed glass vials for counting.

Radioactive Determinations

^{103}Pd in 0.6 m HCL was diluted as indicated and used in all the studies. The isotope has a half-life of 17 days and a 0.362 Mev gamma. Immediately after dosing, whole body counts were made on all animals used in the retention studies. The animals were counted daily for the first few days and then every other day for the duration of the experiment. A 200-channel gamma spectrometer with a 5 in. NaI (Tl) crystal was used for whole body counts. Tissue, urine and feces samples were counted in a well-type refrigerated scintillation spectrometer.

RESULTS

Whole Body Retention

Analysis of the data for whole body retention of ^{103}Pd following a single exposure disclosed that the route of administration of the dose

significantly affected whole body retention. The percent of ^{103}Pd retained with time in the rat following three different routes of administration is presented in Figure 1. Following oral dosing, the retention curve declined very rapidly during the first 3 days to about 0.4% of the initial dose. The initial rapid clearance is attributed to passage of the non-absorbed ^{103}Pd through the gastrointestinal tract. Extrapolation of the second component of the retention curve to the intercept indicated that absorption was less than 0.5% of the initial dose. Retention of ^{103}Pd by the suckling rats following oral administration was similar to the adults, however, the amount absorbed and retained with time was significantly higher.

The amount of ^{103}Pd retained following intratracheal dosing was significantly higher than for oral dosing and also significantly less than for iv dosing. The greatest amount of ^{103}Pd retained with time occurred following iv administration.

Excretion

Radioactive counts of 24 hr urine and feces samples from the rats receiving the ^{103}Pd orally showed that almost all of the ^{103}Pd was eliminated in the feces and only a trace amount was excreted in the urine (Figure 2). With iv administration, ^{103}Pd was eliminated both in the urine and feces in similar quantities. Toward the end of the study, urinary excretion exceeded fecal excretion.

Tissue Distribution

The distribution and concentration of ^{103}Pd was determined for different tissues following oral and iv dosing Twenty-four hours after oral dosing, detectible quantities of ^{103}Pd were found only in the kidney and

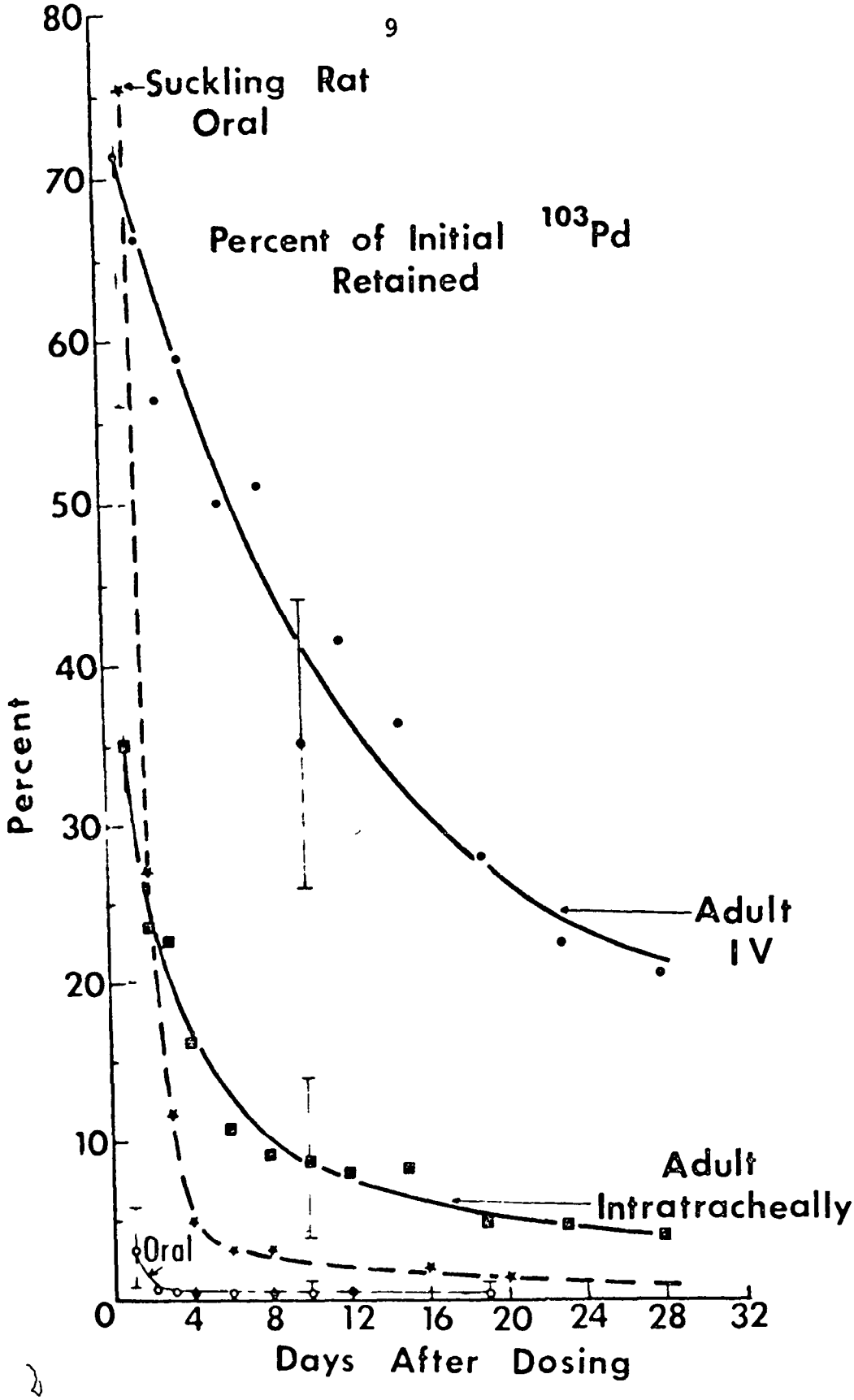

Fig. 1. Whole body retention of ^{103}Pd in adult rats following oral, iv, and intratracheal administration Also shown is whole body retention of ^{103}Pd in suckling rats following oral administration.

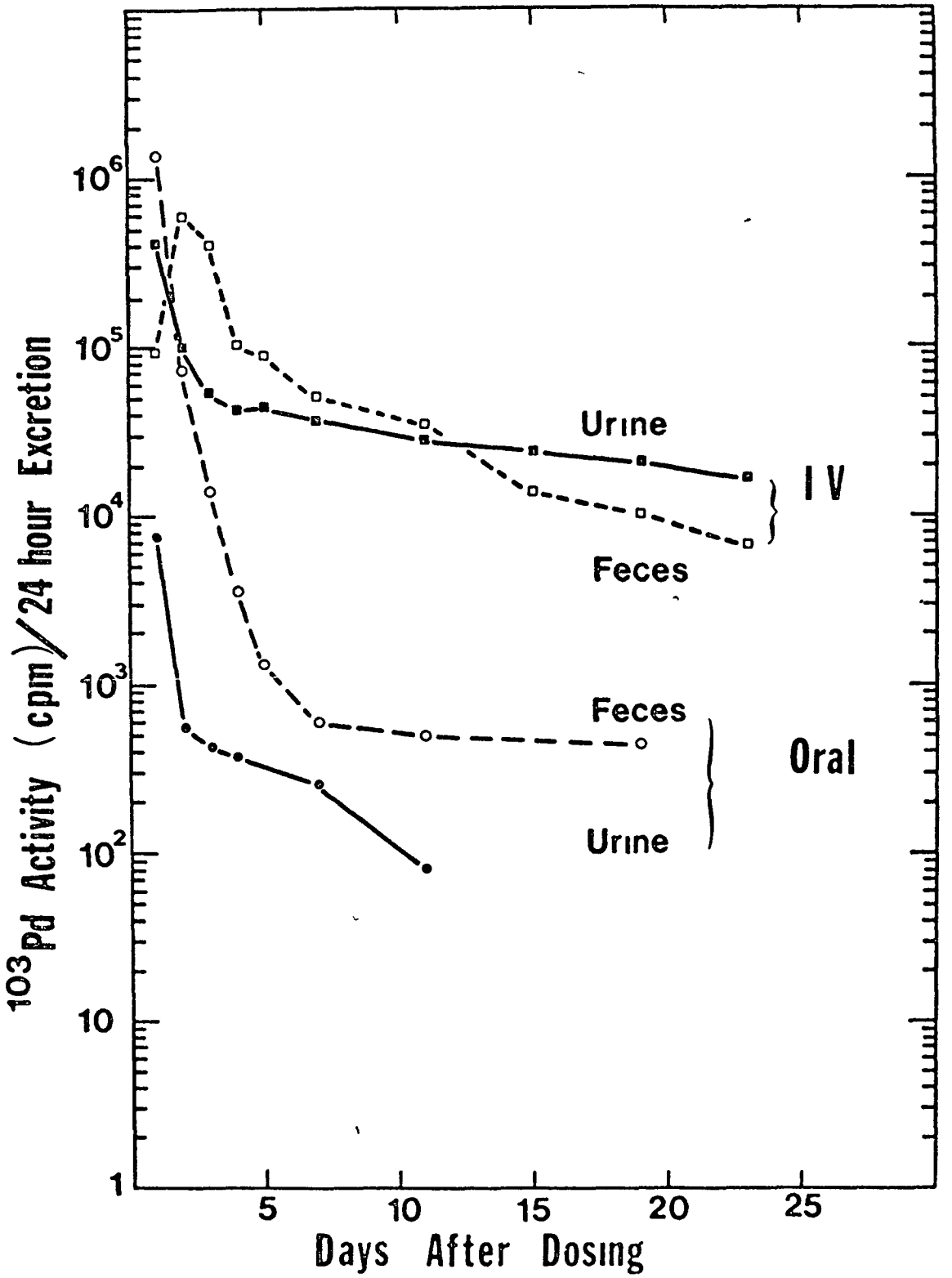

Fig. 2. Excretion of ^{103}Pd following iv and oral administration.

liver. the concentration in the kidney was much greater than in the liver. Twenty-four hours after iv dosing, ^{103}Pd was found in all the tissues analyzed with the highest concentrations occurring in the kidney, spleen, liver, adrenal, lung and bone, respectively.

The rats used in the whole body retention study were sacrificed 104 days post-exposure and the tissues counted. No significant amounts of ^{103}Pd were found in any of the tissues from the group receiving the oral dose. In the iv dosed rats, the highest concentrations of ^{103}Pd were found in the spleen, kidney, liver, lung and bone. For the intratracheally-dosed rats, the lung contained the most ^{103}Pd followed by kidney, spleen, bone and liver.

Maternal/Fetal Uptake

Thirteen pregnant rats (16th day gestation) were given 25 μCi ^{103}Pd iv and sacrificed 24 hrs later. During the 24 hr period, the pregnant rats excreted 44.2% of the initial iv dose. The amount excreted by the pregnant rats was higher than the amount excreted by the fasted adult male rats during the first 24 hr. period. The magnitude of the difference in ^{103}Pd concentration among the maternal organs and also between maternal tissues and the fetuses is best shown by the counts per g tissue and these values are given in Table 1.

Table 1. ^{103}Pd in Maternal Organs and Fetuses

TISSUE	MEAN COUNTS/g
Kidney	588,479
Liver	319,153
Ovary	29,625
Lung	29,211
Bone	18,351
Blood	3,654
Placenta	58,321
Fetal Liver	1,429
Fetus	757

The pattern of distribution and concentration of ^{103}Pd in maternal organs was similar to that previously found in the whole body iv experiment. Most of the fetuses (35) contained a small amount of ^{103}Pd, and the mean value for these fetuses is given in Table 1. However, radioactive counts for 17 fetuses from 5 litters was not significantly higher than background. The same pattern of results was obtained for the fetal livers. The amount of ^{103}Pd found in the fetuses indicated that Pd does not readily move across the placental barrier in the rat.

Within 24 hrs. following the birth of their young, a group of female rats were given 25 μCi ^{103}Pd and the litters counted to see if ^{103}Pd was transferred to the young via the milk The retention of ^{103}Pd by the dams and litters with time following a single iv exposure is shown in Figure 3.

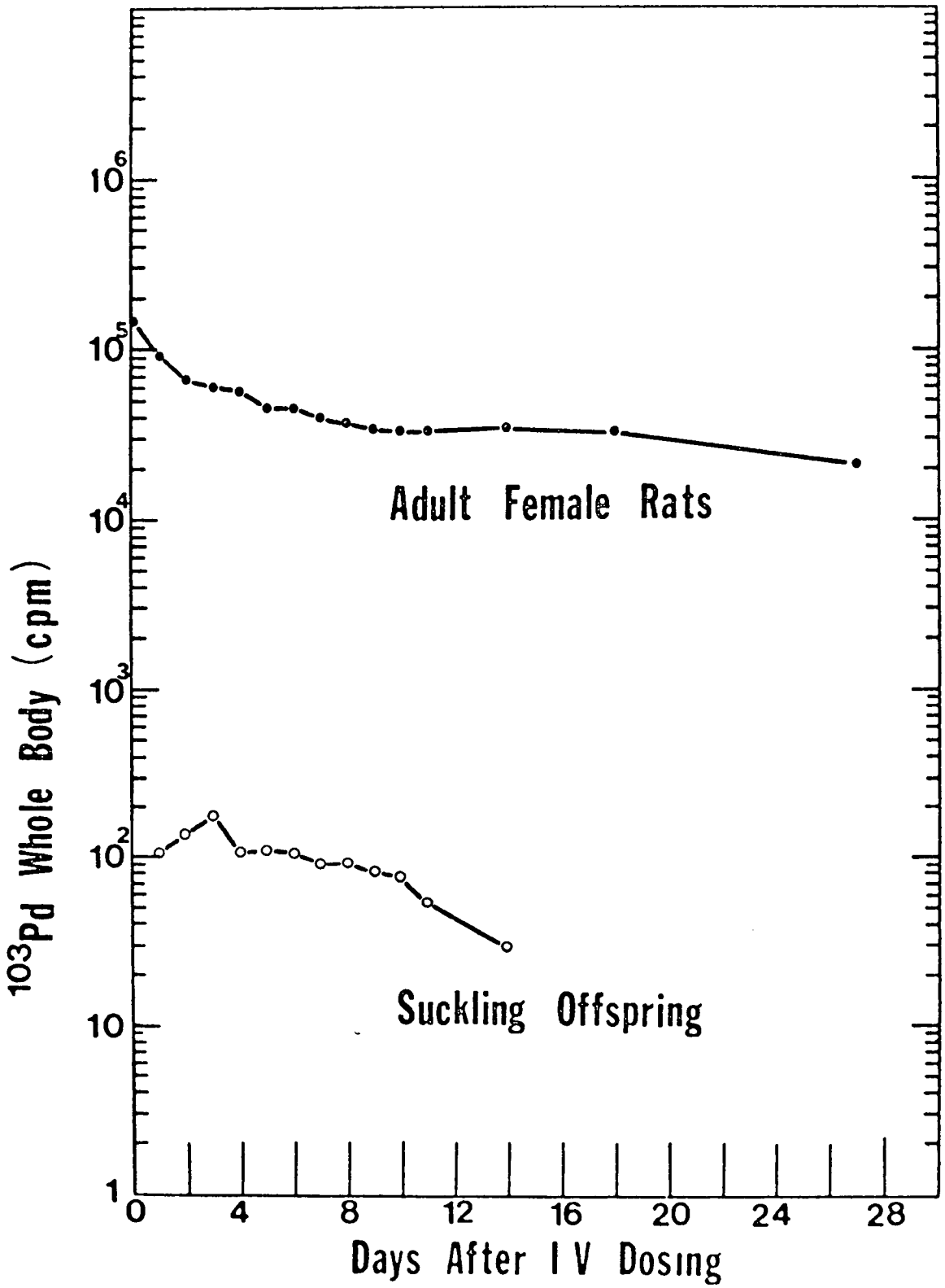

Fig. 3. Whole body retention of ^{103}Pd in nursing female rats following i v. administration and uptake of ^{103}Pd in suckling young via the milk.

It is evident that a small amount of the ^{103}Pd was passed to the young via the milk. Twenty-five days after dosing of the dams, the suckling rats were sacrificed and lung, liver, kidney, bone and spleen taken for analysis. A very small amount of ^{103}Pd (10-50 counts/gram tissue) was found in the tissues. The bone had the highest level of activity followed by the kidney, spleen, lung and liver.

2. Platinum

In discussing the toxicology of platinum, it should be emphasized that much of the information available in the literature deals with the effects of complex platinum salts. The effects of the complex Pt salts appear to differ somewhat from the effects of platinum alone.

Sodium chloroplatinate, a complex platinum salt frequently encountered in industry, has been tested in both rats and guinea pigs (Hofmeister, 1945, Saindelle, 1969). Immediately following the intravenous injection of 20 mg/kg sodium chloroplatinate into a guinea pig, there occurred an intense attack of asthma resulting in death within 3 minutes. Autopsy revealed the lungs to be pale and inflated as in anaphylactic shock. The injection of 1-2 mg/kg resulted in bronchospasm comparable to that caused by 3 µg/kg of histamine. Repeated injections of histamine resulted in reproducible changes in bronchial motility, whereas sodium chloroplatinate exhibited tachyphylaxis upon repeated injection. Aerosol exposure of guinea pigs to sodium chloroplatinate also resulted in an intense asthma attack. Administration of the antihistamine, pysilimine, completely blocked the action of sodium chloroplatinate.

Compared to histamine, sodium chloroplatinate has a long latent period (45 seconds following iv injection and 15 seconds following application to guinea pig ileum). This long latent period, along with the observed tachyphylaxis, suggests that platinum salts do not act by themselves, but instead are involved in the release of a substance from the tissues. Evidence that chloroplatinate may be causing the release of histamine is seen from the fact that 10 minutes after the intracardiac injection of 40 mg/kg of sodium chloroplatinate into rats, the plasma histamine level rose to 1500 µg/L from a norm of 150 µg/L.

Platinosis is a skin or respiratory reaction or disorder resulting from exposure to soluble complex platinum salts. There have also been reports of dermatitis resulting from exposure to platinum oxides and chlorides (Schwarte, 1947) and also to platinum alloys (Scheard, 1955). Skin lesions from platinum exposure have been described as a dry, scaling rash associated with cracking and occasional bleeding Instances of skin reaction have been reported from workers in platinum refineries (Roberts, 1951, Milne, 1970, Parrot, 1969) and by workers in photographic studios, who handled a paper containing potassium chloroplatinite (Karasek, 1911).

Scratch tests on human subjects have provided information suggesting that sensitivity to platinum salts is an acquired reaction precipitated by previous exposure. Prior to employment in a platinum refinery, none of the 24 subjects were sensitive to the test solution above a 1 10^2 dilution. Following employment, workers who showed no signs of platinosis remained insensitive to dilutions above 1 10^2. However, subjects with definite signs of platinosis has positive responses to dilutions of 1 10^3 to 1 10^8. The

degree of sensitivity being more or less dependent on the severity of the subject's symptoms (Roberts, 1951).

Respiratory problems resulting from exposure to complex platinum salts are initiated with irritation of the nose and respiratory tract, coughing, sneezing and running of the eyes Continued periods of exposure may result in an asthma-like condition associated with wheezing, tightness of the chest and shortness of breath The complex platinum salts frequently responsible for platinosis are sodium, potassium, or ammonium chloroplatinate (Levene, 1971).

Symptoms of both dermatologic and respiratory toxicity have been reported for a substantial number of workers exposed to complex platinum salts (Parrot, 1969, Roberts, 1951, Hunter, 1945). It has also been reported that 70% of the exposed staff of a platinum refinery had observable cases of platinosis (Herbert, 1966). Hunter et al. 1945, found that 52 of 91 employees of London platinum refineries had respiratory disturbances. Roberts has stated the opinion that all workers exposed to platinum salts have some degree of platinosis, 60% of which are symptomatic and 40% of which have no obvious symptoms, but who reveal evidence of involvement such as irritated conjunctivae and hypertrophy of the respiratory lymphatics (Roberts, 1951).

With the possibility that platinum may be emitted to the atmosphere and the proven toxicity of certain platinum compounds, it is important to determine the biological fate of platinum

Animals and Treatments

The same procedures were used in this study as in the palladium study except the rats were given 25 μCi of ^{191}Pt.

Radioactive Determinations

The radioactive solution was composed of carrier-free $^{191,193}Pt$ in 0.5 M HCL.

The solution contained at least 50% ^{191}Pt and only the ^{191}Pt gamma was counted. All values were corrected for decay (^{191}Pt has a half-life of 3 days) and the same dilutions and counting procedures were followed as in the ^{103}Pd studies.

Whole Body Retention

The whole body retention of ^{191}Pt following a single exposure was significantly affected by the route of administration The percent of ^{191}Pt retained with time in rats following three different routes of administration is presented in Figure 4. Following oral dosing, the total net gastrointestinal excretion was extremely high resulting in a rapid decline of the retention curve to less than 1 percent at the end of three days. The data indicated that the rapid clearance was due to passage of non-absorbed ^{191}Pt through the gastrointestinal tract. Extrapolation of the second component of the retention curve to the intercept indicated that less than 1 percent of the initial dose was absorbed

The whole body retention of ^{191}Pt following intratracheal dosing was significantly higher than for oral dosing The excretion of approximately 50 percent of the initial dose during the first 24 hours is attributed to mucociliary and alveolar clearance Whole body retention of ^{191}Pt was the

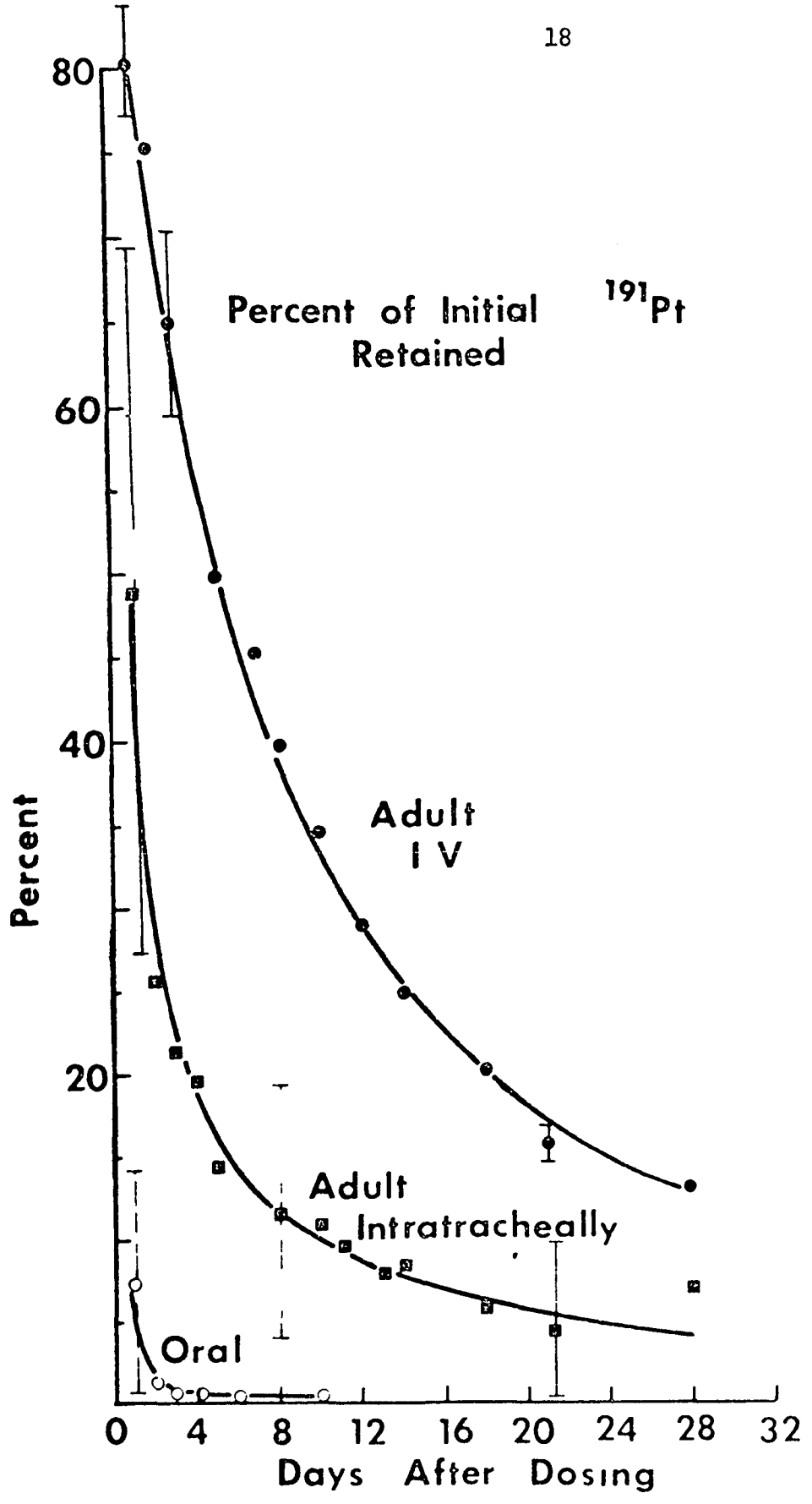

Fig 4 Whole body retention of ^{191}Pt in adult rats following oral, iv, and intratrachael administration

highest following iv dosing, the short half-life precluded an accurate determination of the biological half-life.

Excretion

Radioactive counts of 24 hour urine and feces samples from rats receiving ^{191}Pt orally indicated that almost all of the ^{191}Pt was eliminated in the feces and only a small amount excreted in the urine (Figure 5). These values support the whole body data which showed that total net gastrointestinal absorption was low. Following iv administration, ^{191}Pt was excreted in both the urine and feces. The urine contained a greater quantity of the ^{191}Pt

Tissue Distribution

The distribution and concentration of ^{191}Pt in tissues was determined following oral ard iv dosing After single oral dose, the kidney and liver contained the highest concentrations of ^{191}Pt. The amount of radioactivity found in the other organs was not significantly higher than background The amount of ^{191}Pt found in selected tissues following iv dosing is presented in Table 2. Most of the tissues did not contain levels of ^{191}Pt appreciably higher than that found in blood. However, the fraction of ^{191}Pt in the plasma that was in an "available" form for movement into the various tissues was not determined The large amount of ^{191}Pt found in the kidney suggests that this organ accumulates this element Concentrations higher than the blood values were also found in the liver, spleen and adrenal. The lower count for the brain suggested that either ^{191}Pt can be transferred through the blood -brain barrier only to a limited extent or else much of the circulating ^{191}Pt is complexed to large molecules which do not cross the blood-brain barrier.

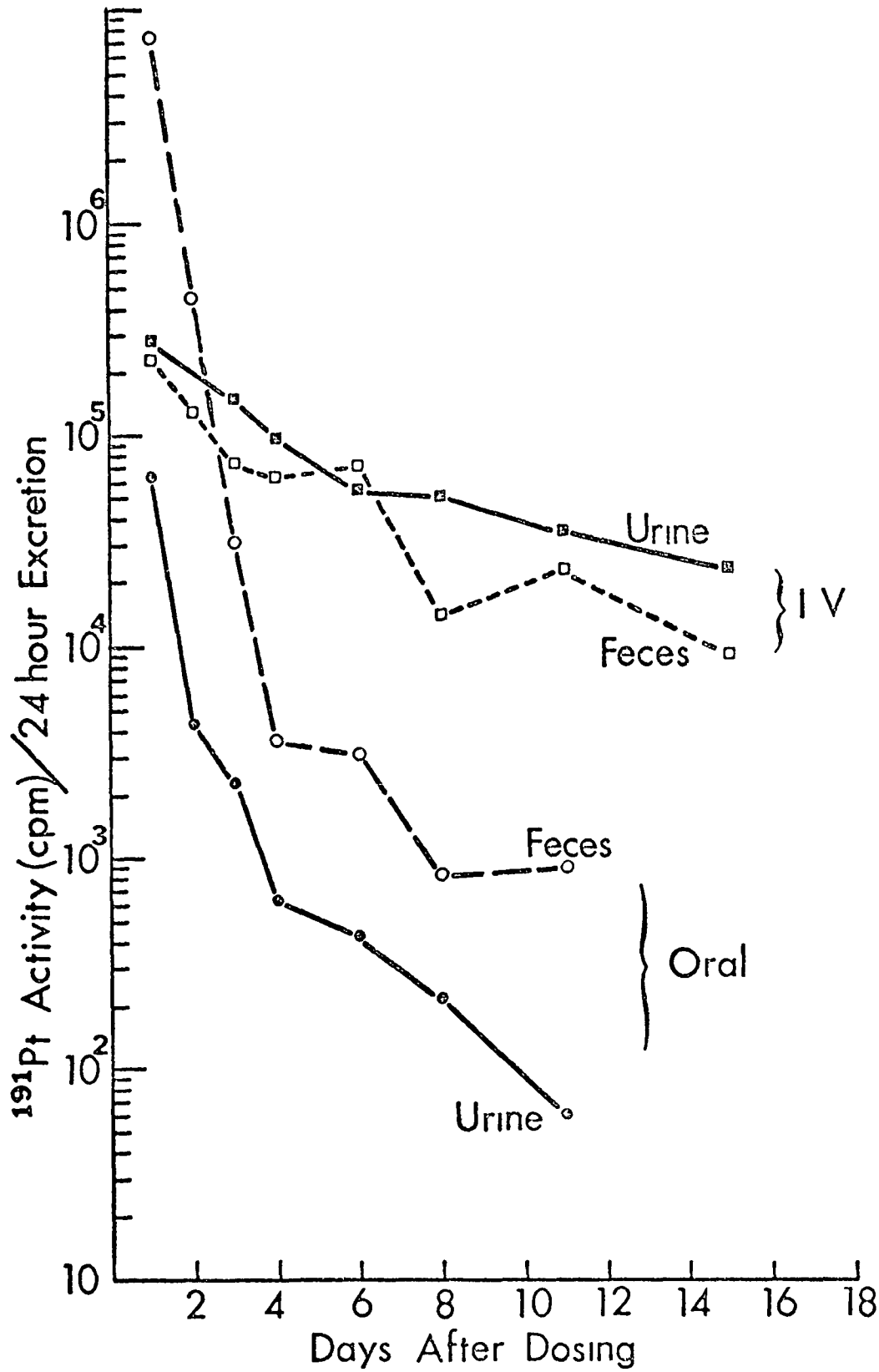

Fig. 5. Excretion of ^{191}Pt following iv and oral administration

Table 2. Counts/g Wet Tissue

	1 day	2 days	3 days	7 days	14 days
Blood	22,147	19,732	21,671	12,774	7921
Heart	11,819	12,201	15,508	8805	4593
Lung	18,432	16,139	17,638	11,180	5770
Liver	36,848	31,274	23,528	25,732	4733
Kidney	162,227	160,656	162.374	138,101	30,195
Spleen	41,085	45,840	44,033	55,764	20,973
Pancreas	22,208	19,487	22,618	14,802	3973
Bone	13,146	12,800	13,184	8932	5440
Brain	1150	2485	1027	595	265
Fat	4487	4501	4576	3201	429
Testis	4186	6540	4545	3873	1431
Adrenal	45,439	42,363	58,596	26,667	6190
Muscle	4798	4671	3930	3441	2146
Duodenal Segment	12,725	6044	6045	4031	1410

Maternal/Fetal Uptake

Fifteen pregnant rats (18th day gestation) were given 25 μCi ^{191}Pt intravenously and sacrificed 24 hours later. During the 24 hour period, the pregnant rats excreted 18 8 percent of the initial dose The amount excreted by the pregnant rats was approximately the same as the amount (19.3 percent) excreted by the adult male rats during the first 24 hour period. The concentration of ^{191}Pt per gram for different maternal tissues and fetuses is given in Table 3.

Table 3 ^{191}Pt in Maternal Organs and Fetuses

TISSUE	MEAN COUNTS/g
Kidney	127,064
Liver	43,375
Lung	17,981
Ovary	14,639
Blood	10,568
Bone	9,193
Brain	792
Placenta	27,750
Fetal Liver	1,421
Fetus	432

The data indicated that there was some transplacental passage of ^{191}Pt, however, there appeared to be placental binding or accumulation ^{191}Pt was present in all the fetuses (60) counted The hemochorial placental barrier of rats is more easily traversed than the more complex placental barriers found in other species of experimental animals

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A.2. THE ACUTE TOXICITY OF PALLADIUM CHLORIDE

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Introduction

As part of the comprehensive evaluation of the inhalation toxicity of catalyst emission compounds, acute toxicity studies with palladium chloride were begun as a rapidly obtained, reliable and inexpensive first estimate of toxicity in order to quantify the upper limits in terms of dose and obtain observations for assessing the biological or pharmacological effects of these compounds.

Results

a. Single dose studies

Using the method of Deichman and LeBlanc[1], the approximate lethal dose (ALD) was determined in 200-300 gm rats, (Charles River COBS) using intravenous, intraperitoneal, and oral modes of administration. In addition, the intravenous ALD was determined in the rabbit and the intratracheal minimum lethal dose was determined in the rat. The results are shown in Table 1.

Table 1. Acute Lethal Toxicity of $PdCl_2$

Species	Approx. LD_{50}	Route
Rat	5 mg/kg slope ∿1.5	IV
Rat	70 mg/kg slope ∿1.5	IP
Rat	>200 mg/kg	Oral
Rabbit	5 mg/kg	IV
Rat	6 mg/kg*	ITR

* Minimum lethal dose

Marked differences are noted among the different routes of administration, ranging from 5 mg/kg for IV to greater than 200 mg/kg for oral.

Using the more precise method of Litchfield and Wilcoxon[2], the intravenous and intraperitoneal LD_{50} (14 days) was determined. Figure 1 shows the log probit plot of the intravenous data. The LD_{50} (14 days) was calculated to be 3.0 mg $PdCl_2$/kg with 95 per cent confidence limits of 2.57-3.49. The slope was found to be 1.43 with 95 per cent confidence limit of 1 15-1.77. The $(CHI)^2$ test indicated that the data are not significantly heterogenous. Following intraperitoneal administration, the LD_{50} was calculated to be 123.0 (91.1-166.1) mg $PdCl_2$/kg with a slope of 1.84 (1.04-3.27), no significant heterogenety was noted (Figure 2).

A limited number of rats from the intravenous and intraperitoneal studies were housed in metabolism cages and several toxicometric parameters were measured during the 14 days observation period. Survivors of an acutely toxic intravenous dose of $PdCl_2$ exhibited a 25 per cent decrease in water intake and urine excretion. Following intraperitoneal dosing a 7 per cent reduction in body weight was observed with up to 80 per cent reduction in food intake. Water intake was reduced markedly initially and then returned to control levels or above. In one rat a 28 per cent increase in urine volume was noted at 14 days after dosing, with a constant decreased specific gravity of 1.030. Proteinuria was noted in all animals following both routes of administration. Elevated urinary ketone bodies were observed in some animals following both routes of dosing.

Following intraperitoneal injection, necropsy findings indicated a chemical type "burn" of the viscera in animals dying within 24 hours.

Gross pathologic examination of intraperitoneally dosed survivors at 14 days showed prominent peritonitis with numerous adhesions involving the liver, intestine, pancreas, and spleen. Involvement did not appear dose related. The kidneys showed a yellowish-tan appearing renal parenchyma with cloudy capsule. The liver in 30 day survivors showed reduced liver mass, and yellowish discoloration with thickened, opaque capsules.

Tissue palladium concentrations were determined by atomic absorption spectroscopy in rats which died following intraperitoneal injection. The concentration for four doses in brain, lung, cardiac muscle, liver, spleen, kidney and testes are shown in Table 2. A dose effect was suggested in some tissue but the sample size was too limited for absolute confirmation. However, the tissue levels in brain, heart, and lung showed that palladium was mobilized from the peritoneal cavity.

In vitro protein binding studies were performed with palladium and platinum chlorides ($PdCl_2$ and $PtCl_4$), using the Toribara ultracentrifugation technique[3] at concentrations up to 200 µg compound/ml using whole plasma or plasma equivalent albumin. Protein binding was greater than 99 per cent at all concentrations. Temperature and pH were found not to affect binding.

Following acutely toxic intravenous doses of palladium chloride, death occurred very rapidly, with a sharp threshold such that if exitus did not occur within five minutes, the animals (both rats and rabbits) survived the 14 day experimental period Rapid death may be due to respiratory arrest, since breathing ceased while a heart beat was still palpable for some time. However, cyanosis was not noted; the animals' oral mucosa and eyes were pink in color. Clonic and tonic convulsions were noted in rabbits and rats.

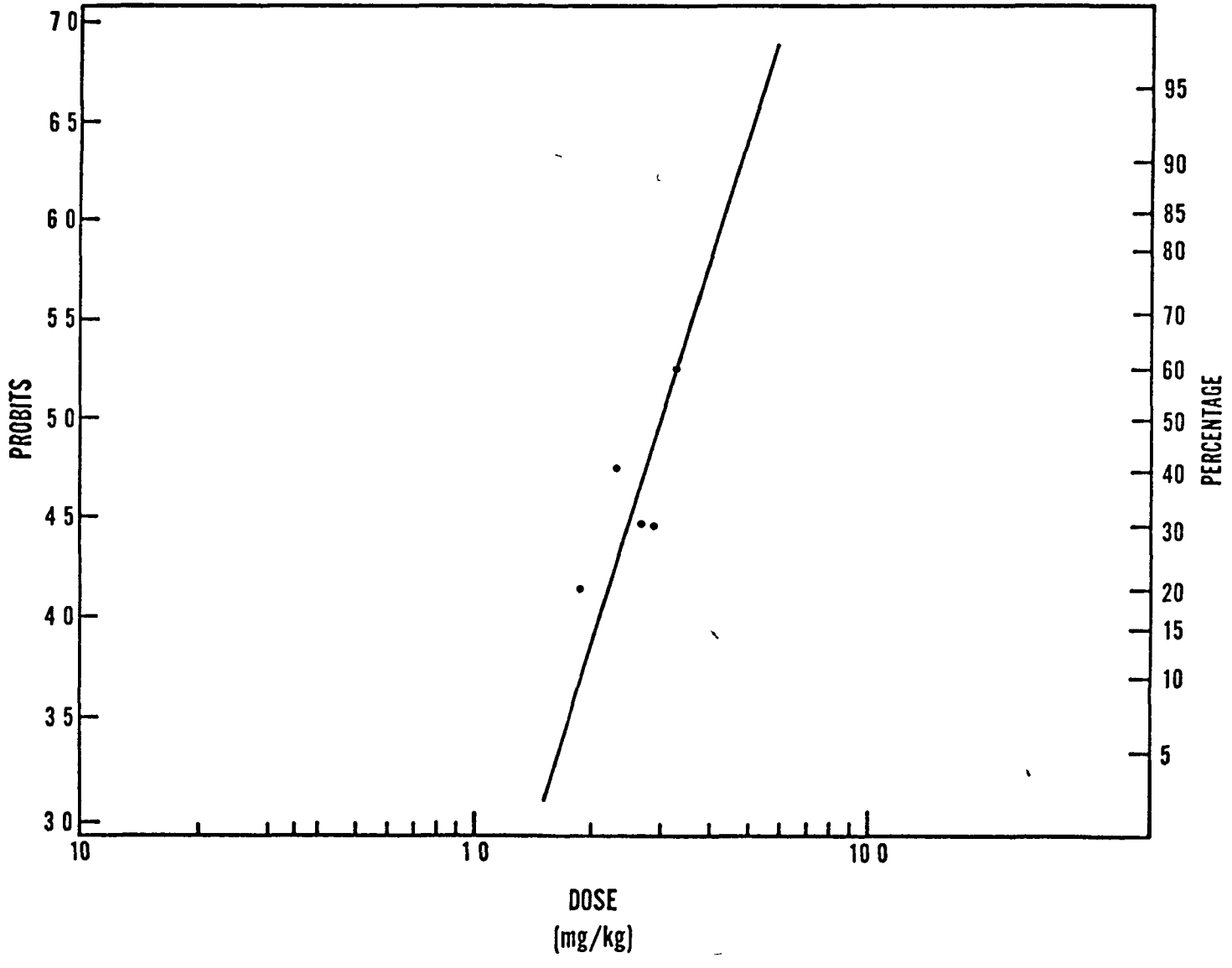

FIGURE 1. INTRAVENOUS $PdCl_2$ MORTALITY DATA PLOTTED BY METHOD OF LITCHFIELD AND WILCOXON (1949)

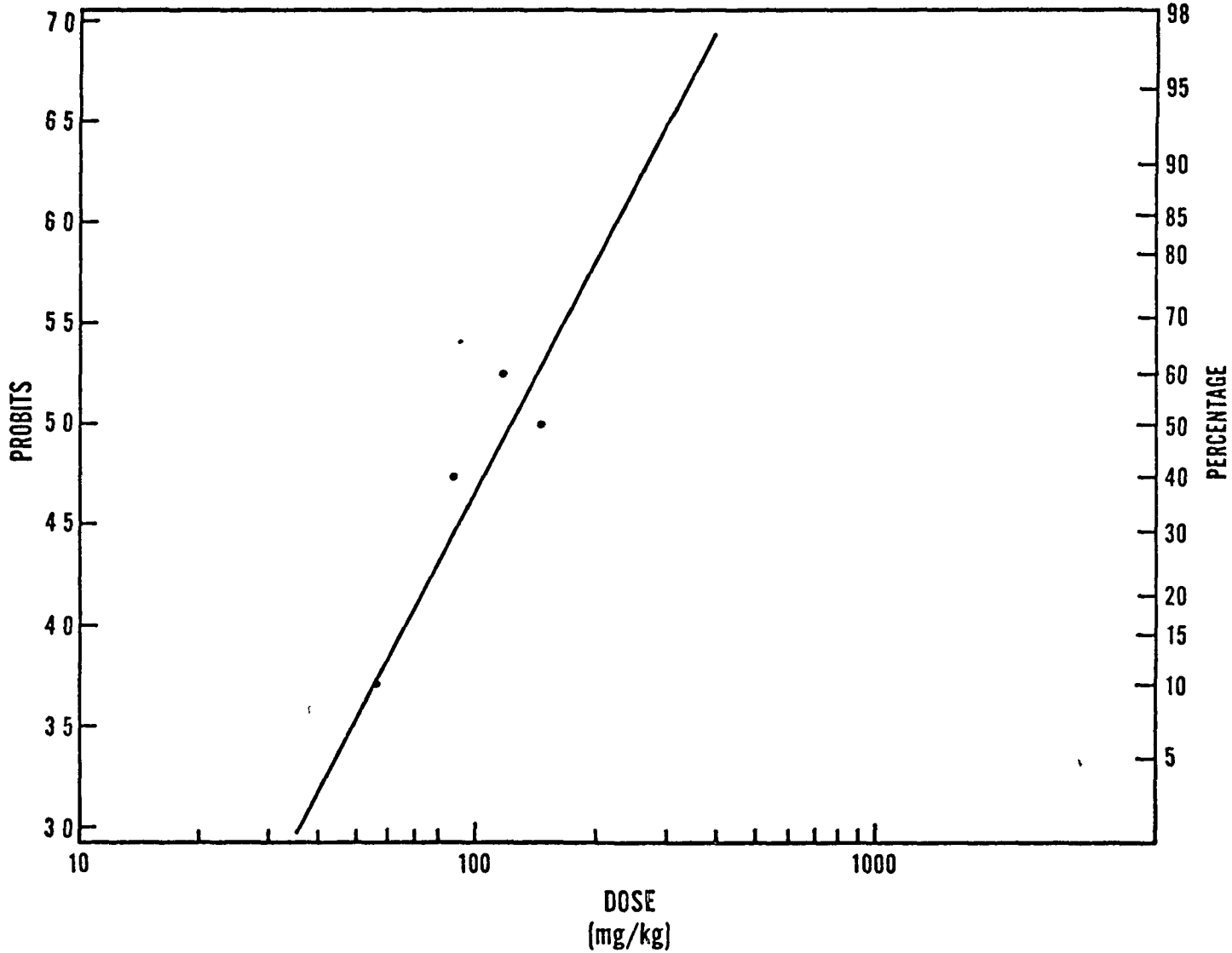

FIGURE 2. INTRAPERITONEAL $PdCl_2$ MORTALITY DATA PLOTTED BY METHOD OF LITCHFIELD AND WILCOXON (1949)

TABLE 2

TISSUE PALLADIUM CONCENTRATIONS IN ACUTELY POISONED RATS FOLLOWING INTRAPERITONEAL ADMINISTRATION (µg/gm Dry Wt.)

Total Dose	Mean Day/Died	Brain	Lung	Heart	Liver	Spleen	Kidney	Testes
30mg	24hr/1	< .04	-	27.32	185 91	330 56	843 39	60 91
	48hr/5	5 42	86 84	37 93	302 68	418 65	476 82	67 58
	72hr/0							
	96hr/0							
24mg	24hr/0							
	48hr/2	5 77	74 46	26 62	201 54	333 02	478 00	42 34
	72hr/2	4.18	78.52	40 12	277 39	272.23	590 86	67.44
	96hr/0							
18mg	24hr/0							
	48hr/1	1 26	41 03	17 57	67.79	317.18	583 94	14.31
	72hr/4	2.18	30 60	11 15	115 60	200 04	202 01	33 56
	96hr/2	4.77	22 86	17 42	51 36	196 80	177.83	31 56
12mg	24hr/0							
	48hr/0							
	72hr/0							
	96hr/1	6.95	3.45	0	37.24	192 09	-	12.70

The sharp threshold for mortality following intravenous administration suggested some compartmental (blood) saturation phenomenon. Therefore, ten rabbits which had survived the first intravenous dose of $PdCl_2$ were re-injected six hours after the first dose with a dose less than or equal to the original dose. All but one died, and the survivor was the largest animal used and had received initially the lowest dose.

b. Multiple dose studies

Toxicity of $PdCl_2$ following daily intravenous dosing

Thirty-three rats weighing 250 g were used in this experiment. Each rat was given 0.5 mg Pd in .1 ml (2 mg Pd/kg), daily in the tail vein for five days. Deaths occurred almost immediately following injection. The mortality during the five day period is given in Table 3.

Table 3. Cumulative Intravenous Toxicity of $PdCl_2$

Date	Amount Injected IV	Total Accum. Dose mg/kg	No. of Acute Deaths	Accum Deaths
11/26/73	.5 mg	2.0	0	0/27
11/27/73	.5 mg	4.0	2	2/27
11/28/73	.5 mg	6.0	2	4/27
11/29/73	.5 mg	8.0	4	8/27
11/30/73	.5 mg	10.0	3	11/27

An additional group of six rats was given 0.5 mg Pd on the first day and 1 mg on the second, five of the six animals died almost immediately.

Discussion

While the goals of this task are to determine the inhalation toxicity of catalytic components, other routes were examined for a more comprehensive evaluation. Intravenous studies were initiated because of their ease, inexpensiveness, and rapid information return regarding systemic and comparative intoxication. Intraperitoneal studies were also initiated for the above reasons, and also because of the similarity of mobilization between intraperitoneal and pulmonary depots Oral administration was studied since material deposited in the lung is cleared in part to the gastrointestinal tract. Intratracheal dosing is a reasonable model for inhalation studies.

Palladium chloride was selected for initial study because palladium compounds are principal contenders for use as catalysts in automotive exhaust control devices and, therefore, the toxicity and biological effects of palladium ion are of prime interest as a form of the metal which might distribute within the organism.

The large difference between the intravenous, intraperitoneal and oral acutely toxic doses suggest a difference in distribution of the palladium ion depending on the initial depot. This is supported by the tenacious macromolecular binding (> 99 per cent protein bound), the extensive peritoneal pathology after IP administration, and the sharp threshold for acute intravenous toxicity.

The signs accompanying intravenous toxicity were suggestive of acute central respiratory depression, respiration ceased before cardiac arrest occurred. Convulsions were noted in some animals which could possibly have been other than agonal Further studies are needed to elucidate the mechanism of acute toxicity. However, the tenacious protein binding, coupled with the sharp threshold and the repeat dosing experiment with rabbits and rats, suggest saturation of some binding sites before distribution to the ultimate target site(s).

Two additional effects were observed that merit comment. Respiratory arrest was not accompanied by frank cyanosis. This would suggest some effect of palladium on metalloporphyrin proteins Secondly, a pilot study was performed to determine the effect of palladium chloride on whole blood in vitro. The addition of this salt to whole heparinized blood caused clotting in the sample. Further studies are necessary to ascertain the significance of these findings.

Mortality after intraperitoneal administration appears to involve a chemical peritonitis, although other direct toxicity may occur, as in the kidney, since mobilization of the palladium occurred as shown by the tissue analysis.

Some comment is necessary regarding the discrepancy between the intraperitoneal ALD_{50} as determined by the method of Diechman and LeBlanc[1] and the LD_{50} as determined by the procedure of Litchfield and Wilcoxon[2]. Assuming the LD_{50} to be the most precise value (123 mg/kg), the value of the

ALD_{50} (70 mg/kg) represents a 43 per cent underestimation. The principal reason for this discrepancy, although the difference is not particularly alarming, is thought to be due to the chemistry of $PdCl_2$. The ALD_{50} was determined using aged solutions which have been shown to be more toxic than the fresh solutions. Whereas the LD_{50} was performed with freshly prepared solution of $PdCl_2$. In fact, before we were aware of this anomolous chemical behavior of palladium chloride in solution, an LD_{50} determination was performed according to the method of Litchfield and Wilcoxon[2] with aged solution. This procedure produced a value for the LD_{50} in good agreement with the ALD_{50} method of Diechman and LeBlanc[1].

These studies suggest that if palladium ion is formed after either inhalation or ingestion, a great potential for intoxication exists because of the enormous affinity of this metal ion for biological macromolecules.

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A.3. COMPARATIVE TOXICITY OF NOBLE METAL COMPOUNDS ON LACTIC ACID DEHYDROGENASE AND GLUTAMIC OXALACETATE TRANSAMINASE IN VITRO

S. D. Lee and R. M Danner

An in vitro study was conducted to study the relative toxicity of palladium and platinum compounds on two enzymes glutamic oxalacetate transaminase (GOT) and lactate dehydrogenase (LDH) The concepts of correlation between metal-ion toxicity, enzyme inhibition and serum enzyme levels as related to cellular damage in certain organs represent the hypothesis for this study

1 In vitro enzyme test system was as follows heparinized rabbit blood plasma was pooled and 5 ml portions were sealed and stored at -20°C until use. The test solutions were prepared from stock solutions which contained one milligram of metal compounds, which was dissolved in dilute hydrochloric acid and made up to a final volume of 10 ml with sodium acetate buffer (pH 7.4, 1.0M). The test system used in this experiment was similar to that of Christensen (1971/1972) with minor modifications The concentrations of stock solutions was 1.0 mg/ml. Concentrations of 1×10^{-5} through 1×10^{-3} µg/ml were prepared and tested.

Various reaction combinations were prepared

Control = 0 3 mg plasma, 0.1 ml acetate buffer (pH 7.4, 1.0M, 100 µl), test sample = 0.3 ml plasma, 0.1 ml test solution containing noble metal compounds, reagent blank = 0.3 ml acetate buffer and 100 µl test solution, enzyme blank = 0.3 ml plasma and 4N HCl (100 µl), standard = Versatol E (0.3 ml) and 0.1 ml acetate buffer

The test solutions were incubated for 30 min at room temperature (25°C) Following incubation, the enzyme activities were determined using DADE reagent se[redacted] All analyses at each concentration of test solution were performed in duplicate and average values determined. The enzyme activities were expressed in International Units/ml for LDH and Reitman-Frankel Units/ml for GOT following correction for enzyme and reagent blanks

Figure 1 shows the inhibitory effects of palladium chloride ($PdCl_2$), dimer of propionyl palladium chloride $(C_3H_5\text{-}PdCl)_2$ and potassium chloro-palladate (K_2PdCl_4 on serum LDH $PdCl_2$ exhibited a "stair-case" type inhibition pattern and was the most toxic among the three Pd compounds tested, it reached 80% inhibition at 250 mg/l $(C_3H_5PdCl)_2$ was less toxic than $PdCl_2$, it leveled at a plateau of 35% inhibition between 100 mg/l and 250 mg/l K_2PdCl_4 was the least toxic of the three compourds, it showed a mere 5% inhibition up to 150 mg/l and reached 20% inhibition at 250 mg/l.

Figure 2 shows a comparative inhibitory effect of $(C_3H_5PdCl)_2$, $PdCl_2$, K_2PdCl_4, $PtCl_4$ and $HgCl_2$. $HgCl_2$ was used as a reference compound since its toxic effect is well known. The relative toxicity of the five compounds tested was ranked as follows

$$HgCl_2 > (C_3H_5PdCl)_2 > PdCl_2 > PtCl_2 > K_2PdCl_4$$

These tests were performed to ascertain what type of effects one might expect if sufficient quantity of Pt and Pd compounds were found to be emitted into the ambient air. The in vitro data on inhibition of LDH and GOT may serve as a useful information as additional data are determined relative to the emissions of these compounds

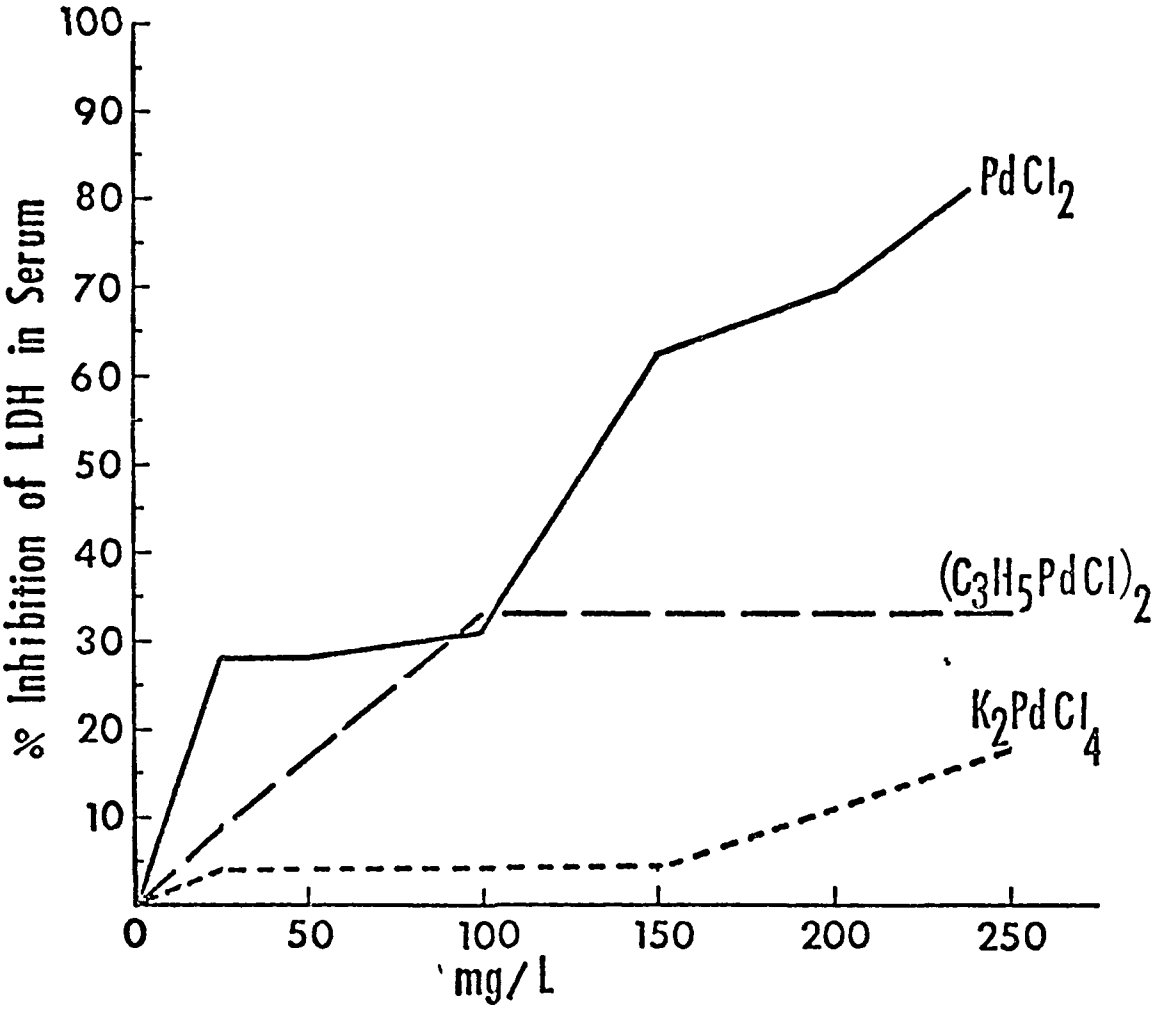

Figure 1. Comparative in vitro inhibition of Pd-compounds on Serum LDH

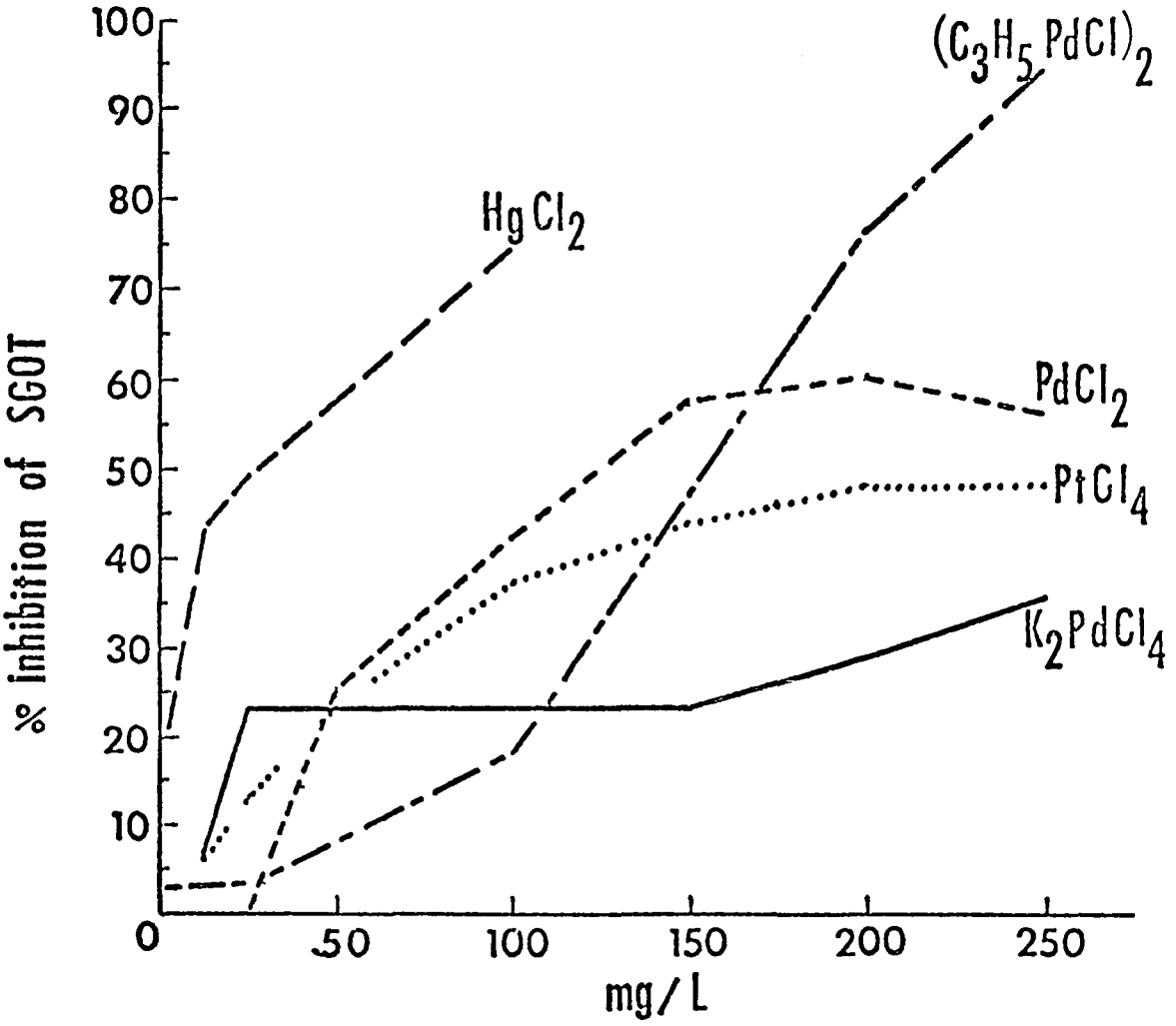

Figure 2. Comparative in vitro inhibition of SGOT

A.4. EFFECT OF NOBLE METAL COMPOUNDS ON PROTEIN SYNTHESIS IN VARIOUS ORGANS OF RATS

S D. Lee and R M. Danner

Experiments were conducted to detect early biochemical effects of intragastric administration of noble metal compounds ($PdCl_2$ and $Pt[SO_4]_2$) on protein synthesis in various organs as determined by the rate of incorporation of ^{14}C-leucine. Experimental animals (rats) were given $PdCl_2$ (1 mg/kg body weight) 24 hrs before sacrifice. Control animals were given saline solution One hundred forty μCi/kg body weight of ^{14}C-leucine was injected through the tail vein of all rats (control and treatment groups) and allowed to metabolize for one hour before sacrifice, The ^{14}C content of purified protein in liver, kidney, lung, heart and blood serum were examined.

Each of the excised organ samples was homogenized with 0 25 M sucrose 3:1 v/w. An aliquot of the homogenate was used to precipitate protein with 10% trichloroacetic acid v/v The precipitate was washed twice with 5% trichloroacetic acid v/v and then twice with 95% ethanol The concentration of protein was determined by the Biuret method. Radioactivity levels were measured in a Packard Liquid Scintillation Spectrometer The observed values were expressed in terms of dpm/mg protein and per cent alteration with reference to control

As can be seen in Table I, no change was observed in kidney ard lung, and only a slight decrease in liver. However, there was a marked increase in ^{14}C-leucine incorporation into the heart and blood serum protein. The increases were 137% ard 49% in heart and blood serum, respectively.

A different pattern of ^{14}C-l-leucine in rats given 0 5, 1.0 and 5.0 mg per kg body weight of $Pt(SO_4)_2$ was observed.

As depicted in Table II, $Pt(SO_4)_2$ caused a different response in ^{14}C-l-leucine incorporation into protein of five organs examined The most pronounced change was observed in the lung where a definite dose-response was observed with increasing concentration incorporation of ^{14}C-l-leucine rose 10.3% for 0.5 mg/kg, 22.7% for 1.0 mg/kg and 109 4% for 5 mg/kg body weight, respectively.

The incorporation of ^{14}C-l-leucine in kidney showed a reverse trend +16.9% for 0 5 mg, 6 2% for 1 0 mg/kg and no change for 5 mg/kg body weight, respectively. The changes in the brain showed similar pattern as the kidney There was a 30% increase at 0.5 mg/kg body weight for liver and no other apparent changes were indicated. Treatment with 5.0 mg/kg body weight of $Pt(SO_4)_2$ resulted in a 9% decrease About 30% and 26% increase for 0.5 mg and 1 o mg/kg levels were observed, respectively Apparently, $Pt(SO_4)_2$ at the concentrations used in this study did cause a significant disruption in protein synthesis in organs tested

Table I. Effect OF $PdCl_2$ on ^{14}C-Leucine Incorporation Into Protein

	dpm/mg Protein				
	Liver	Kidney	Lung	Heart	Blood Serum
Control	1,484 (4)*	1,708.0 (4)	1,566.5 (4)	955 (4)	1,877.3 (3)
Experimental	1,361 8 (4)	1,728.2 (5)	1,540 6 (5)	2,265 (4)	2,796 5 (4)
% Change	- 8.0	+ 1.2	- 1 7	+ 137 2	+ 49.0

( ) * Denotes number of animals

Table II Effect of $Pt(SO_4)_2$ on ^{14}C-l-Leucine Incorporation Into Protein

Organ / Dose mg/kg B W.	cpm/mg Protein Corrected for Organ Weight				
	Liver	Kidney	Lung	Heart	Brain
0	1056 (6)*	2332 (6)	1732 (6)	1355 (6)	882 (6)
0.5	1374 (4)	2807 (5)	1911 (5)	1356 (5)	1142 (5)
% Change	+ 30 1	+ 16.9	+ 10.3	0	+ 29.5
1.0	1088 (4)	2477 (4)	2239 (4)	1326 (4)	1111 (4)
% Change	+ 3.0	+ 6.2	+ 22.7	- 2.1	+ 26.0
5.0	1110 (4)	2331 (4)	3627 (4)	1233 (4)	949 (4)
% Change	+ 5.0	0	+109.4	- 9.0	+ 7.0

( )* = number of animals used.

A 5. DERMAL IRRITANCY OF SEVERAL Pd, Pt and Pb COMPOUNDS AND OF MMT

K.I. Campbell, E L. George, L L. Hall and J.F. Stara

A necessary aspect of general toxicologic characterization of potential environmental pollutant substances is the evaluation of dermal irritancy. A series of such tests were performed on several palladium and platinum compounds, for their relevancy to catalytic automotive emission control devices, and on two lead compounds and the gasoline antiknock additive, 2-methyl cyclopentadienyl manganese tricarbonyl (MMT).

The test procedure used was essentially that in standard use by the National Institute of Occupational Safety and Health,[1,2,3] a modification of the official Food and Drug Administration procedure [4] In each test six (6) healthy, male albino rabbits weighing 2 to 3 kg were used Up to seven (7) pairs of sites 2 x 2 cm were used on the closely clipped dorsolateral aspects of the trunk of each animal, the sites on the right side being abraded and those on the left remaining intact. Test materials in the solid (powder) state were applied in 0 1 gm quantity per site, mixed with about 0 1 ml deionized water and spread over the site, liquid materials were applied directly in 0.1 ml quantity. Each application was covered immediately with a gauze patch and further secured with tape (and overwrap in one test) and use of a leather restraining harness. After 24 hours, harnesses and coverings were removed, and test sites were washed with mild soap, rinsed, and dried. The skin reactions were then evaluated and scored, and again scored 48 hours later. Skin reactions were evaluated and scored using a grading system summarized in Table 1

The assigned rating was calculated as the average of means of the 24 and 72 hour scores for the test group, rated separately for intact and abraded skin, as illustrated in Table 2. Ratings were interpreted according to the scheme summarized in Table 3.

The materials tested, the dermal irritancy (intact skin) and cellular toxicity (abraded skin) responses observed, and the corresponding interpretations are shown in Table 4. Results were interpreted conservatively, i.e. based on the test in which the most severe responses were observed Many of the test materials showed a delayed healing of the abrasion lines themselves, in addition to or regardless of the standard response criteria.

Severity of response to some of the compounds tested more than once was quite variable Skin character and hair growth patterns among rabbits in the specified weight range were somewhat variable, and these could be factors in irritancy responses and evaluations. We recommend selection of rabbits for uniformity on these additional criteria. Close but gentle (atraumatic) clipping in preference to shaving, and over-wrapping in preference to taping for security of patches, are also recommended. In addition to tests for dermal irritancy, tests for sensitization should also be performed. Sensitizations may be far more serious or chronic than direct irritation, they may develop at lower and more common levels of exposure, and opportunity for development may be greater by virtue of extended or repeated exposure by ingestion and inhalation as well as cutaneously

Table 1

Evaluation of Skin Reaction to Test Material

Reaction	Grade Value and Designation	
	Intact Skin	Abraded Skin
No irritation	0 (Non-irritant)	0 (Non-toxic)
Erythema (regardless of degree)	1 (Mild irritant)	1 (Mild cellular to
Erythema and edema confined to test area	2 (Irritant)	2 (Cellular to i
Erythema and edema extending beyord test area	3 (Strong irritant)	3 (Strong cellula to
Eschar (Deep reaction involving dermis)	4 (Corrosive)	4 (Corrosive)

Table 7

Example Calculation of Test Rating

	Intact Skin Reaction (Dermal Irritancy)				Abraded Skin Reaction (Cellular Toxicity)			
nimal Number	24 Hr	72 Hr	Total	Mean	24 Hr.	72 Hr	Total	Mean
1	1	1	2	1.0	2	3	5	2.5
2	0	1	1	0.5	1	2	3	1.5
3	2	1	3	1.5	2	2	4	2.0
4	1	0	1	0.5	2	1	3	1.5
5	1	2	3	1.5	2	2	4	2.0
6	0	1	1	0.5	1	1	2	1.0
N=6				5.5				10.5

Dermal irritation rating for intact skin = 5 5/6 = 0.9. In this example, a non-irritant.

Primary irritation rating for abraded skin = 10 5/6 = 1.8. In this example, a mild cellular

TABLE 3

Interpretation of Skin Test Ratings

	Rating	Interpretation
Intact Skin	0 - 0 9	Non-irritant, probably safe for intact human skin contact
	1 - 1.9	Mild irritant, may be safe for use, but appropriate protective measures are recommended during contact
	2-- 4	Too irritant for human skin contact, avoid contact
Abraded Skin	0 -0 9	Non-toxic to cellular components of abraded skin, probably safe for human skin contact
	1 - 1.9	Mild cellular toxins, may be safe for abraded skin contact provided protective measures are employed
	2 - 4	Cellular toxins too irritant for abraded skin contact, avoidance of contact is advised

MIXED REACTIONS

Intact Skin	Abraded Skin	
0 - 0 9	0 - 0 9	Safe for human skin contact
	1 - 1 9	Safe for intact human skin contact, may be safe for abraded skin contact when protection is maintained
	2 - 4	Safe for intact human skin contact with abraded skin should be avoided
1 - 1.9	1 - 1.9	May be safe for intact and abraded skin contact when protection is maintained
	2 - 4	May be safe for intact human skin contact when protection is maintained, but contact with abraded skin is to be avoided
2 - 4	2 - 4	Unsafe for intact and abraded human skin contact, avoid contact

Table 4 - Rated Responses to and Interpretation of Direct Dermal Irritancy and Cellular Toxicity Tests in Rabbits

Material Tested	Severity Rating* Intact Skin (Irritancy)	Abraded Skin (Cellular Toxicity)	Interpretation+
Deionized water (negative control)	0	0	1) Safe for human skin contact
Glacial Acetic Acid (ethanoic) acid (Positive control)	2.6	3.2	2) Unsafe for human skin contact
Potassium chloropalladite, K_2PdCl_4	0 (0)	1.6 (1.9)	3) Safe for intact human skin may be safe for abraded skin when protection is maintained
Potassium chloropalladite, $K_2[PdCl_6]$	0 (0)	1.6 (2)	4) Safe for intact human skin abraded skin contact should be avoided
Palladium chloride, $PdCl_2$	0 (0 1)	0 6 (1)	5) Safe for intact human skin may be safe for abraded skin when protection is maintained
Ally palladium chloride dimer, $(C_3H_5PdCl)_2$	0 8	1.8	6) Unsafe for human skin contact
Dichlorodiammine palladium (II)Trans, $Pd(NH_3)_2 Cl_2$	0 (0)	0 2 (0 5)	7) Safe for human skin contact
Ammonium chloropalladite, $(NH_4)_2PdCl_4$	1 5 (3 1)	2.5 (3 7)	8) Unsafe for human skin contact
Ammonium chloropalladat $(NH_4)_2PdCl_6$	2 8 (4)	3.2 (4)	9) Unsafe for human skin contact
Palladium monoxide, PdO	0	0	10) Safe for human skin contact
Platinum (II) dichloride, $PtCl_2$	0 2	0 6	11) Safe for human skin contact
Platinum (IV)tetrachloride, $PtCl_4$	1 8 (2.7)	2 6 (3.8)	12) Unsafe for human skin contact
Platinum(IV)dioxide, $Pt O_2$	0	0	13) Safe for human skin contact.
Lead chloride, $Pd Cl_2$	0	0	14) Safe for human skin contact.
Lead monoxide Pb O	0	0.1	15) Safe for human skin contact.
2 - Methylcyclopentadienyl manganese tricarbonyl (MMT)	0 1	0 8	16) Safe for human skin contact

*Rating in parentheses indicates the most severe test result where tested more than once, those without indicate the single test rating or average of 2 or 3 test ratings

+Based on most severe or single test result

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A.6. DERMAL ABSORPTION OF ^{191}PLATINUM^{+4} IN HCl SOLUTION

K. Campbell, E George, W Moore, W. Crocker, F. Truman

In conjunction with tests of dermal irritancy of platinum compounds an experiment to assess transcutaneous absorption of ionic platinum was performed In each of 5 rabbits, 10 µl of a solution containing $^{191}Pt^{+4}$ in 0.5M HCl was spread over a closely clipped 1 cm square area of dorsal skin in the scapular region The nuclide dose was 8 36 µCi, a Packard gamma scintillation spectrometer (Model 5375) was used for counting Samples of blood before application and at 4, 24, 48 and 72 hours post-application, and 72-hour terminal samples of skin (incorporating the site of application), liver and kidney were counted. Counts were corrected for background and decay, expressed as counts per minute (CPM) per gm of sample (except for the skin specimen for which only total count was pertinent), and the fraction of the original applied dose was calculated

Results showed that at the 72 hour terminal period, on the average, 53.41% of the original dose was in or on the skin at the site of application and that very small fractions appeared in the blood or in the tissues. Of the sequential blood samples, the earliest (at 4 hours post-application) contained by far the greatest fraction of the applied dose (0 0074%), subsequent samples contained less than 1/10 as much Among the tissues at sacrifice, the concentration of activity in kidney was about 2.7 times that in liver and 14 3 times that in blood. The results are summarized in Table 1. Data of this experiment do not permit conclusions as to total amounts absorbed vs lost from the skin, the fractional distribution to other tissues, and the amounts excreted, they do suggest early minor transcutaneous absorption, with distribution to blood, liver and kidney There was no visible sign of dermal irritation at the site of application.

Table 1. Tissue Levels of ^{191}Pt Activity Following Dermal Application of $^{191}Platinum^{+4}$ in HCl Solution

Specimen	CPM/gm mean	Proportion of Dose Applied, Dec. Fract.x10^{-6} Mean	Range	Remark
Blood				
Pretreatment	0	0	0 - 0	n=5
4 Hr. post-treatment	92.4	74.0	0 - 240	"
24 Hr. " "	1.4	1.24	0 - 3.2	"
48 Hr. " "	6.8	6.60	0 - 30	"
72 Hr. " " (terminal)	2.1	1 82	0 - 8	"
Skin, terminal	594,493 Total*	534,100	274,000 - 732,200	Ave. 53.41% of orig dose
Liver, terminal	11.35	10.25	3 - 16	n=4
Kidney, terminal	30.3	27.0	6 - 48	n=5

*Based on entire skin sample.

A 7. OCULAR IRRITATION OF TWO PALLADIUM COMPOUNDS IN RABBITS

D Hysell, S Neiheisel and D Cmehil

The test was performed as outlined in the Code of Federal Regulations, Title 21, part 191 12, revised as of April 1, 1973 Six albino rabbits, having no known ocular abnormalities, were restrained and 100 mg of the test material was deposited on the surface of the right eye The left eye was maintained as a control. The animals were examined for ocular inflammation 24, 48 and 72 hours following application of the material

In the case of PdO (Table 1) no reaction was noted in any of the six rabbits In one animal, the test material was still present in the conjunctival sac at the end of 72 hours, but was completely covered with a thick mucous material

All six animals receiving PdCl showed a severe corrosive type lesion of the conjunctiva with severe inflammation of the cornea and anterior chamber of the eye (Table 1) This was noted at 24 hours and persisted throughout the test period.

These test results would indicate that at the dosage levels used, PdCl was a severe irritant, PdO was not

Table 1

Compound	Fraction of animals showing reactions at specific test intervals		
	24 hours	48 hours	72 hours
PdO	0/6	0/6	0/6
PdCl	6/6	6/6	6/6

A.8. NEUROPHYSIOLOGY SUMMARY OF Pt AND Pd

J Lewkowski, T Wessendarp,
W Moore and J Stara

The visual evoked potential in the rat is being utilized as a screening technique to test the relative short-term effects of various toxic agents Over 120 anesthetized rats have been exposed via intravenous injection in the past year The resultant changes in the visual evoked potential has been analyzed using various methods including computer averaging techniques

The results have indicated that this screening technique may be important in assessing the significant acute effects of various pollutants on central nervous system function The table below indicates the mean threshold dose of a particular cation which elicited a reproducible change in the visual evoked potential in 50% of the animals studied.

VISUAL EVOKED POTENTIAL SCREEN

Ion	Mean Reproducible Dose-Effect Threshold (mg/kg)
Co	0 010
Cd	0 10
Pd	0 40
Cr	0.80
Ba	2 0
Mn	2 0
Pt	Minimal effect

Therefore, the relative short-term effect of the intravenous administration of these cations on the rat visual evoked potential may be ranked as follows

Co > Cd, Pd, Cr > Ba, Mn > Pt

Assuming a similar blood level of Pd and Pt, it would appear that Pd has a greater effect on the central nervous system function under the experimental conditions of this particular screening technique It should be noted that this effect may not necessarily be a direct effect on central nervous system function. Further experimentation is underway to determine whether these effects are direct or are due to indirect factors, such as changes in blood pressure or some other physiological changes

In addition, more quantitative and less subjective methods to determine these thresholds are currently being utilized in an attempt to more precisely determine the relative central nervous system toxicity of these metals

A.9. A PRELIMINARY REPORT ON THE CARDIOVASCULAR ACTIONS OF PALLADIUM

M. J. Wiester

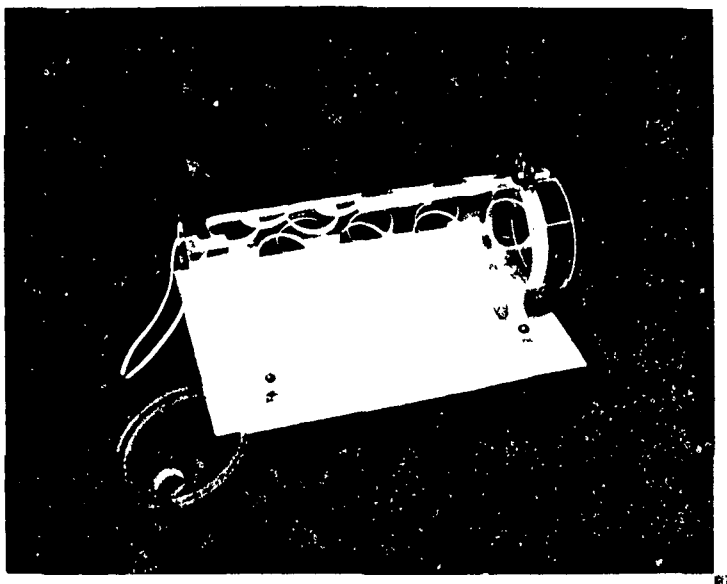
Introduction

Palladium chloride has been shown to be extremely toxic when given intravenously (I.V.). Rabbits, rapidly injected with 0.6 mg/Kg quickly die with damage chiefly to the heart.[1] The nature of the heart damage was not further defined and there is very little other information in the literature addressing this subject. The purpose of this study was measure the effects of different palladium chloride solutions on heart rate, ECG pattern, blood pressure, cardiac contractility (dp/dt) and breathing for one hour following intravenous injection.

Methods

Sprague Dawley ♂ rats (300 ± 50g) were surgically prepared one day prior to use. Surgery consisted of catherization of the abdominal aorta with tubing (#50 P.E.) for measurement of blood pressure and tubing (#10 P.E.) was inserted into the femoral vein to accomodate I.V. injections Both catheters were guided through the subcutaneous tissue to the back region and via a puncture wound through the skin to the outside. Six small silver electrodes, fitted with micro-strip connector pins, were inserted under the skin and sutured. Electrodes were arranged laterally so that four were near the limbs to record the ECG and two were on the lateral surface of the rib cage for respiratory measurements. After surgery, the rats were returned to their cages and given food and water ad libitum (Purina Lab Chow and tap water).

For testing, an unanesthetized animal was placed in a plastic tubular holder for the duration of the experiment and sensor leads fed to a recorder (Figure 1). The measurement system is diagrammed in Figure 2. After a



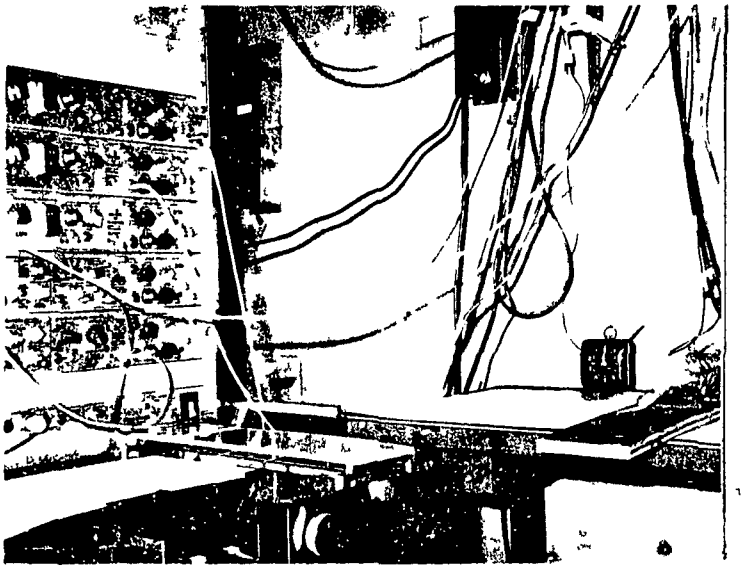


FIGURE 1. PLASTIC RAT HOLDER. HOLDER WITH RAT AND POLYGRAPH LEADS

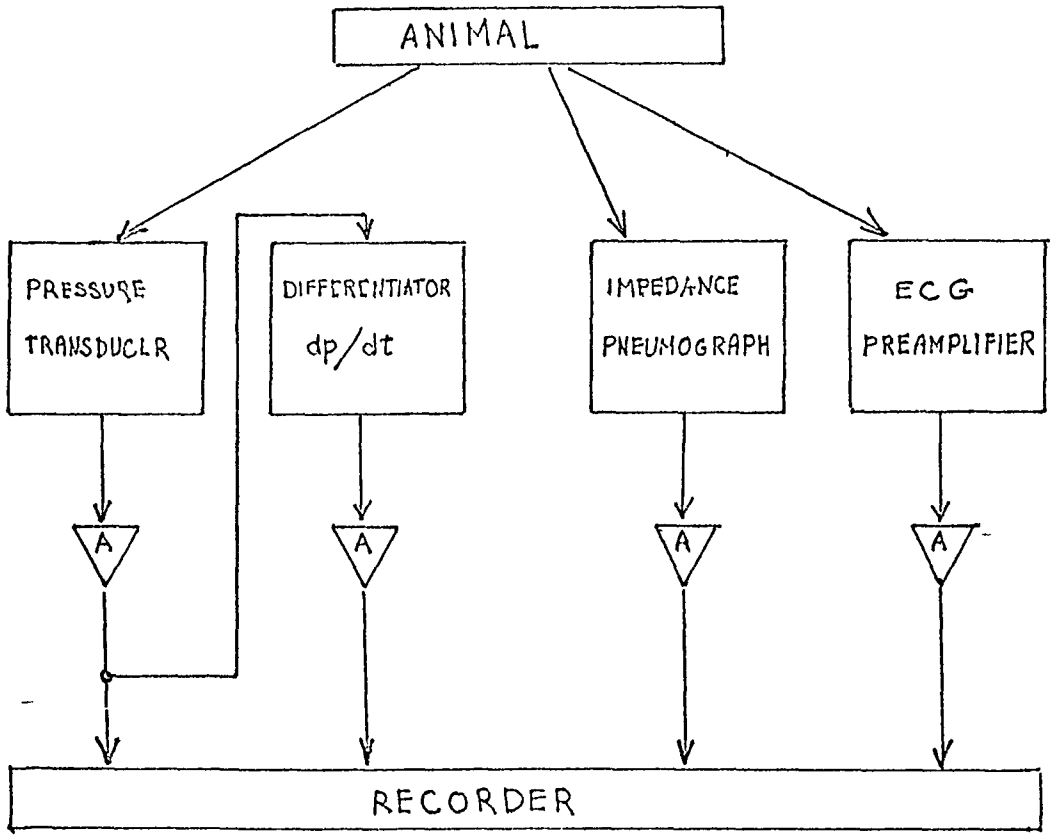


FIGURE 2 Block Diagram of Recording System

Pressure Transducer	For measurement of arterial blood pressure - calibrated with a Hg manometer (Miller Instrument)
Differentiator	For a record of maximum rate of change of aortic pressure - time constant of 1 msc Calibrated with an oscilloscope
Impedance Pneumograph	Monitors rate and relative depth of respiration (Narco Bio-Systems)
ECG Preamplifier	(Grass) Lead 2 was recorded
Polygraph	(Grass 7C)

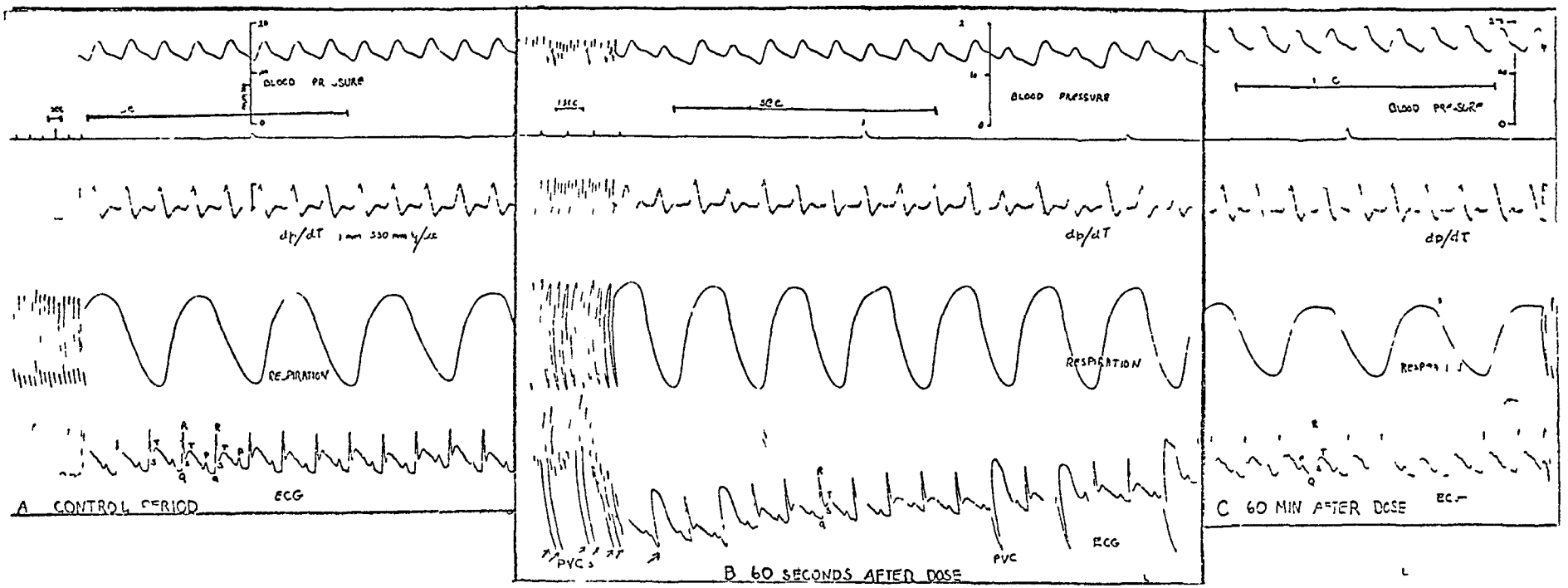


Fig 3 Rat #4 A Polygraph recording 25 minutes into the control period Aortic blood pressure is 165/125 Electronic differentation of the blood pressure signal is displayed as dp/dt The upward deflection shows maximum rate ofpressure development, which for this pressure pulse is 3,000 mmHg/sec dp/dt reflects the contractile state of cardiac muscle The respiration record shows the rate and relative depth of breathing The ECG is derived from lead 2 For this lead a prominant P wave and R wave can be defined The Q, S, and T waves are somewhat less specific However, the pattern is dependable and remains unchanged throughout control periods Heart Rate = 460 beats/min

B This section shows the measurements immediately following the I V injection of 2 04 mg/kg $PdCl_2$ Gross abnormalities can be seen in the ECG PVC's are not frequent enough to cause a detrimental fall in blood pressure Breathing was not altered Similar irregularities continued for approximately 3 minutes The animal survived

C One hour following the injection blood pressure had increased to 185/140 mmHg, dp/dt = 3660 mmHg/sec , the ECG showed no gross abnormalities, heart rate = 408 beats/min and breathing was unchanged

thirty minute stabilization or control period the palladium solution was injected and washed in with saline. The total volume of the dose and wash solution was 1 ml. and total infusion time was one minute. Effects of the injection were then observed for 60 minutes. Control animals were injected with 1 ml. of saline and treated the same.

Results and Comments

Palladium chloride exerted an immediate cardiovascular effect in the unanesthetized rat. The most pronounced effect was seen on the electrical integrity of the heart. A total of ten animals were dosed in amounts ranging between 1.14 - 5.9 mg $PdCl_2$/Kg and in each instance, premature ventricular contractions (PVC) were noted within one minute after dose initiation. PVCs were never seen during the 30 minute control periods or in control animal experiments (5 rats). Doses between 1.14 - 1.75 mg/Kg resulted in mild episodes of PVCs following the injection with no consequential fall in blood pressure. These arrhythmias continued for 3-4 minutes then the ECG stabilized. This stability, however, was dependent on the quiet state of the animal. If the rat moved or showed signs of distress, PVCs reappeared. Rats that received doses between 1.75 - 5.9 mg/Kg experienced gross alterations in the ECG pattern following injection. If the cardiac arrhythmias were intermittent or of such a nature as to allow adequate filling and pumping, then the animal survived (Figure 3). These surviving animals were able to maintain sufficient blood pressure levels during the critical 3-4 minute period following injection.

These surviving rats also reestablished a stable ECG during the one hour observation period, and like the low dose animals, were susceptible to arrhythmias, if they became agitated. Rats that succumbed after

receiving $PdCl_2$ intravenously suffered gross alterations in the ECG accompanied by a precipitous fall in blood pressure. After the aortic pressure fell, breathing became erratic and the ECG continued to deteriorate (Figure 4). Death usually occurred within four minutes after injection. Additional ECG abnormalities, other than PVCs, that were seen after injection of $PdCl_2$ were extra p waves, large S waves and various degrees of A-V block.

Rats surviving a $PdCl_2$ injection developed elevated blood pressures which persisted throughout the one hour observation period. Systolic pressure increased 20-50 mmHg and diastolic 10-20. Heart rates correspondingly decreased, and dp/dt changed very little.

Intravenous $PdCl_2$ appeared to have no initial effect on respiration. Changes in breathing were seen, however. the changes followed gross cardiac arrhythmias and falling blood pressures. If the rat reestablisned a steady and productive heartbeat, thus, survived the injection, breathing returned to control values and remained stable.

Results from preliminary experiments described above indicate that $PdCl_2$, when injected I.V., acts as a non-specific cardiac muscle irritant as well as a peripheral vasoconstrictor. Since the chloride salt strongly dissociates in solution ($PdCl_2 \rightleftarrows Pd^{++} + 2\ Cl^-$) the palladium ion itself may be the irritant. Effects seen might be due to the release of catecholamines or to stimulation of adrenergic receptors located in the cardiovascular system by the metal ion.

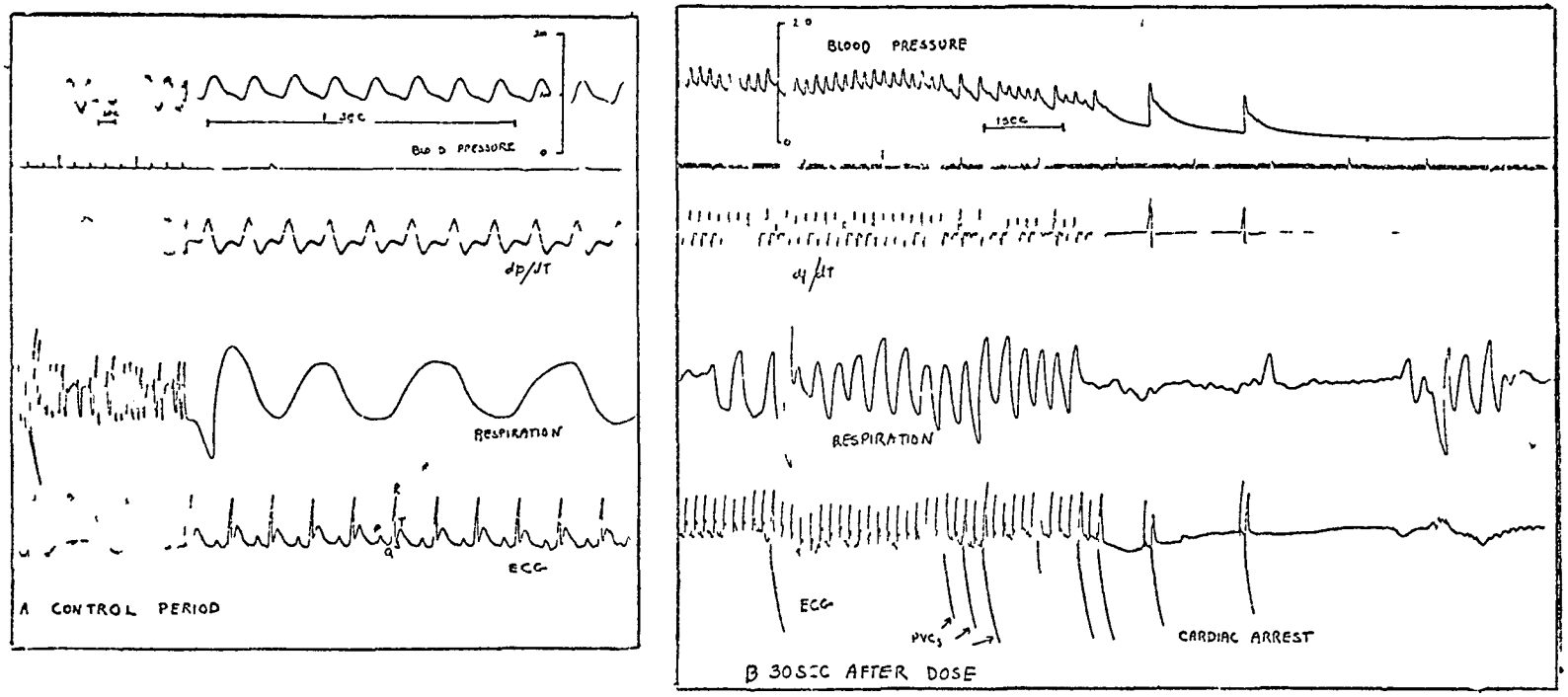


Fig 4 Rat #7 A Recording 30 minutes into control period - blood pressure = 125/90 mmHg dp/dt = 3,200 mmHg/sec Heart rate = 450 beats/min , ECG normal pattern Breathing frequency = 132/min - irregular pattern due to animal movement

B Recording showing gross abnormalities in the ECG, declining blood pressure and death of the animal immediately following I V injection of 2 75 mg/kg $PdCl_2$

1. Orestano, G. The Pharmacologic Actions of Palladium Chloride. Boll. Soc. Ital. Biol. Sper. 8 1154-1156, 1933

WHOLE EXHAUST EMISSION STUDIES

B.1. AUTO EXHAUST FACILITY MODIFICATION

R. G Hinners and J. K Burkart

Introduction

The auto exhaust generating system has recently been modified at this laboratory and the following will update other papers[1,2] describing the earlier facilities for the production of irradiated and nonirradiated gasoline engine exhaust-air mixtures It is also intended as a reference for biologically oriented papers discussing health effects of completed studies The Toxicity Assessment of Mobile Emissions (TAME) project represents a series of acute and subacute bio-effect studies which test exposures of experimental animals to whole automobile exhaust emissions with fuel additives ard/or with or without a catalytic converter Briefly, the exhaust gases are generated by an engine-dynamometer unit and mixed with clean conditicned air in a dilution system to produce the desired concentration The exhaust gas mixture is divided with one part flowing direct to animal exposure chambers, and the remainder flowing through irradiation chambers to other animal chambers. The changes include an air dilution tube for the immediate mixing of the entire raw exhaust emissions with conditioned air and a large mixing chamber after the dilution tube. Information is also provided on air supply, engine cycle, fuel supply and other minor changes that have been made.

Dilution Tube

The effluent from the engine exhaust system is passed into an air dilution tube, thrcugh flexible stainless steel tubing, connected to the muffler The dilution tube is 23 in in diameter and made from 10 ga SS

plate, rolled and welded. Dilution air enters the tube through a 90° elbow from a remote supply source. Located between the flanges of these two tube sections is a mixing baffle plate, with a 7-1/4 in. diameter hold bored in the center The incoming dilution air under pressure, is forced through this hole to mix with the raw exhaust The tailpipe exhaust inlet elbow enters 90° to the tube axis and is bent 90° again, so that the flow axis of the exhaust outlet coincides with the center line axis of the dilution tube The exit end of the 2-in diameter SS exhaust elbow is in the same plane as the baffle Located on top and outside the dilution tube, at the baffle plate, are two quick-disconnect couplings One allows the end of the flexible 2-in. I D. exhaust pipe from the muffler to connect with the dilution tube and the other connects to the outside atmosphere. A blank plug is installed in the disconnect to the dilution tube when the exhaust is vented outdoors System back pressure at this point is 4 in. water This feature provides the capability of varying the modes of engine operation for aerometry and allows interruption of animal exposures.

By closing a damper in the air supply line, the dilution ratio can be controlled. To retain the particulate matter in suspension and prevent condensation, it is necessary to dilute the whole exhaust with at least 8 parts of air to 1 part of exhaust For each pound of fuel burned, approximately a pound of water is formed and some condensation occurs if the exhaust is not immediately diluted with dry air Also to prevent condensation, the outside of the dilution tube is insulated since engine room temperature often exceeds 90°F and dilution air temperature averages 50°F and 67% relative humidity The main portion of the dilution tube consists of two 7 ft. long flanged sections before reducing through a transition to a 6-in. diameter and entering the mixing chamber

Mixing Chamber

The diluted auto exhaust enters the mixing chamber, formerly used as an irradiation chamber, through a 6-in diameter SS pipe opening in the side wall An elbow discharges the exhaust in front of and parallel with a tube-axial fan, controlled at a low rpm by a Zero Max unit, to mix the entering auto exhaust with the chamber atmosphere

The chamber is 23-1/2 ft long, by 4 ft wide, and 8 ft high, with a volume of 683 ft^3 The sides consist of a framework of aluminum structural members holding metal panels to replace the plastic windows The aluminum sheet metal panels are clamped and sealed by means of pressure screws and gasketed channels. Previous studies with a reference fuel, to which had been added methylcyclopentadienyl manganese tricarbonyl (MMT) as an antiknock additive required darkness, due to the light sensitivity of the MMT. The top, bottom, and ends of the chamber are formed of 1/4 in thick aluminum plate welded on both sides at all seams to prevent leakage

At the end of the chamber opposite the entry port is a 6-in diameter line with a motorized damper control vented to the atmosphere. Another 6-in. diameter outlet pipe from the chamber supplies the exhaust either to irradiation chambers or to raw exhaust animal exposure chambers A pressure sensor, which is adjustable and located downstream of the chamber exit line, controls the motorized damper in the vent line to maintain 2-in of positive water pressure in the chamber

Irradiation Chambers

The photochemical reactions that result from the exposure of the diluted raw exhaust to artificial sunlight take place in five irradiation chambers

Fluorescent lighting panels composed of blue lamps, black lamps and sun lamps outside the chamber pass intense ultraviolet radiation through windows of Teflon FEP fluorocarbon film. One irradiation chamber is needed to provide the atmosphere for each animal exposure chamber Normal flow through the irradiation chambers is 11 cfm, which results in 15 air changes per hour in the animal exposure chambers In some instances, however, the flow has been reduced by one-half that of normal, which, of course doubles the irradiation time One of the original irradiation chambers used in previous exhaust studies has been converted into a mixing chamber which is described separately

At a volume of 683 ft^3 and 11 cfm flow, 43 minutes is needed to achieve 50% of inlet concentration when "building up" from zero Approximately five times 43 minutes (3-1/2 hours) are needed to reach equilibrium at the inlet concentration, decay time is also 3-1/2 hours

Air Supply

The air purifier unit provides, at maximum, 550 cfm of CBR (chemical, biological, radiological) filtered and conditioned air Inside building air is passed through a cooling coil to lower the temperature to 40°F (saturated at coil outlet), there is no reheating or humidification. Therefore, if the relative humidity of the outside air drops below 36 grains of moisture per pound of dry air, the relative humidity in the final exposure chamber will also vary Most of the time, there is no problem maintaining constant relative humidity, but occasionally on very dry days, there is a change

The humidifier is turned off, because of the constant need for cool dry air to mix with hot wet raw exhaust. Exposure chambers on control air are supplied from a separate CBR filtered source, with controls set to maintain 72° ± 2°F and 55 ± 5% relative humidity in the animal chambers. The same air is also ducted to the air filter inlet of the engine being used for the study since a change in humidity effects the NO_x emissions from the engine

Engine Cycle

The dynamometer driving schedule for the Chevrolet engines consists of a repetitive series of idle, acceleration, cruise, and deceleration modes of fixed time sequences and rates The following Table I and Figure 1 is the modified "California Cycle" used in the fuel emission studies.

Table I

Mode	Speed, M P H	Time, seconds
Idle	0	20
Acceleration	0 to 30	14
Cruise	30	15
Deceleration	30 to 15	11
Cruise	15	15
Acceleration	15 to 49	29
Peak	49 to 50	1.5
Deceleration	50 to 0	31.5
	Total	137 sec.

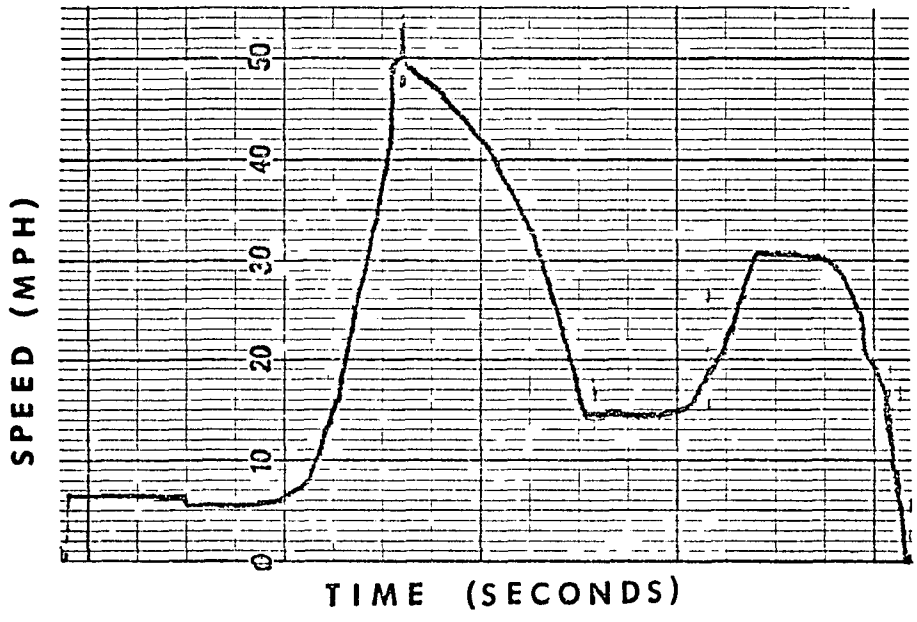


FIGURE 1 CYCLE SPEED TRACE
(137 SECONDS)

Replacement of the California Cycle with the IA-4 cycle controller was considered at one time. However, after consultation with other experts in the field, it was decided to continue with the California Cycle because the exhaust is being further diluted to prescribed levels and both cycles are very similar since they reflect transient as well as cruise operation. The key to this research is comparative toxicity either cycle is satisfactory to achieve this goal A simple repetitive cycle that is easily controlled over long periods of time (weeks) is of prime importance to toxicologic investigations

Fuel Selection

The gasoline selected for use in the Chevrolet engines as a standard reference, baseline fuel for evaluation of engine, fuel and additive variables was American Oil Co. Unleaded 91 Octane Test Fuel, Intermediate Grade Indolene Clear As a referernce it was important that it be of quite precise and reproducible composition and character, including absence of lead and other additives (except as specifically noted), and similar to high-volume regular market gasoline This gasoline has been used for such purposes in research and development by industry and other agencies The lubrication oil selected was Texaco Havoline 30W, API service specification SE. Table II shown below, represents a comparison and product analysis of the two gasoline deliveries used for exhaust emission studies during 1973.

Table I. Fuel Properties

Shipment No.	#1	#2
Date Delivered	3/30/73	10/29/73
Quantity, gallons	2,000	1,500
Octane No., research	91.4	91.3
Octane No., motor	82.9	82.5
Lead Atm. Abs., gm/gal.	0.01	0.01
Phosphorus, gm/gal.	0.002	0.00
Sulfur, wt. %	0 04	0.05
Aromatics, Vol %	25.4	23.5
Olefins, Vol. %	11.8	9.9
Gum, Existent, mg/100 cc	0.8	1.0
Gravity, °API	61.4	61.5
Oxidation Stability, minutes	600+	600+
Ried Vapor Pressure, lbs	9.1	9.0

Note. Shipment #1 used for studies G, H, I and J. Shipment #2 used for study K with Thiophene added to produce 0.10% by weight sulfur

Fuel Storage and Handling

Local fire and safety regulations require flammable liquids to be stored outside the building, so two underground fuel storage tanks were installed on the property near a blacktop surface driveway. To promote chemical stability of the fuel during storage, the tanks are maintained under slight positive pressure with nitrogen supplied from cylinders and

controlled by a pressure regulator. A double acting pressure and vacuum relief valve on the vent outlet compensates for changes due to fuel being pumped out or temperature increase, which would alter the pressure of the nitrogen gas cover Each tank is of 2000 gallon capacity and equipped with an electric fuel pump rated at 15 gallons per minute

Outside the building wall and next to the engine room is an 18-gallon marine fuel tank setting on a weight scale and connected to a remote electric fuel gauge located in the instrument panel. Transportation of the test fuel from the main underground storage supply to the one-day supply tank is effected by a mobile safety dispenser cart made especially for transporting flammable liquids The 60-gallon-capacity cart carries the Underwriters Laboratories' approval as a portable flammable liquid tank and is equipped with transfer pump and grounding reel. The cart also has a drain, and removing the pump gives access to a 4" handhole for reaching and cleaning the tank interior between fuel changes. Similarly, the 18-gallon marine tank can easily be inverted for cleaning when required.

An alteration in the composition of the reference fuel for a study is made by the addition of the required amount of chemical to a full cart batch. Thus studies requiring the testing of fuel additives such as methylcyclopentadienyl manganese tricarbonyl (MMT) or thiophene to increase the sulfur content can be conducted by mixing only the amount of fuel necessary.

References

1. Hinners, R. G Laboratory Produced Automobile Exhaust Facility. Biomed. Sci. Instrum. 1 53, 1963

2 Hinners, R G , Burkart, J K and Contner, G L Animal Exposure Chambers in Air Pollution Studies. Arch Environ Health. 13 609-615, (Nov) 1966.

B.2. CATALYST EXHAUST EXPOSURE STUDIES

R G. Hinners and J.K Burkart

During 1973, animal exposure studies were conducted in ETRL, NERC-Cincinnati, to assess the relative health hazard of automobile exhaust emitted from engines equipped with and without catalytic converters using similar engine settings. Automotive exhaust catalysts were developed to lower exhaust emissions of carbon monoxide, hydrocarbons by oxidation, and oxides of nitrogen by reduction. They are the three pollutants specifically listed in the Federal Clean Air Act of 1970. The regulations also require that no pollution control device shall emit "noxious or toxic" substances Three possible conditions could result in such emissions 1) As the hot catalysts promote the oxidation of carbon monoxide and hydrocarbons in automotive exhaust, converting them to carbon dioxide and water, it may simultaneously convert the organic sulfur compounds present in all gasoline into sulfuric acid mist and eventually sulfates 2) The metals used in the converter, such as platinum and palladium, may be emitted under conditions of catalyst degradation from the exhaust pipe in fine particles and be suspended in the air. 3) The total emissions may be altered and may produce different quantities or new species.

In order to perform the assigned tasks, this laboratory recently acquired and installed two new engines equipped with catalytic converters from General Motors Company* and the Ford Motor Company * (See Table 1)

Table 1

FORD - 400 C.I D (1975 Prototype) R-6 Engine with R-14 Calibration and the Following Controls

(1) EGR (exhaust gas recirculation)

(2) Air Pump

(3) Fluidic spark delay valve

(4) Various temperature sensing triggers

(5) Catalytic converter of monolith, noble metal oxidation type. Two converters of this type are required, one for each bank of cylinders. (Catalyst by Matthey-Bishop Co)

GENERAL MOTORS - 350 C I.D. (1973 Production Engine)

(1) EGR

(2) Air Pump

(3) Catalytic converter, pelletized type, noble metal oxidation catalyst. One converter after Y pipe (Catalyst by Engelhard Co.)

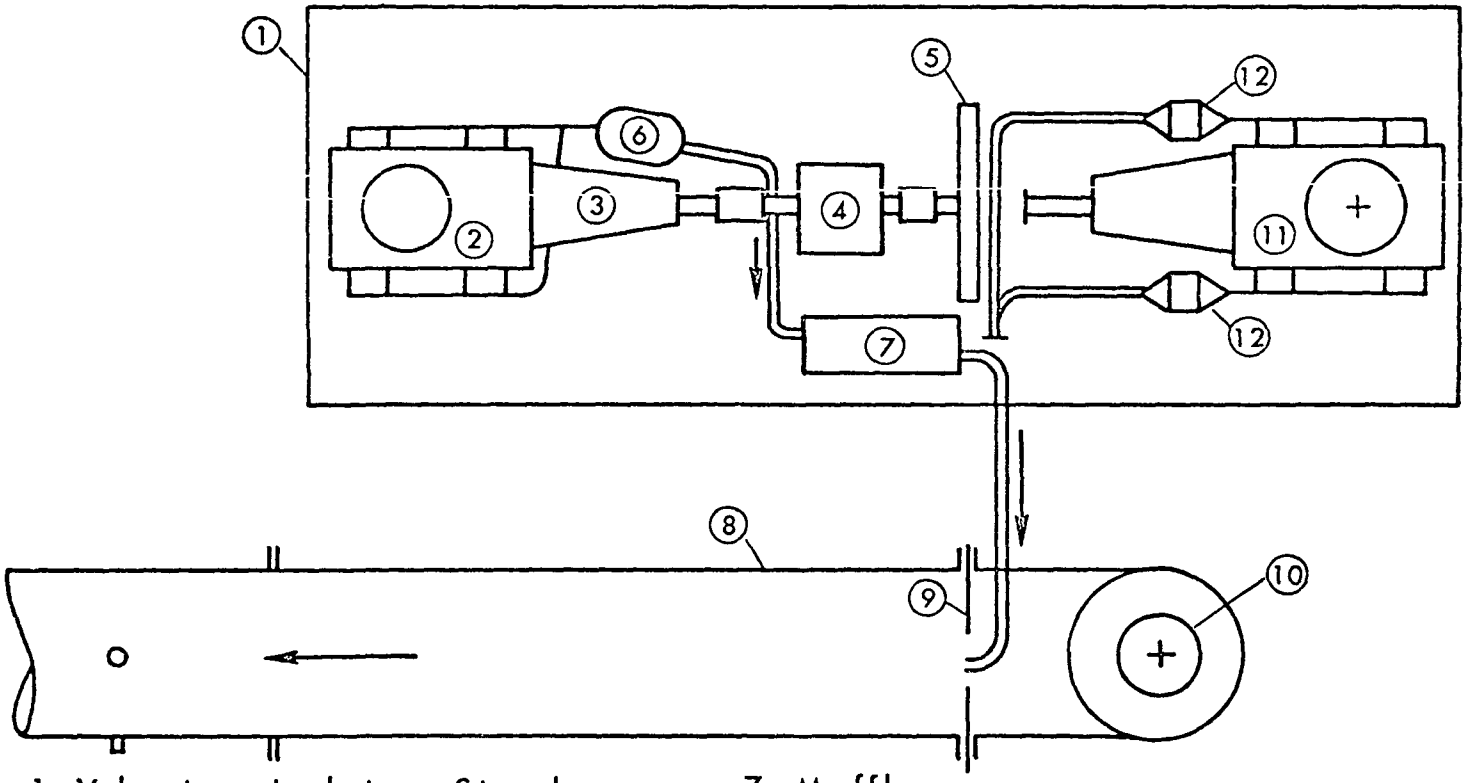
The schematic plan view of the engine-dynamometer unit and dilution tube is presented in Figure 1.

During TAME studies H, I, J and K, the 1973 Chevrolet engine was operated continuously for 7 days using the California Cycle Comprehensive data for comparison of study engine operating conditions is given in Table 2

TAME 'K' was designed to test emissions and bioeffects of a high-sulfur gasoline free of other undesirable substances such as lead. Thiophene was added to produce a sulfur content of 1000 ppm in the control fuel, Indolene Sulfur compounds present in gasoline are mainly in the form of polysulfides and thiophene compounds with an insignificant amount of hydrogen sulfide

It was reported and confirmed by our testing procedures that the addition of oxidation catalysts to the automotive exhaust system causes an increase in the emitted particulate material (consisting mainly of hydrated sulfuric acid droplets) as a result of the oxidation of organic sulfur compounds in gasoline. Recent national averages of the sulfur content are between 210 and 260 ppm for premium gasoline, and between 390 and 440 ppm for regular gasoline The "Indolene" motor fuel used at the ETRL facility had a sulfur content 0.04% by weight or 400 ppm.

DOUBLE ENGINE-DYNAMOMETER UNIT
(PLAN VIEW)



1 Vibration Isolating Stand
2 1973 Chev V-8 350 C I D
3 Turbohydromatic Transmission
4 Dynamometer Absorption Unit
5 Flywheel
6 Catalytic Converter
7 Muffler
8 Dilution Tube
9 Baffle Plate
10 Dilution Air Supply
11 1975 Ford V-8 400 C I D
12 Catalytic Converters

FIGURE 1

	TAME H	TAME I	TAME J	TAME K
Dates	9/10 - 17	10/10 - 17	10/24 - 31	11/14 - 21
Fuel	Ref. Only	Ref. Only	Ref. Only	Ref. + Sulfur
Engine	'73 Chev. w/catalyst	'73 Chev. No catalyst	'73 Chev. w/catalyst	'73 Chev. w/catalyst
Engine Hrs.	62-230	255-425	444-615	675-841
Study Hrs.	168	170	171	166
Eng. Miles	4600	8500	12,300	16,820
Cumm Catalyst Hrs.	244	244	465	632
Catalyst Miles	4880	4880	9300	12,640
Total Fuel (lbs)	1533	1545	1601	1495
Fuel, Lb/Hr.	9.10	9.08	9.40	9.02
Exh. Oxygen (%)	4.9	N.A	4.2	4.7
Air/Fuel Ratio	--	14 4 cycling 12 4 idle	--	--
Oil Consumption, qts.	1-1/8	1/2	1/4	1/4
Dilution Ratio	8.0/1	9.6/1	8.7/1	9.5/1
Dilution Air Flow Average, SCFM	318	305	310	324
Dilution Tube Temp. Average, ^{O}F	106	101	114	101

Table 2. Comparison of Study Engine Operating Conditions

B.3. DESIGN AND SYSTEM PERFORMANCE FOR STUDIES OF CATALYTIC EMISSIONS

J. Burkart and R Hinners

All of the catalyst studies in this report were performed with the 1973 Chevrolet engine, new headpipe, standard muffler and fabricated stainless steel tailpipe New road load data supplied by the EPA Motor Vehicle Emissions Lab were used, they are equivalent to an increase of inertial weight from 3400 lbs (used on 1972 Chev) to 4000 lbs. No attempt was made to adjust idle mixture, as in earlier TAME A through G studies and carburetor "limiters" remained in place. In TAME 'H' the engine was run "as received" except for setting idle speed, dwell and timing. Maintenance performed before TAME I, J and K consisted of oil and filter change, new points, condenser, spark plugs and setting hot idle speed, dwell and timing In addition, before TAME 'K' new spark plug wires were installed

For each of the continuous one week studies shown in Table 2 of the article B-2, approximately 3400 miles were accumulated on the California cycle Separate cumulative engine miles and catalyst miles are reported since the catalyst was removed in TAME 'I' and additional steady speed runs (without animal exposures) were made to characterize emissions The dilution ratio is determined by the ratio of average tailpipe CO_2 to dilute CO_2

Because the variability of tailpipe CO_2 throughout the cycle is small, the problem of obtaining a proportional sample is negligible. Samples for CO_2 detection flow at a constant 1 liter per minute thru a refrigerated cooler, dessicant dryer and paper filter to the Beckman IR Model 315. This instrument, converted for CO_2, is calibrated 15% CO_2 full scale and zeroed

on dilution air, however, because of the cooler some CO_2 loss in the condensate was unavoidable

For all studies a continuous trace at constant sample flow on two-Mosley (2 pen) recorders was made of the following emissions

(1) tailpipe CO

(2) dilute CO

(3) tailpipe THC

(4) tailpipe CO_2

The above, along with "spot" checks of tailpipe oxygen and dilute CO_2, monitored engine and dilution system operation.

TAME schematic Figure 1 shows sampling points throughout the system starting with engine (E), catalytic converter (C) and standard muffler (M). The numbers will be referred to for aerometry sample identification except when exposure chambers are sampled, the chamber number and treatment (I, NI, clean air) is used Average total particulate losses on a percentage basis are also shown starting with 100% at point 5 in the dilution tube An overall loss of 39% occurs by the time the NI chamber is reached. The diagram shows only part of the exposure chambers receiving autoexhaust and there are control chambers which receive filtered air from a separate supply

During the studies, the entire tailpipe volume was mixed with the quantities of air given in Figure 2 and resulted in the dilution tube temperatures shown The dilution air temperature for all studies ranged from 48° to 55°F. Figure 3 depicts tailpipe conditions of exhaust oxygen content and average catalyst temperature (on center line of tailpipe one-inch from catalyst oftlet) At the tailpipe, the seven day trend during the catalyst studies (H, J, K) was oxygen decrease, CO_2 increase and catalyst temperature increase.

Table 1 shows the GM catalyst efficiency when TAME 'I' (w/o catalyst) average emissions are used as the basis for comparison It is noted that the catalyst is more efficient in terms of CO than for HC under the hot cycling condition.

Some initial loss of efficiency may be due to the higher oil consumption during TAME 'H', also by the end of that study #3 plug had "fouled."

Table 1 GM CATALYTIC CONVERTER EFFICIENCY

	H	I	J	K
Average Tailpipe Concentrations* (PPM)				
Carbon Monoxide	56	5376	400	380
Hydrocarbons (as methane)	96	1056	191	171
Total Per cent Reduction Below TAME I				
Carbon Monoxide	99%	X	93%	93%
Hydrocarbons	91%	X	82%	84%

*Calculated from dilute concentrations multiplied by dilution ratio.

Basic specifications for the 1973 Chevrolet engine are shown below in Table 2.

Table 2 Chevrolet Engine System

	1973
Displacement	350 C.I.D.
Compression Ratio	8.5/1
Carburetor Type	Roch. 2GV 1-1/2
Carburetor No.	#7043114
Distributor*	# 1112168
Mech. Adv. Unit	C 4815
Vacuum Adv. Unit	C6020(46914)
Dwell	30°
Initial Timing	8oBTC
Maximum Vacuum Advance	14°
Emission Control Equipment	Air Pump
	Rich tune (A/F ~14.5/1)
	Timed port Vac. Adv.
	EGR 11633 (LF 7040437)

Data for exposure chamber temperature and relative humidity are presented in Figure 4 The temperature profiles appear favorable, however, the relative humidity for chambers receiving exhaust were consistently above 60 per cent relative humidity while the reverse was true for control air chambers

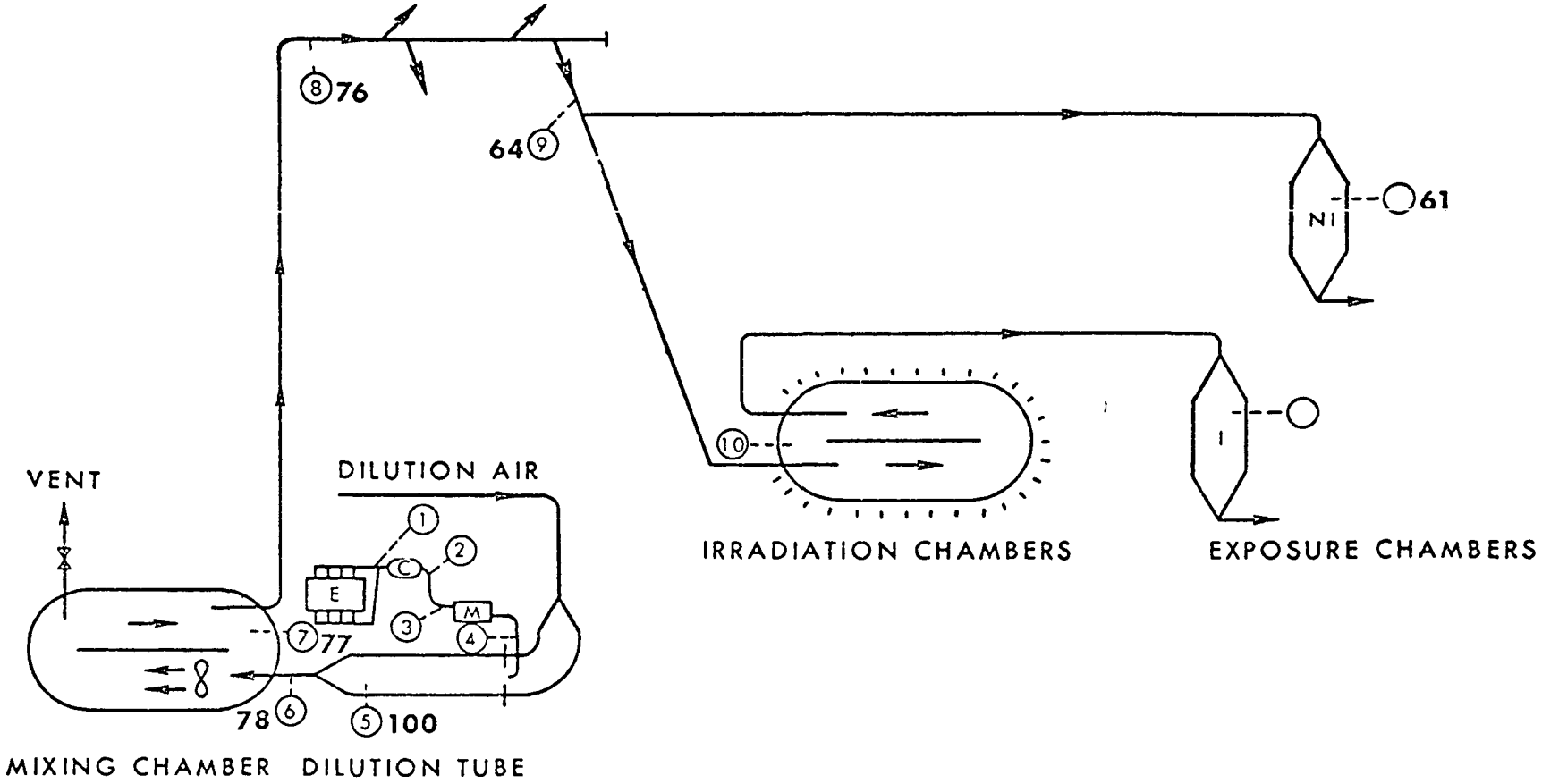


FIGURE 1. TAME SCHEMATIC SHOWING SAMPLING POINTS ... ○

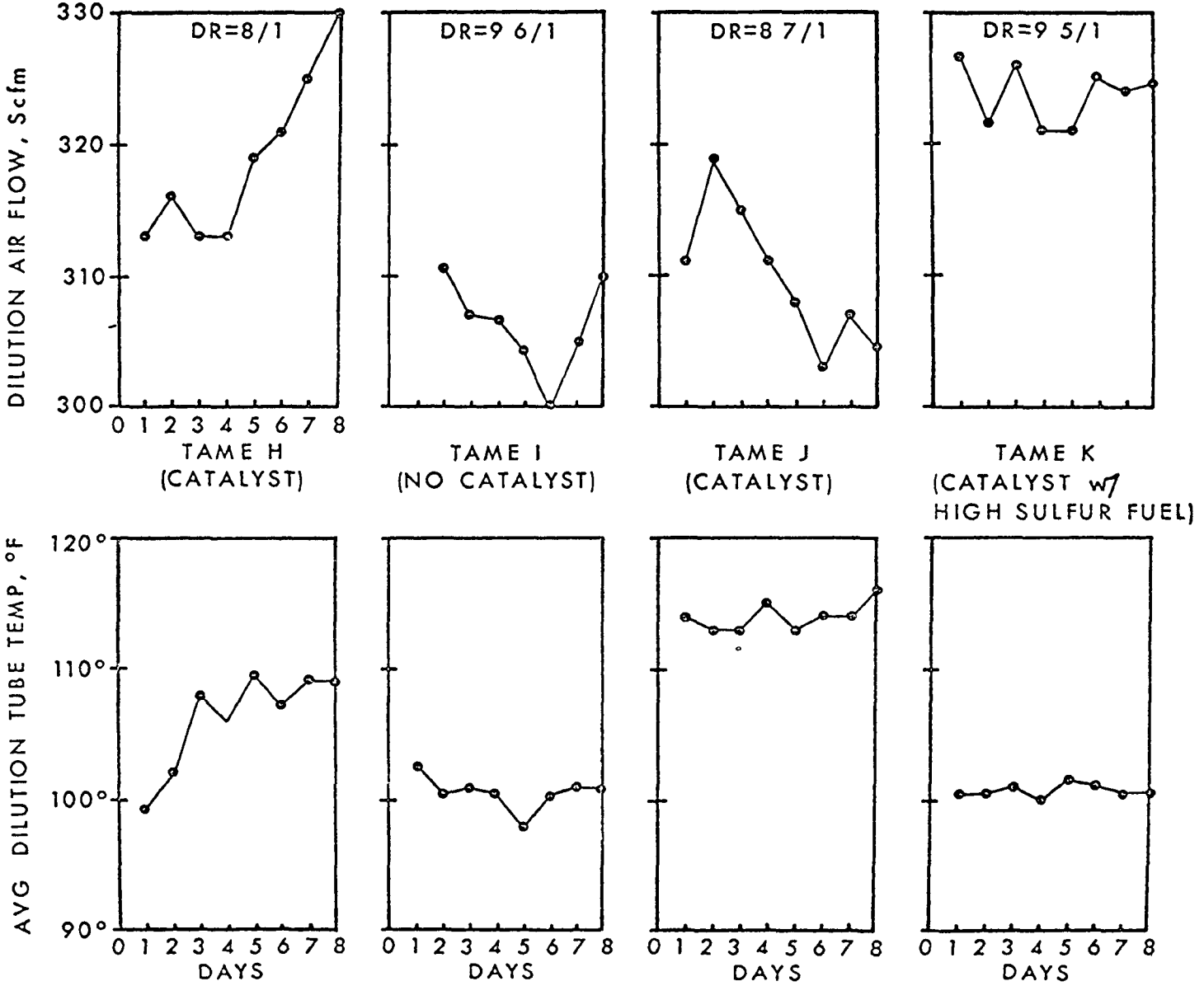


FIGURE 2- DILUTION AIR FLOW AND DILUTION TUBE TEMPERATURE

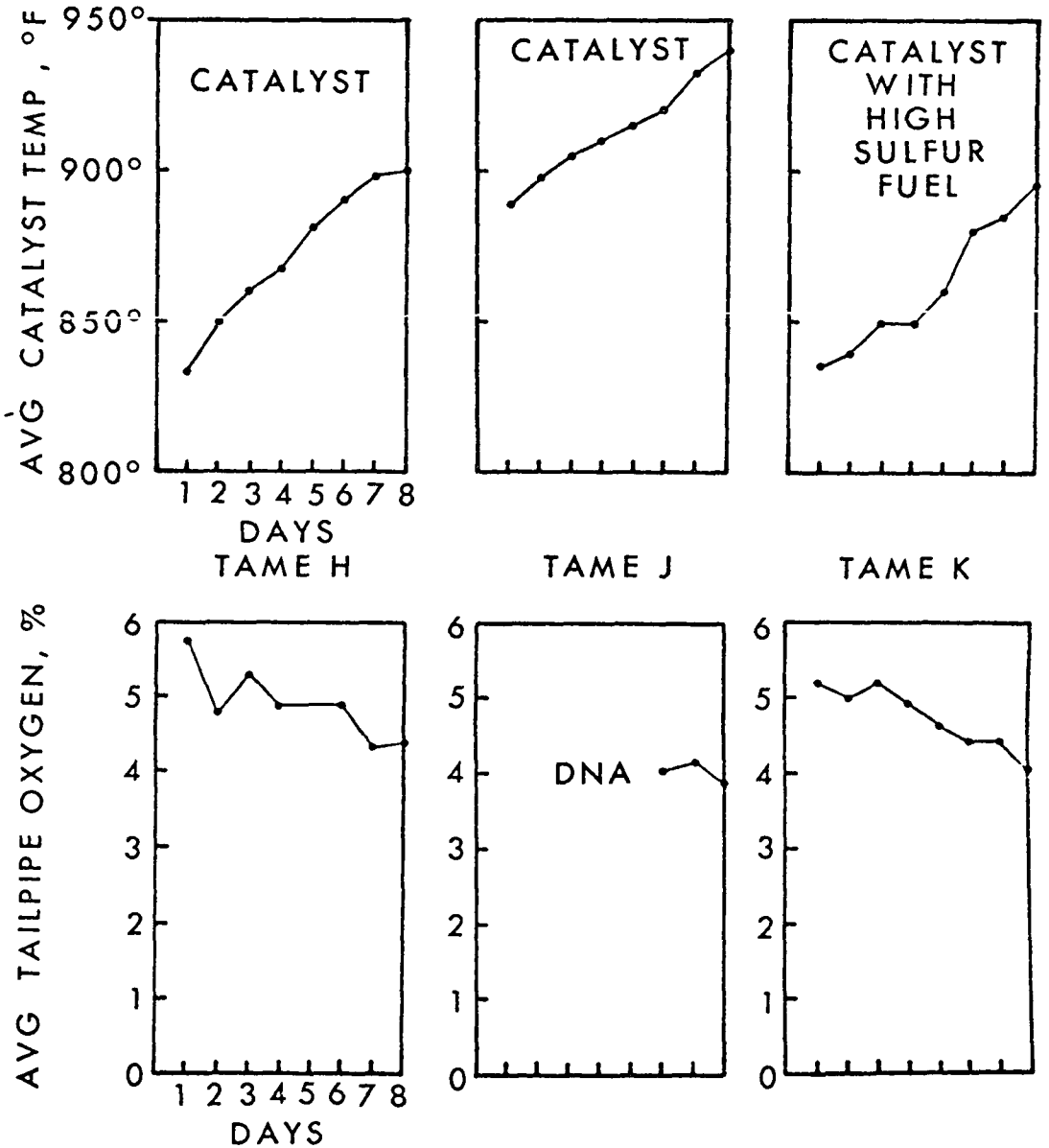


FIGURE 3 TAILPIPE CONDITIONS

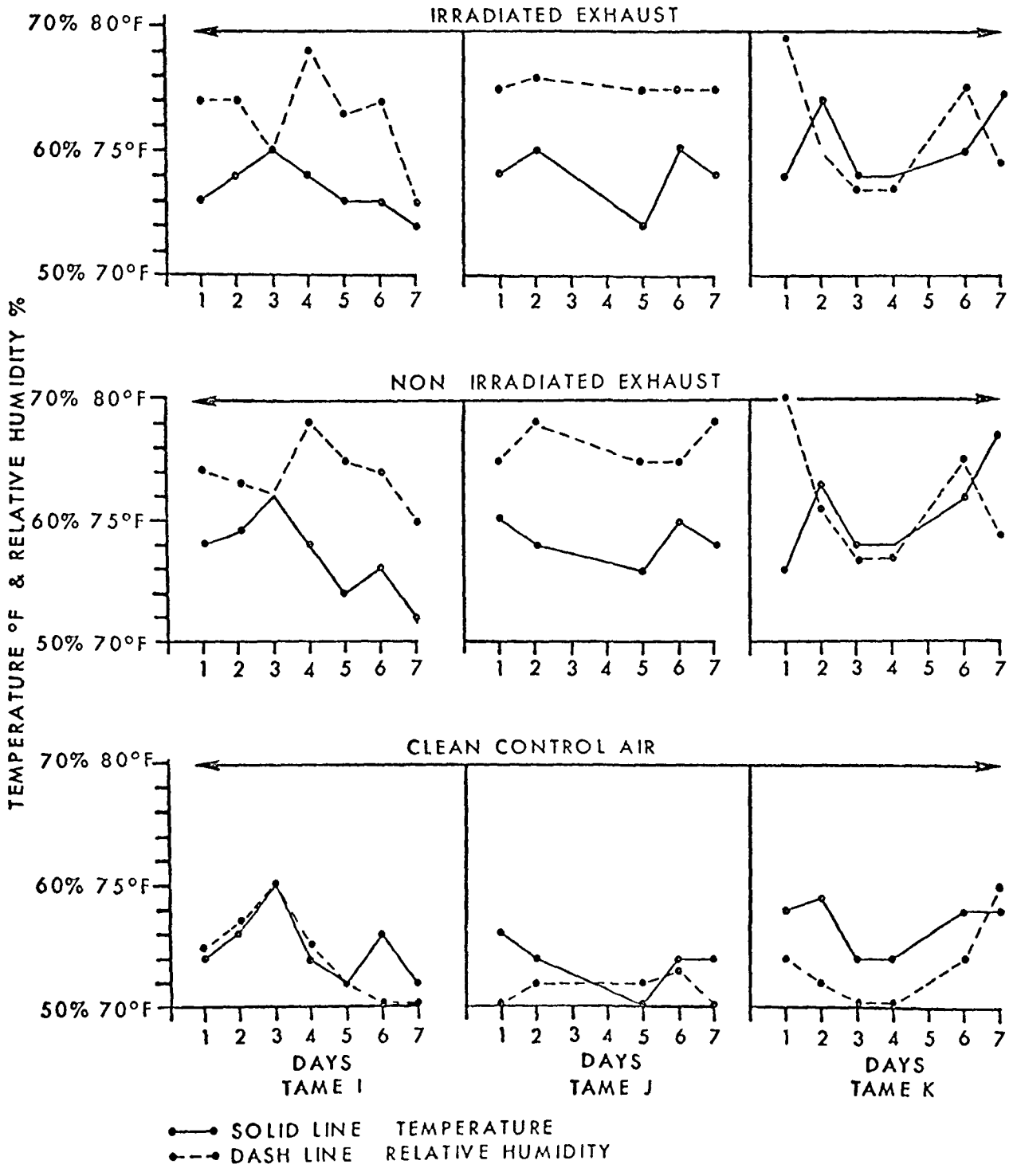


FIGURE 4 EXPOSURE CHAMBER TEMPERATURE & RELATIVE HUMIDITY

B.4. EXHAUST EMISSIONS DURING STEADY SPEED RUNS WITH THE CATALYTIC CONVERTER IN THE EXHAUST SYSTEM

M. Malanchuk, N Barkley, G Contner,
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Introduction

In preparation for studies on the exposure of animals to the exhaust emissions from catalytic-equipped systems, preliminary runs were made with the 350 C I.D. Chevrolet engine operating at constant speeds. Information was sought that would indicate the levels of constitutents different from those of previous runs made under different engine operating conditions The data were needed particularly with reference to sulfur compounds and acidity of the emissions Preparations were made to test for sulfates, sulfur dioxide, sulfuric acid and nitrate components Sample procedures were adapted with such changes as were considered expedient to get quantitative results.

Experimental Procedure

The main effort was directed to the sampling of particulate in order to establish the nature of the anticipated changed character of the particulate.

Since membrane-type filters used to collect aerosol from the catalytic converter system deteriorated from the corrosive action of the sample, quartz fiber material was used and found favorable for such samplings. Not only did the material resist breakdown, but also in the aqueous extraction medium it did not show any resulting changes in the nature of the solution (e.g pH) upon standing for as long as 20-30 hours.

The sample filters were handled in two different ways Every filter was weighed immediately after sampling Some were permitted to stand overnight to equilibrate in the room atmosphere (70-75^{o}F, 40-60% R.H.) until the weight

had stabilized. These final weights were used to calculate the particulate concentrations in the sampled atmospheres Other filters, immediately after they were weighed following the sampling, were placed in a measured volume of distilled, deionized water. Conductance and pH measurements were then made to determine ion concentrations mainly, the acidity of dissolved samples. The aqueous extracts were also used for analysis of particular ion radicals like the sulfate and nitrate groups

Analytical procedures included the barium chloranilate method[1] and also nephelometry for sulfate, the phenol-hypochlorite reaction[2] and ion-specific electrode for ammonia and ammonium compounds, and the hydrazine reaction[3] for nitrate

Bubblers containing distilled water or a weak acid solution were used to scrub sampled atmospheres for nitrate - and for ammonium-producing components. A sampling train of bubblers similar to that used in stack sampling[1] was arranged for separation of SO_2 from SO_3 in atmospheres drawn from the exhaust pipe before and after the catalytic converter and from the animal exposure chambers. The first bubbler in the train, containing isopropanol, collected SO_3 The succeeding two bubblers, containing hydrogen peroxide, collected the SO_2 and converted it to the sulfate form. In some cases, the follow-up bubblers contained tetrachlormercurate instead of peroxide to trap the SO_2 for analysis by the West-Gaeke method [4]

None of the animal exposure chambers from which the atmosphere was sampled contained any animals. These chambers previously were hosed down thoroughly with hot water to minimize, if not eliminate, sources of contaminating deposits.

Results

The effects of different engine speeds and of different concentrations of sulfur in the fuel are seen in the concentration values of exhaust emission components in Table I.

Values of gaseous components are listed first - carbon monoxide (CO), total hydrocarbons, as methane (THC), nitrogen oxides (NO_x) with a breakdown into nitric oxide (NO) and nitrogen dioxide (NO_2), the aliphatic hydrocarbons of the C_4-C_5 grofp examined, olefins of the C_2-C_4 group, and acetylene Values of particulate material are listed as total particulate and the sulfate and nitrate concentrations in that particulate

The first three column groups of concentration values were obtained from the operation of the engine with the base fuel, Indolene gasoline, and the use of the catalytic converter unit in the immediate exhaust system. The last two column groups show the concentrations obtained when the Indolene gas was "spiked" with an organic sulfur compound to double the concentration of sulfur in the fuel, in one case, the catalytic converter unit was retained in the exhaust system, in the second case, the unit was removed before the run was started

The samples were mainly collected from the animal exposure chambers receiving the diluted exhaust emissions that had been exposed to the irradiation lights, I, and those chambers receiving diluted emissions not treated to the irradiation effects, N-I. Some samples were collected from the exhaust system in the immediate range of the engine, viz , the particulate samples identified as diluted exhaust (Dil'd Exh)

In order to relate the values from the higher engine speeds and the runs with higher gasoline sulfur to the "base" 15 mph with its 7 5/1 dilution, the actual values of those runs have been adjusted to equivalent values for a 7.5/1 dilution which are given in brackets. Thus, for total particulate weight in the diluted exhaust, it is seen that the significantly different values of 6 10 mg/M^3 at 30 mph and of 8.40 mg/M^3 at 50 mph become nearly the same value as the 5 03 mg/M^3 at 15 mph when the dilution adjustments are made for the 6 2/1 and 4.9/1 at 30 mph and 50 mph, respectively.

Since the data applies to only single runs at each of the five different sets of conditions (except the "base" 15 mph with regular Indolene fuel, there were two runs for which the average values were calculated), the information must be considered as tentative Duplicate runs will be made to establish reproducibility and to confirm the results presented

Nevertheless, large differences in the results between runs were seen which may be acceptable for what they indicate. The large increase in the nitrogen oxides at the highest speed of 50 mph was to be expected (see columns 1-3 of Table 1). There was a concomitant decrease in hydrocarbons. Particulate levels remained about the same with the changes in engine speed Apparently particulate sulfate concentrations also remained the same at different speeds, however, they seem to make up most of the total bulk of the particulate Nitrate was present at much loser concentrations.

With the high sulfur (2 x) Indolene gas as fuel, the carbon monoxide and hydrocarbons (total and individual) levels remained the same (column 4). The big difference was seen in the total particulate and sulfate contents, about 1-1/2 - 2 times as much.

The engine operating with the high-sulfur fuel but without the catalytic converter had, as expected, much higher levels of carbon monoxide and hydrocarbons The nitrogen oxide levels were at similar concentrations as those for the 15 mph with regular Indolene fuel. The particulate levels, column 5, were only 1/3 to 1/4 of those for the catalytic converter, column 4. Sulfate in the particulate was almost negligible by comparison.

Measurements of acidity of the particulate from the catalytic-equipped exhaust emissions was, without exception, high enough to allow for all the sulfate to be considered present as sulfuric acid That is to say, that the hydrogen ion concentration as ($2H^+$) that was measured with a pH meter was greater than the sulfate (SO_4-) concentration, sometimes by a factor as high as three times. Such high acidity was found unaccountable on the basis of the anionic components measured and of the total particulate determined, so that additional study is needed to resolve that phenomenon. An indication of relative acidity levels with different operating conditions is shown in Table 2. The particulate, cclumn 5 of Table 1, in the no converter condition, on the other hand, was almost completely neutral as measured by pH meter on its aqueous extract.

Measures of sulfur components, SO_x, in the undiluted exhaust before and after the catalytic converter showed 50-90 per cent decrease of sulfur across the converter That is to say, that there is a considerable hold-up of sulfate in the catalyst bed itself

TABLE 1. COMPARISON OF EXHAUST EMISSIONS, STEADY SPEED RUNS

		Regular Indolene Gasoline With Catalytic Converter					High-Sulfur Indolene w/catalytic	w/o catalytic
		15 mph	30 mph		50 mph		15 mph	15 mph
Exhaust Dilution Ratio		7.5/1	6 2/1	(7.5/1)	4.9/1	(7 5/1)	8.1/1 (7.5/1)	8/0/1 (7.5/1)
CO, ppm·								
Exp. Ch	N-I	7	8	(7)	10	(7)	7 (8)	491 (522)
	I	7	8	(7)	10	(7)	7 (8)	
THC, ppm								
Exp. Ch:	N-I	9	9	(8)	4	(3)	8 (9)	93 (99)
	I	8	9	(8)	4	(3)	8 (9)	
NO_x, ppm								
Exp. Ch:	N-I	20.0	28.4	(23.5)	102.0	(66.6)		21.1 (22.5)
	I	19.4	31.8	(26.3)	104.8	(68.5)		20.3 (21.6)
NO, ppm								
Exp. Ch:	N-I	14.7	21.2	(17 5)	71.9	(46.9)		13.3 (14.2)
	I	13.2	21.8	(18 0)	63.6	(41.6)		9.6 (10.2)
NO_2, ppm								
Exp. Ch:	N-i	5.3	7 2	(6 0)	30.1	(19.7)		7.8 (8.3)
	I	6.2	10.0	(8.3)	41.2	(26.9)		10.7 (11.4)
Aliphatics ppm								
C_4-C_5 Exp. Ch:	N-I	0 107	0.113	(0 093)	0.031	(0 020)	0.109 (0 118)	
	I	0 108	0.113	(0.093)	0.026	(0.017)	0.115 (0.124)	
Olefins ppm								
C_2-C_4 Exp. Ch:	N-I	0 450	0.465	(0.385)	0.204	(0.133)	0.433 (0.467)	
	I	0.426	0.456	(0 377)	0.195	(0 127)	0.447 (0.483)	
Acetylene ppm								
Exp. Ch:	N-I	0.025	0.018	(0 015)	0.012	(0.008)	0.018 (0.020)	
	I	0.024	0 020	(0 017)			0.028 (0 030)	
Particulate, mg/M^3 -								
Dil'd Exh		5.03	6 10	(5 04)	8.40	(5.49)	6.46 (6.97)	1.92 (2.05)
Exp Ch	N-I	2.24	5.00	(4.14)	6.25	(4.08)	7.63 (8.24)	2 50 (2.66)
	I	1.98	4 72	(3 90)	5 71	(3.73)	5.79 (6 25)	2 33 (2 48)
Sulfate, mg/M^3-Dil'd Exh.		4.33	6 18	(5 11)	6.93	(4.53)	13.16 (14.20)	0 54 (0 58)
Exp. Ch	N-I	4.80	5 00	(4 14)	4.90	(3.20)	11.86 (12 80)	0 49 (0 52)
	I	3.01	3.94	(3.26)	2 72	(1.78)	10.65 (11.50)	0 49 (0.52,
Nitrate, mg/m^3-Dil'd Exh.		0.32	---	(---)	0.47	(0.31)	0.36 (0.39)	0 28 (0.30)
Exp. Ch	N-I	0.01	---	(---)	0.00	(0.00)	0.01 (0.01)	0.04 (0.04)
	I	0.01	---	(---)	0.31	(0.20)	0.01 (0.01)	0.17 (0.18)

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B.5. EXHAUST EMISSIONS FROM CATALYST-EQUIPPED ENGINES

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Introduction

Some early studies in the automobile industry have indicated that oxidation-type catalysts in auto exhaust systems generated high levels of sulfuric acid aerosol, as much as 0.1 gram of the acid per vehicle mile It was hypothesized that the engine combustion process converted organic sulfur compounds in the gasoline into sulfur dioxide, and that the dioxide was oxidized by the catalyst to sulfur trioxide which reacted with water vapor in the exhaust to produce sulfuric acid droplets.

Therefore, unlike the constant engine speed runs as described in the previous article, cycling speed runs more nearly simulating automobile operation in the streets were used for the animal exposure studies Measurements of exhaust emission components were made to determine the levels of such toxic components to which the animals in the studies were exposed The effective changes in exhaust composition were determined when the catalytic converter unit was added to the exhaust system, and when high-sulfur fuel was substituted for the reference Indolene gasoline

Emission components present in relatively high concentrations were monitored in much the same way as in previous runs

Sampling and Analytical Procedures

The instrumentation and methods used for key components are summarized in Table I Atomic absorption spectrophotometry was used for trace metal determinations in particulate.

Table I. 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)	Chemiluminescence spec , 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		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"	Chemiluminescence spec.			

*EPM - Exhaust or primary exhaust air mixture, EC - exposure chamber

Particulate samples were collected on pure quartz fiber filters after early membrane filters deteriorated from exposure to the high reactivity of the collected sample of catalyst-treated emissions.

Bubbler and impinger samples of the atmospheres were used for collecting ammonia- and sulfur-based gases.

Results

Table II lists the concentrations of various engine exhaust components measured during the series of studies of the catalytic converter system Individual hydrocarbons measured by gas chromatography are shown in Table III, the aromatic compounds were not measured after it was discovered that those concentrations were so low in the catalyst-equipped system atmosphere as to be near or below detection level.

TAME J and TAME H (see Hinners report) were run under the same engine operating conditions, i e they were "duplicate" runs However, reference to the data of Table I shows considerable differences in values between the two runs. When TAME H was performed, the engine was probably not fully broken in and the catalyst was quite new, the piping system for conducting the emissions to the exposure chambers probably had not as yet attained equilibrium conditions of surface exposure characteristics (i e. mainly deposition of particulate and adsorption of organic vapors) for the new engine system. TAME J, run at a later date, when a more stable system should have been established, was considered to have the more accurate atmospheric component values than TAME H. Therefore,the concentration values from TAME J were used for comparison to those from TAME I for the purpose of evaluating the effect of the catalytic converter upon the make-up of the

auto exhaust emissions reaching the animal exposure chambers. That comparison is emphasized by the large percentage reduction values of several atmospheric components due to the use of the Pt-Pd coated, pelleted catalyst converter (Table IV), and also seen in the greatly reduced concentrations of individual hydrocarbons, TABLE III

Since the dilution of the raw exhaust with clean air was not as great in TAME J (8.7/1 as in TAME I (9.6/1), the reduction values listed in the third column of Table IV were adjusted by a factor appropriate to the differences in dilution values, about ten per cent of the TAME J values, to obtain the more accurate "normalized" values listed in the fourth colum

A barely detectible concentration of platinum, 0.029 $\mu g/m^3$, was measured in the diluted emissions of the animal exposure chamber

A barely detectible concentration of platinum, 0 029 $\mu g/m^3$, was measured in the diluted emissions of the animal exposure chamber - this result, of course, for a system using a catalytic converter unit that was quite new and that was shown to be adsorbing a large proportion of the sulfur gases in the exhaust gases

On the basis of an average flow of 1 M^3/min of raw exhaust produced at a calculated average speed of 22 mph on the engine dynamometer, it was estimated that the 0 029 μg Pt/m^3 represented a loss of nearly 0 62 μg/Pt/mi

If it is estimated that there is 0 04 troy ounce of the noble metal in the catalytic unit (1 244 gm, i e) then 0.5 x 10^{-4} per cent of the platinum was lost per mile Such a loss over 50,000 miles of operation would mean a total loss of 2 5 per cent of the platinum originally present.

Conclusions

The incorporation of the oxidation-type catalyst in the exhaust system resulted in drastic changes in the exhaust emissions

a. The effectiveness of the catalyst was revealed in the large reduction of carbon monoxide, total hydrocarbons and various individual organic compounds (such as acetylene)

b. An almost total elimination of aldehydes was achieved.

c. In TAME I (without catalyst), the high value of particulate in the irradiated atmosphere along with the low value of nitric oxide, NO, and the measurpd presence of ozone indicated that much more photochemical reaction of hydrocarbons occurred than in TAME J (with catalyst) That activity was greater in the case of the olefins than in the acetylene, and negligible for the aliphatics.

d. Gross evidence (color, weight stability) of the particulate in TAME I, indicated that the nature of the sample was mainly organic The particulate in TAME J, on the other hand, was strongly acidic, liquid in nature and lost significant weight upon standing Analysis showed sulfate to be the primary constituent Such facts suggested the presence of sulfuric acid as the major component in TAME J particulate.

More detailed reports of sulfate and acid measurements are given in the related articles, "Sulfate Emissions from Use of High-Sulfur Fuel, TAME-K" and "Exhaust Emissions During Steady Speed Runs with the Catalytic Converter in the Exhaust System."

TABLE II.

ENGINE EXHAUST EMISSION VALUES FOR CATALYTIC CONVERTER SYSTEM STUDY

			TAME-H 10-16 Sept. '73	TAME-I 10-16 Oct. '73	TAME-J 24-30 Oct. '73	TAME-K 14-21 Nov. '73
Exhaust Dilution Ratio			8/1	9.6/1	8.7/1	9.5/1
CO, ppm.						
	Exp. Ch	N-I	7	651	46	40
		I	8	559	41	38
THC, ppm						
	Exp. Ch.	N-I	12	110	22	18
		I	13	95	22	18
NO_x, ppm.						
	Exp. Ch.	N-I	11.0	11.9	12.9	12.6
		I	11.0	5.1	12.6	11.2
NO, ppm.	Exp. Ch.	N-I	8 5	6.7	11.1	10.8
		I	8.0	00.5	9.6	9.7
NO_2, ppm	Exp Ch·	N-I	2.5	5.2	1.8	1.8
		I	3.0	4.6	3.0	1.5
Aldehydes ppm						
	Exp. Ch	N-I	-----	10.20	0.08	0.18
		I	-----	14.62	0.10	0.11
Methane, ppm						
	Exp. Ch	N-I	-----			6.53
		I	-----			6.13
Aliphatics ppm C_4-C_5						
	Exp. Ch	N-I	-----	1.30	0.61	0.44
		I	-----	1.32	0.58	0.39
Olefins ppm C_2-C_4						
	Exp. Ch.	N-I	-----	13.24	0.89	0.91
		I	-----	9.23	0.79	0.82
Acetylene,	Exp. Ch	N-I	-----	3.28	0.03	0 04
		1	-----	3.06	0.03	0.04
Ozone, ppm						
	Exp. Ch	N-I	-----	0.0	-----	-----
		I	-----	0.4	-----	-----
Particulate, mg/M^3						
Dil'd. Exh.				1.08	1.02	5.97
	Exp. Ch	N-I	1.86	0.69	0.96	6 53
		I	2.05	3.19	1.09	5.85

Table III.

Gas Chromatographic Measurements of Hydrocarbons, ppm

Component	TAME-I		TAME-J		TAME-K	
	N-I	I	N-I	I	N-I	I
n-Butane	0.61	0.61	0.30	0.29	0.21	0.18
i-Butane	0.08	0.08	0.05	0.05	0.03	0.03
n-Pentane	0.20	0.23	0.09	0 09	0.05	0.05
i-Pentane	0.41	0.40	0.17	0.15	0.15	0.13
Acetylene	3.28	3.06	0.03	0.03	0.04	0.04
Ethylene	6.85	5.10	0.82	0.72	0.81	0.74
Propylene	1.81	0.71	0.04	0.04	0 06	0.04
Butane-1	0 26	0.08	Bld	Bld	-	-
Isobutylene	0.63	0.20	Bld	Bld	-	-
1,3-Butadiene	0.41	0.08	Bld	Bld	-	-
Methane					6 53	6.13

Bld - Below level of detection

TABLE IV.

COMPARISON OF EXHAUST EMISSIONS, TAME-I AND -J

		TAME-I 10-16 Oct. '73	TAME-J 24-30 Oct. '73	% Conc'n Reduction I → J	Normalized % Reduction Value
Exhaust Dilution Ratio		9.6/1	8.7/1		
CO, ppm					
Exp. Ch:	N-I	551	46	91.7	92.4
	I	559	41	92.7	93.3
THC,PPM					
Exp. Ch	N-I	110	22	80.0	81.9
	I	95	22	76.9	79.0
NO_x,ppm					
Exp. Ch	N-I	11.9	12.9		
	I	5.1	12.6		
NO, ppm Exp. Ch	N-I	6 7	11.1		
	I	0.5	9.6		
NO_2,ppm Exp. Ch	N-I	5 2	1.8		
	I	4.6	3.0		
Aldehydes,ppm					
Exp. Ch	N-I	10.20	0 08	99.9	99.9
	I	14 62	0 10	99.9	99 9
Aliphatics, ppm· C_4-C_5					
Exp. Ch	N-I	1.30	0.61	53.1	57.7
	I	1.32	0.58	56.1	60.0
Olefins, ppm C_2-C_4					
Exp. Ch	N-I	13 24	0 89	93.3	93 9
	I	9.23	0 79	91.4	92.2
Acetylene, ppm					
Exp. Ch	N-I	3.28	0 03	99.1	99 2
	I	3.06	0.03	99.0	99 1
Ozone, ppm					
Exp. Ch	N-I	0.0			
	I	0.4			
Particulate, mg/M^3					
Dil'd. Exh.		1 08	1 02		
Exp Ch	N-I	0.69	0.96		
	I	3.19	1 09		

B.6. SULFATE EMISSIONS FROM USE OF HIGH-SULFUR FUEL, TAME-K

M. Malanchuk, N. Barkley, G. Contner and M. Richards

Introduction

To supplement the data on exhaust emissions from catalyst-equipped systems studied in which regular Indolene fuel was used, in study TAME-K a high-sulfur content gasoline was substituted In that study a quantity of thiophene was added to the reference Indolene fuel to provide a sulfur level twice as great, 0 10 percent, as that normally present. A more detailed analysis of the particulate was made in order to establish the concentration of sulfate and of the expected high acidity.

Experimental Procedure

The high acidity of the aerosol produced in the exhaust emissions from oxidative catalytic equipped systems was indicated in preliminary runs of the 350 C I D Chevrolet engine. Aerosol collected from an exposure chamber onto an electrostatic precipitator plate was a water-white liquid and proved to be very acid by pH-paper test. Also, membrane-type filters used to sample the exposure chamber atmospheres remained an undiscolored white and deteriorated upon standing several hours, sometimes to the point of breaking into fragments.

Therefore, quartz fiber filter material (Pallflex type 2500-QAO) was used to collect aerosol samples at all the sampling points of the piping system. Every filter was weighed immediately after sampling Some were weighed again after several hours or overnight standing to allow for equilibration with the room atmosphere and stabilization of the sample weight. Others that were used for aerosol acidity measurements were (total

or portions of) then placed without delay after the early weighing into a beaker of a specified quantity of distilled, deionized water At least 30 minutes was allowed for water extraction of the sample before the initial measurements of conductance and of pH were made Final measurements of ion concentration were made 16-40 hours later.

The aqueous extracts were subsequently used for determination of sulfate (SO_4^-), of ammonium (NH_4^+), and of nitrate (NO_3^-) Sulfate was analyzed by the barium chloranilate method[1], ammonium by phenolhypochlorite reaction,[2] and nitrate by hydrazine reduction.[3]

Alternate analytical methods were used in some cases to confirm the concentrations determined. A nephelometric method was adapted to sulfate measurement, and ion specific electrode applied to ammonium measurement. Those methods had limited use for the present group of samples because of sensitivity and reproducibility requirements.

Gas samples for nitrate - and for ammonium - producing components in the atmospheres were collected by absorption into distilled water or into a weak acid solution For separation of SO_2 from SO_3, the procedure[1], of drawing the gas through a bubbler containing isopropanol and then through hydrogen peroxide, H_2O_2, solution or through tetrachlormercurate, TCM, contained in two follow-up bubblers was applied The ammonium and the nitrate product concentrations were determined by the same methods cited for the aerosol analysis. The SO_3 sulfate in the first bubbler and the SO_2 sulfate in the peroxide bubblers of the three bubbler-train were analyzed by the chlorarilate method The SO_2 in the TCM absorption liquid was analyzed by the West-Gaeke method.[4]

The animal (population) occupancy of an exposure chamber was noted by the number of cages and activity wheels A cage might have three adult rats, or a litter of recently-born rats with their mother, or a group of four hamsters An activity wheel was associated with a single mouse. A record of the animal occupancy was kept for comparison with levels of ammonium and acidity of the particulate in the atmosphere of the exposure chamber.

Results

A condensation of the analytical values for several ionic components is given in Table 1. Gas and particulate values are listed for the diluted atmosphere sampled immediately after the exhaust pipe and for the atmospheres in each of several exposure chambers.

Total particulate for TAME-K is shown in the fourth column of values of Table 1 of the article, "Exhaust Emissions from Catalyst-Equipped Engines." The sulfate value for the diluted exhaust pipe emissions, 46.5 μ mol/M^3, in Table 1 of this article represents almost 75 per cent of the weight of the total particulate, 5.97 mg/M^3. Considering the very highly acid nature of the aerosol (particulate), one must assume that the sulfate is most likely, totally, sulfuric acid. On the basis of an average emissions volume generated by the engine of 1 M^3/min and an average of 22 m.p.h equivalent road speed for the engine operation (California cycle), the total particulate value of the diluted exhaust was calculated as approximately 0.16 gm/mile.

Actually, the acidity is so high that at the present time it is unaccountable in terms of the amount of aerosol reported Further work is required to explain this phenomenon The point should be made that those

filter samples which were not used in the extraction scheme did lose, after standing overnight, as much as 50 per cent of their weight sometimes. It was this final weight upon which the total particulate calculations were made.

Although values are given for (NH_4^+) and (NO_3^-) in the gas phase, Table 1, it is assumed that the analytical procedures are accounting mainly for ammonia (NH_3) and for the contribution of nitrogen dioxide (NO_2) to these ion concentration values. The SO_4^- in most cases is probably a fine mist of acid aerosol in the submicron size range of 0 1 micron or less.

The particulate analyses of Table 1 show that the aerosol in the diluted exhaust pipe emissions (first column) is a highly acid sulfate. The aerosol in exposure chamber #15 (second column) is non-acidic and contains ammonium ($2NH_4^+$) nearly quantitative to the sulfate (SO_4^-) measured. It is not unreasonable to think, therefore, in terms of the acid such as sulfuric acid H_2SO_4 or of the salts such as ammonium sulfate $(NH_4)_2SO_4$ The amount of sulfate measured in the exposure chambers themselves represented an average of at least 15 per cent of the sulfur present in the fuel.

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Table 1

TAME K - Atmospheric Component Concentrations, $\mu mol/M^3$

		Exh.Pipe Emissions Diluted	Exp.Ch#15 Irrad. 15-10 cages	Exp Ch #18 Non-Irrad. 2-0 cages	Exp.Ch.#6 Non-Irr. No Animals	Exp.Ch.#22 Irrad. 4-2 cages + 6 wheels	Exp.Ch.#24 Non-Irrad 2 cages + 6 wheels	Exp.Ch.#23 Non-Irrad. 12 cages	Exp.Ch.#17 Irrad. 6 wheels
GAS	(NH_4^+)		0 4	0.1		0.22	0.33		
	(NO_3^-)		7.3	2.4		8.70	6.25		
	($SO_4^=$)		15 0	28 0					
	(SO_2)		2.0	0					
PARTICULATE	($2NH_4^+$)	1.6	38.6	4.6	1.1	7.9	21.9	41.2	11.4
	(NO_3^-)	0.2	1.2	0	0	0	0.2	0	0.6
	($SO_4^=$)	46.5	32.8	34.3	35.0	34.2	31.3	25.5	31.3
	($2H^+$)	170.6	0.2	122 9	109.9	54.7	-	0	97.0

B.7. COMPARISON OF THE BIOLOGICAL EFFECTS OF ACUTE EXPOSURE TO WHOLE EXHAUST EMISSIONS FROM AN AUTOMOBILE ENGINE EQUIPPED WITH AND WITHOUT A NOBLE METAL CATALYTIC CONVERTER

D. Hysell, W Moore, L Garner, D. Crehil, S. Neiheisel,
H. Ball, Y Yang and J Stara

This study was undertaken to compare the biological effects which might result from an acute exposure to catalyst treated exhaust (TAME J) vs. nontreated exhaust (TAME I). The exposure facility and emission chemistry are discussed in detail in other reports (See Hinners, et al. and Malanchuk et al). The experimental animals included young adult male rats and hamsters, lactating female rats and their suckling young Biologic parameters studied included mortality, body weight, hematology and blood chemistry, and pathology.

Body Weight and Mortality

The animals in this portion of the study were exposed to treatment atmospheres 24 hrs/day for 7 days There were 7 treatment groups consisting of clean air (CA), irradiated exhaust (IH), and non-irradiated exhaust (RH) for both TAME I and J and a carbon monoxide control (CO) with CO levels comparable to those encountered in the emissions produced in TAME I Each treatment group consisted of 10 lactating female rats and their 2-week old litters (10 suckling rats/litter) which were weighed at the beginning and end of the study Each treatment group was examined several times daily for possible mortality

The results indicate that the IH and RH treatment groups in TAME I showed the most severe changes in weight of lactating female (Fig. 1) and infant rats (Fig. 2), ard survival rate of infant rats (Fig. 3) There appeared to be minimal changes in infant survival in the high CO group (Fig 3). There

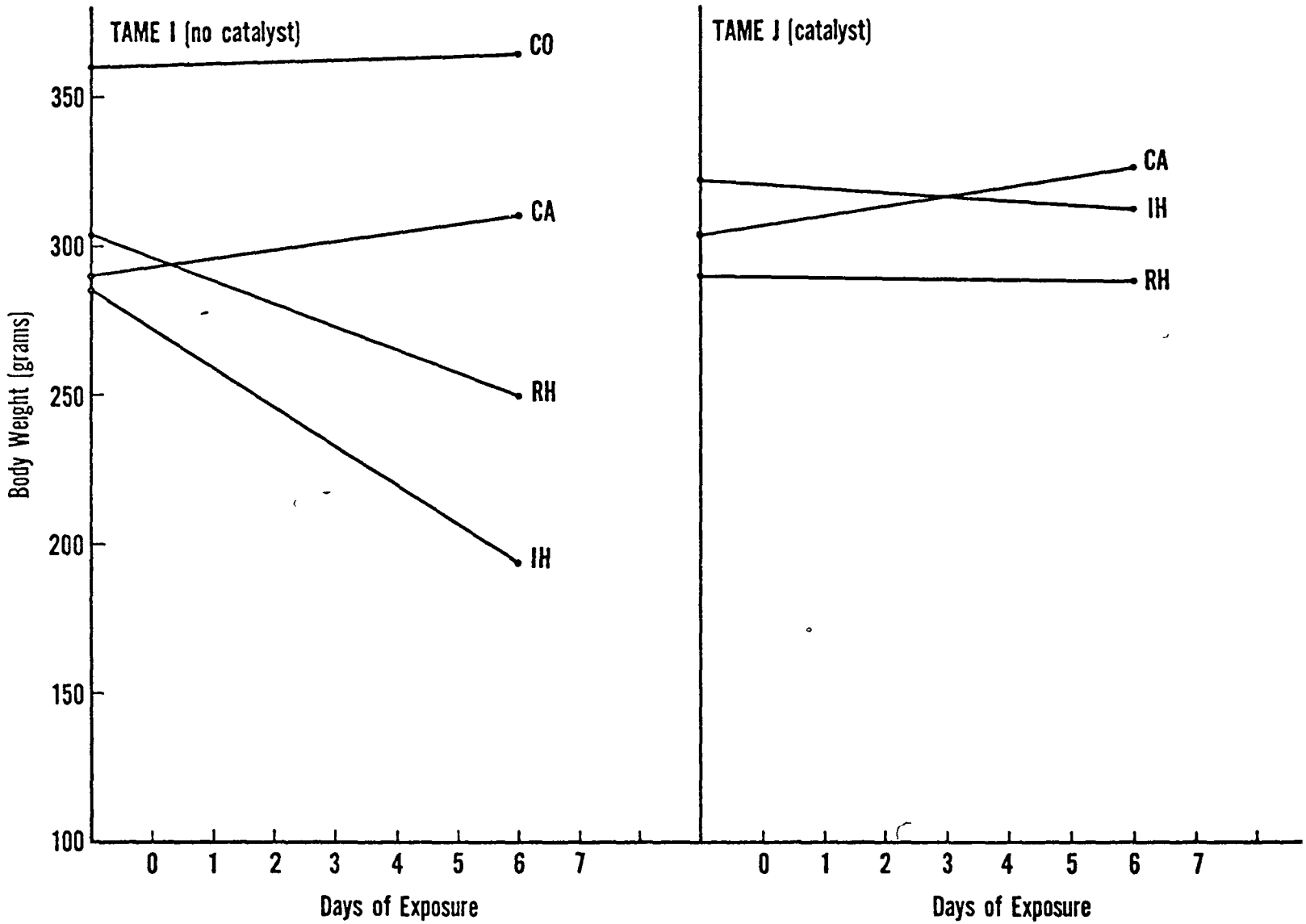

FIGURE 1. BODY WEIGHT OF LACTATING FEMALE RATS

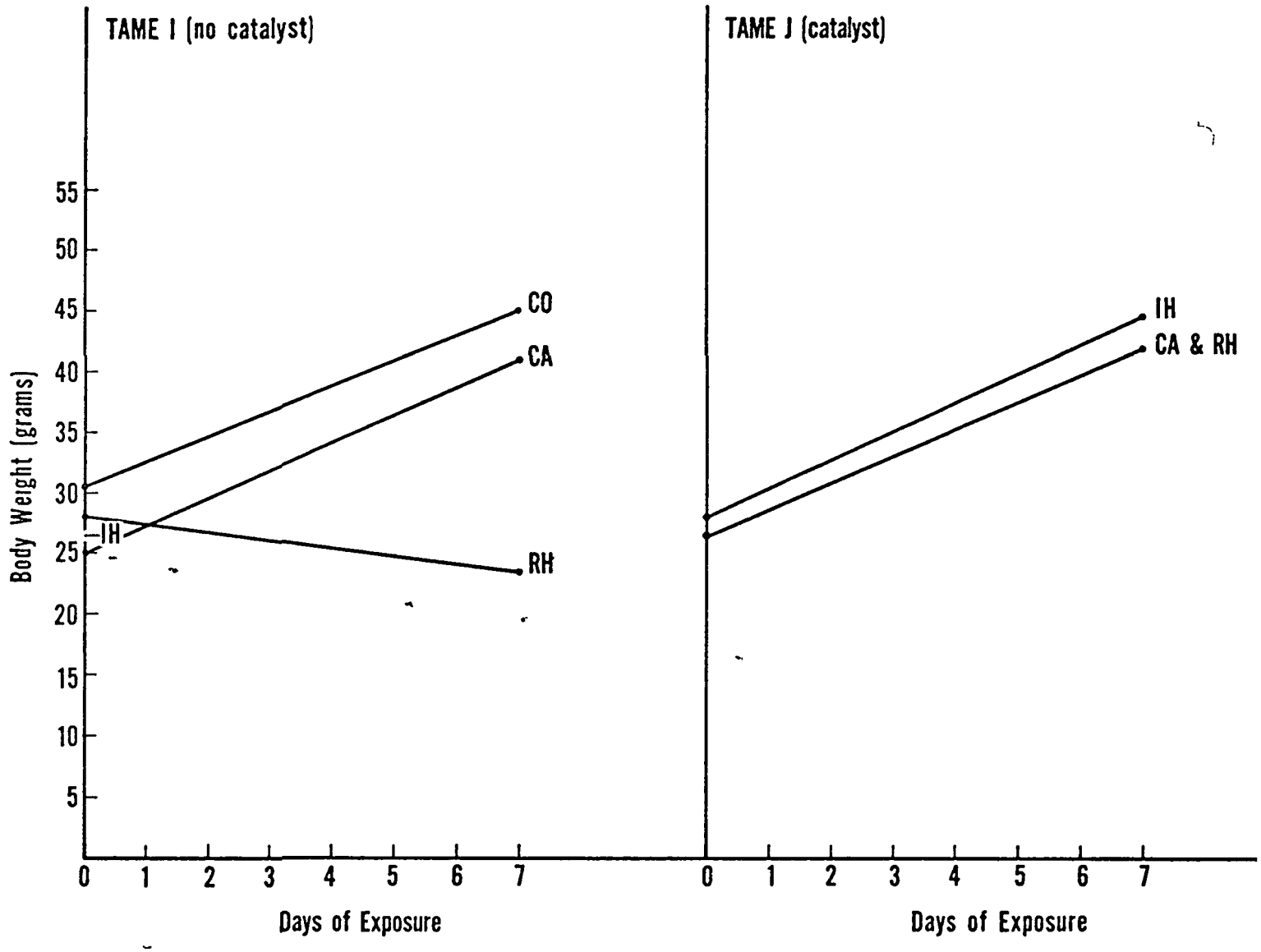

FIGURE 2. BODY WEIGHT OF INFANT RATS

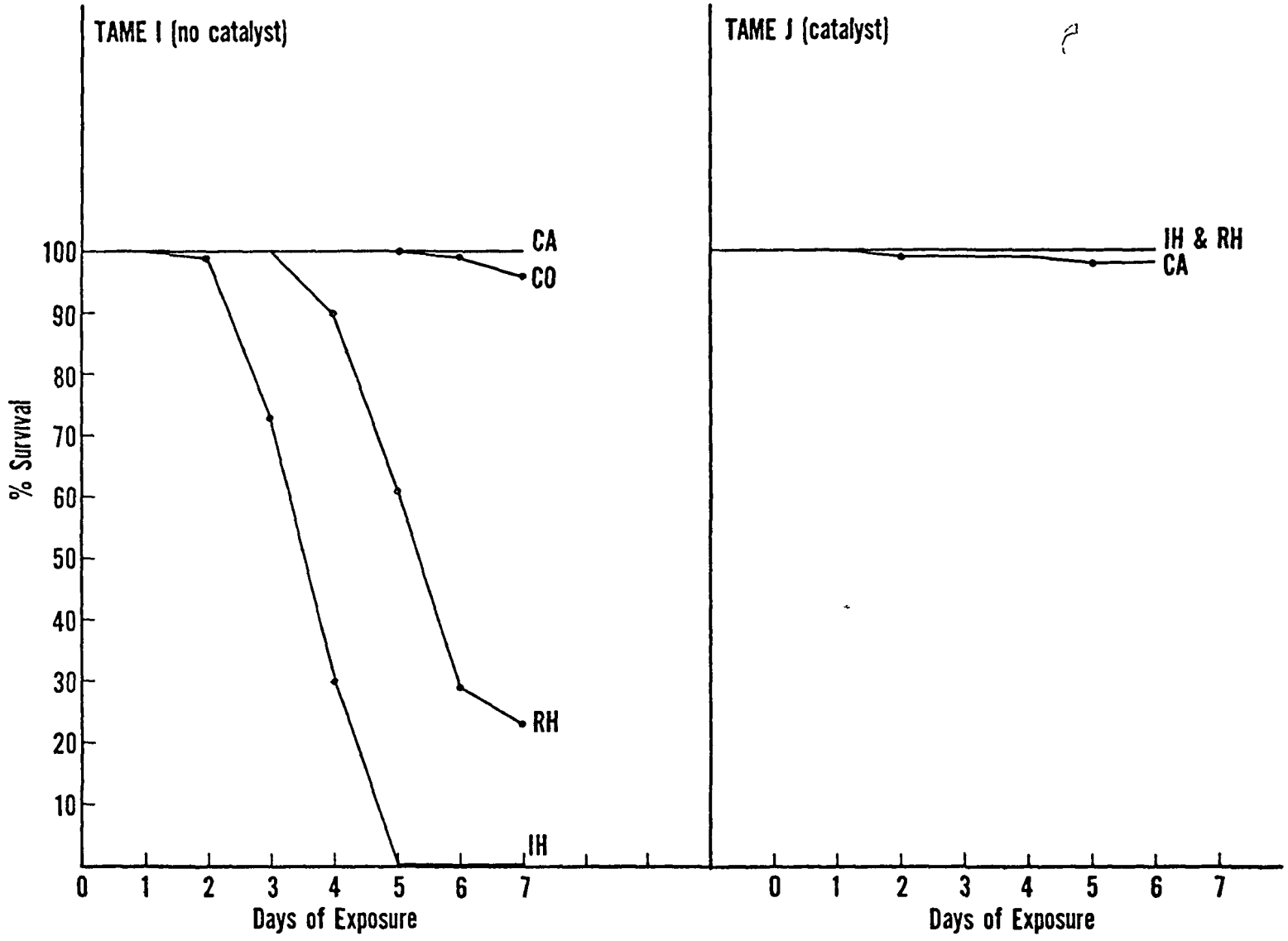

FIGURE 3. SURVIVAL OF INFANT RATS

were no apparent effects in TAME J with the possible exception of weight loss in IH and RH lactating female rats (Fig 1).

Hematology and Blood Chemistry

For this portion of the study, adult male rats were maintained in six treatment atmospheres (CA, RH, IH for both TAME I and J) 24 hrs./day. Five animals per group were removed on days 1-5, anesthetized and exsanguinated by abdominal aorta catheterization. The clinical laboratory determinations included hemoglobin (HB), hematocrit (HCT), red (RBC) and white blood cell (WBC) counts, platelet counts, reticutocyte counts, white blood cell differential, partial thromboplastin time (PTT), prothrombin time (PT), fibrinogen determination, total serum protein, alkaline phosphatase, SGOT, SGPT, blood urea nitrogen (BUN), Na, K, Cl, and Ca. Standard laboratory procedures were used in these determinations The results are presented in Tables 1 and 2.

Examination of the results indicates that in TAME J, the only statistically apparent treatment effect was an increase in total serum proteins in exhaust exposed animals. In TAME I there were statistically significant treatment effects in both raw and irradiated exhaust exposures on total protein, platelet count, RBC and WBC counts, white cell differential, alkaline phosphatase, hemoglobin, hematocrit, partial thromboplastin time, SGOT and SGPT levels The irradiated exhaust exposure also produced a treatment effect on levels of BUN fibrinogen. It should be emphasized that while the treatment effects were statistically significant, the data may not be physiologically significant They do, however, indicate a potential hazard to certain organ systems with prolonged exposure. In explaining the effects, the RBC related changes and alkaline phosphatase levels could relate to the high levels of CO, the WBC

TABLE I.

Treatment Mean Values for Selected Hematologic Parameters in Male Rats

		Without catalyst	With catalyst
RBC/cmm (x 10^6).	CA [a]	7 074	7.141
	RH [b]	7.616	7.004
	IH [c]	7.784	7.073
WBC/cmm (X 10^3):	CA	9.1	9.3
	RH	11.9	9.0
	IH	12.0	8.7
Platelets/cmm (x 10^6)	CA	0.95	0.93
	RH	1.07	0.97
	IH	1.10	0.92
Lymphocyte neutraphil ratio	CA	5.3	5.3
	RH	1.7	5.1
	IH	1.0	5.3
HB (gm %).	CA	14.9	14.6
	RH	16 5	14.7
	IH	16.7	14.5
HCT (%).	CA	41.6	40.8
	RH	46.6	41.0
	IH	47.4	40.1

a: Clean air control atmosphere

b: Nonirradiated exhaust atmosphere

c: Irradiated exhaust atmosphere

TABLE II.

Treatment Mean Values for Selected Blood Chemistry Parameters in Male Rats

		Without catalyst	With catalyst
Total protein, (gm %)	CA [a]	6.0	5.8
	RH [b]	6.3	6.1
	IH [c]	6.8	6.0
Alkaline phosphatase (Int. Units)	CA	79.1	80.9
	RH	54.2	91.4
	IH	40.8	83.4
SGOT (R-F Units)	CA	161.6	169.7
	RH	185.3	174.4
	IH	196.7	174.8
SGPT (R-F Units):	CA	48.5	40.0
	RH	60.5	40.7
	IH	53.7	42.0
Fibrinogen (mg/dl).	CA	170	175
	RH	165	175
	IH	220	170
BUN (mg %).	CA	23.8	22.5
	RH	21.0	21.8
	IH	28.3	21.4
PTT (seconds).	CA	19.9	19.9
	RH	21.7	19.3
	IH	22.4	19.2

a. Clean air control atmosphere

b: Nonirradiated exhaust atmosphere

c: Irradiated exhaust atmosphere

changes would suggest a rather severe acute inflammatory response, the other changes relate to hepatic and/or renal dysfunction.

Pathology

Tissues from the adult male rats used in the Hematology and Blood Chemistry Section of this study were saved in 10% formalin for pathology. In addition, an equal number of adult male hamsters were exposed and tissues saved for pathology Sections of hematoxylin and eosin stained lung, liver and kidney were examined microscopically for abnormalities.

In TAME I, 2 of 5 IH hamsters showed acute inflammatory pulmonary changes after one day exposure. The lesion was characterized by an infiltrate of polymorphonuclear neutraphils (PMN) into alveoli at the level of the terminal bronchioles After 2 days, the lesions consisted of prominent macrophage and PMN exudate in alveoli at the level of the terminal bronchioles plus a patchy acute purulent bronchiolitis and pneumonia with some ulceration of bronchiolar epithelium. By the end of 5 days, the IH animals had a subacute purulent bronchiolitis and pneumonia (Fig. 4). The alveoli at the level of the terminal bronchioles had thickened septae with some crescentic epithelial caps. Many were plugged with an admixture of fibrin, macrophages and PMN.

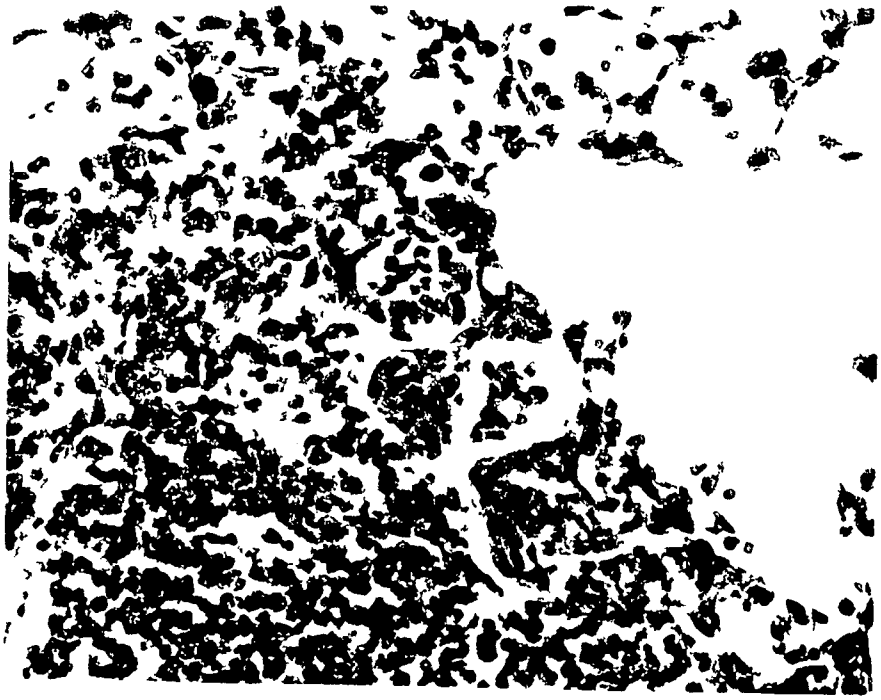

Figure 4. Subacute purulent bronchiolitis and pneumonia in hamsters exposed to irradiated exhaust in TAME I for 5 days

In the RH hamsters, the pulmonary changes were not apparent until day 2, and thoughout the study the changes were confined to the alveoli at the level of the terminal bronchioles. The lesions were not as severe as in the IH group and tended to be more proliferative than exudative. By the end of 5 days, there were some alveoli with thickened septae with an increase in alveolar macrophages (Fig. 5).

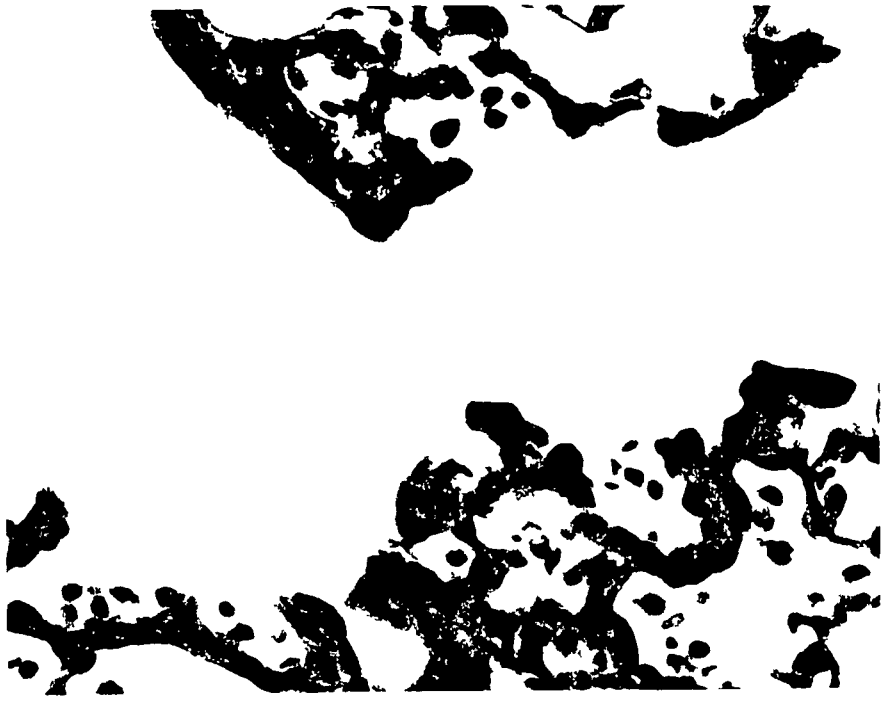

Figure 5. Pulmonary changes in hamsters exposed 5 days to non-irradiated exhaust in TAME I.

In both the RH and IH hamsters from TAME I, no treatment related changes were noted in liver and kidney until 5 days of exposure. In the IH group, all 5 animals showed vacuolar change (possibly lipidic degeneration) in hepatic parenchymal cells (Fig. 6). Two RH animals showed similar changes.

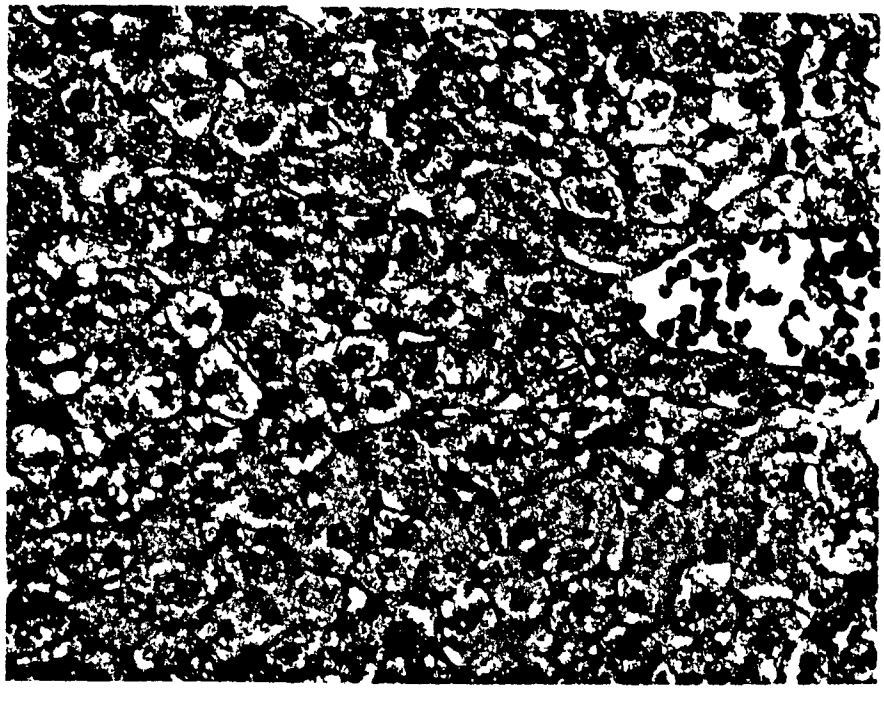

Figure 6. Cytoplasmic vacuolar change in livers from hamsters exposed 5 days to exhaust in TAME I.

Three of the IH animals also had similar vacuolar changes in renal tubular cells (Fig. 7).

Figure 7. Renal tubule cytoplasmic vacuolar change in hamsters exposed 5 days to irradiated exhaust in TAME I.

Pulmonary changes noted in TAME I rats paralleled those noted in the hamsters but were not apparent as early in the study and were not as severe. Extramedullary hematopoiesis was present in the livers of the IH and RH rats by day 4 probably as a result of the high CO levels.

Tissues from TAME J have not yet been examined. For the purpose of comparison, TAME H (identical to TAME J in design) results are included. No pulmonary changes were noted in IH and RH rats. The changes noted in hamster pulmonary tissue were rather mild and mainly present in the IH group only after 2 days of exposure. The lesion was confined to the alveoli at the level of the terminal bronchioles and consisted of an increase in macrophages, thickened septae and some crescentic epithelial caps. The changes were rather typical of lesions ascribed to exposure to NO_2. No changes in liver or kidney were noted.

B.8. EFFECT OF EXHAUST EMISSION FROM CATALYTIC CONVERTER ON ARYL HYDROCARBON HYDROXYLASE

L. Hall, I. Washington, J. Adams, K. Campbell and Y Yang

Introduction

Chemical carcinogenesis is of considerable concern in relation to exposure to environmental pollutants. Due to the association of microsomal metabolism with carcinogenesis the effect of catalytical modified auto exhaust on aryl hydrocarbon hydroxylase (AHH), one of the mixed function oxidase responsible for the biotransformation of some known potent carcinogens was determined

METHODS

Male Syrian hamster retired breeders [>8 mos. old] were exposed continuously for five days to automobile exhaust using the system described by Hinners et al (ETRL) These exposures were carried out in the three studies, TAMEs I, J, K, described elsewhere in the Catalytic Report

In each study after five days continuous exposure to either clean air (CA), nonirradiated (NI), or irradiated (I) exhaust, the hamsters were sacrificed with pentobarbital (IP), exsanguinated, and the puncture, and the lungs were removed in toto and quickly immersed in cold saline (4°C) The lungs from three animals were then trimmed of bronchi and connective tissue, weighed,and placed in cold 0.15 M KCl for homogenization Aryl hydrocarbon hydroxylase was assayed as described by Dixon et al [1] using a homogenate concentration of 25 mg/ml and an incubation period of 60 minutes. The results are expressed as the fluorescence equivalent to picomoles of 3-hydroxy-benzo pyrene formed/min/mg tissue

RESULTS

Table 1 shows the hamsters'lung AHH activity following exposure to either clean air, nonirradiated, or irradiated exhaust in TAMEs I, J and K.

Statistical analysis of the data showed that significant enzyme reduction occurred as a result of exposure to both nonirradiated and irradiated atmospheres in the reference study (TAME I - without catalyst) Exposure to catalyst-modified exhaust (TAME J) resulted in a depression of mixed function oxidase (MFO) activity in both experimental groups, but statistically significant ($p \leq .05$) depression was noted only in the group exposed to irradiated exhaust The use of high sulfur fuel with the catalyst (TAME K) produced a depression following exposure to irradiated exhaust, although the decrease did not reach statistical significance at the $p < .05$ level.

Due to the innate variability in enzyme activity of control animals between experiments, the data were normalized to percent of control for further comparison between studies Following exposure to nonirradiated exhaust a reduction in AHH activity of 52, 12 and 0 per cent was seen in TAMEs I, J, and K, respectively Following irradiated exhaust exposure, the depression in TAME I was 55 percent, 28 percent in J, and 18 percent in K Duncan's multiple range test revealed that TAMEs J and K effects were similar and different from TAME I

Table 2 shows the lung weight/body weight ratios for the three studies Only in TAME I (without catalyst) was a significant increase in the ratio noted The severity of this exposure was also evident in the mortality of some of the other experimental animals, which did not occur in the other studies.

Table 1. Aryl Hydrocarbon Hydroxylase in Hamster Lung[1]

	I	J	K
Clean Air	0.01192	0.01789	0.01134
Non-irradiated Exhaust	0.00579*	0 01671	0.01150
Irradiated Exhaust	0.00529*	0.01352*	0.00974

1 Activity expressed as equivalent to the formation of 3-hydroxy-BP in picomoles/min/mg tissue

* Significantly different from control at $p \leq .05$

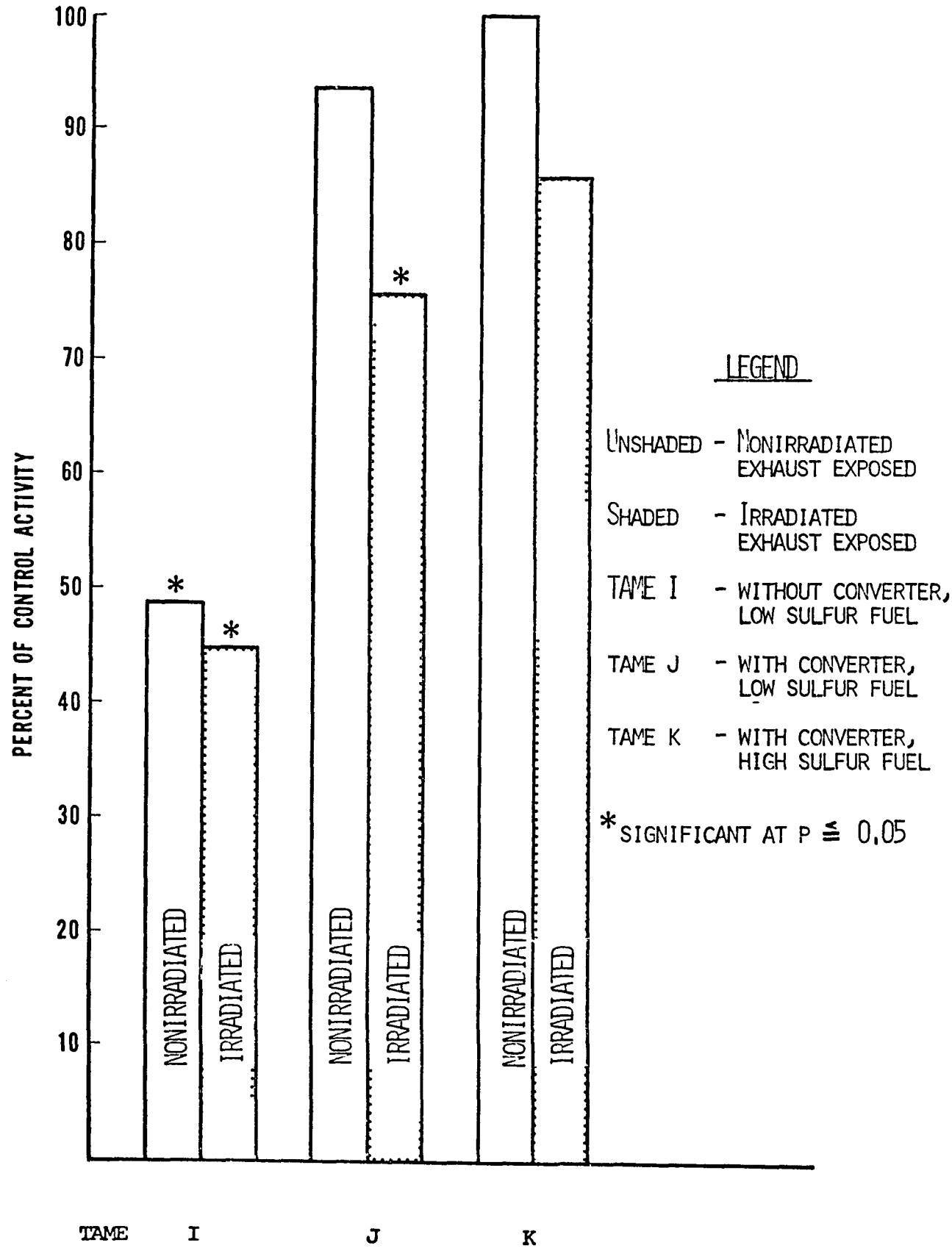

FIGURE 1. DEPRESSION OF LUNG ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN EXHAUST-EXPOSED HAMSTERS

Table 2. Lung Weight/Body Weight Ratio in Hamsters Exposed to Auto Exhaust

TAME	n.	CA	Non-Irrad.	Irrad
I	12	.536	.696*	.725*
J	12	.505	.498	.492
K	12	.521	.531	.528

* Significantly different from control at $p \leq .05$

DISCUSSION

TAME I, the reference study, showed profound toxicological and biological effects as manifested by death, lung weight/body weight ratio changes, and depression of AHH activity. However, no gross effects were noted in TAMEs J and K. Ozone (0.4 ppm) and NO_2 (4.6 ppm) was measured in the exhaust chamber atmospheres in TAME I. Palmer et al. [2] noted a 33 per cent decrease in hamster lung AHH activity following a three-hour exposure to 0.75 ppm ozone, but no effect on bronchial AHH was noted for NO_2 concentrations up to 50 ppm for three hours.[3] Thus ozone probably had significant effect on AHH activity in TAME I. The contribution by the other exhaust components, many with known biological effectiveness, is not known. No ozone was detected, and no significant change in NO_2 concentration was found in TAME J or K which would account for the depressed AHH activity. The biological effectiveness as reflected in AHH activity, therefore, may reside in the organic fraction of the atmospheres. Further work is necessary to resolve this problem.

Two additional comments are needed. The lack of statistical significance in the AHH depression in the NI atmosphere of TAMEs J and K and in the irradiated exposure in TAME K is thought to be due to the small sample size (4/treatment) rather than to lack of effect. Depression of AHH activity has been a consistant finding following exposure to auto exhaust in several studies (ETRL Annual Report, 1972).

The apparent lack of AHH induction is curious, since Holt and Keast[4] found induction of AHH in the lungs of mice exposed to cigarette smoke, which contains several components such as NO_2, aldehydes and polycyclic hydrocarbons, also present in the exhaust. The significance of the depression and lack of induction is not clear, but due to the association of this enzyme

system with carcinogenesis it seems important to determine the impact of effects on aryl hydrocarbon hydroxylase and its relationships to cancer initiation and promotion.

In summary, the use of the catalytic device significantly reduced but did not eliminate the depression of lung aryl hydrocarbon hydroxylase by exposure to auto exhaust Whether this residuum of biological activity in catalytic modified exhaust reflects the lower part of the dose response spectrum, or the formation of potent new chemical species, is not yet clear Additional research is necessary for insight into this aspect of catalyst evaluation.

ACKNOWLEDGMENT

We acknowledge with gratitude the sample of 3-hydroxy-benzpyrene from Dr. H.V. Gelboin, National Cancer Institute, that was used to standardize our work.

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B.9. BIOCHEMICAL EFFECTS OF EMISSIONS FROM AN AUTOMOBILE ENGINE WITH AND WITHOUT CATALYTIC CONVERTER

S. D. Lee, V. N. Finelli, L. McMillian, and R. M. Danner

As a part of the toxicologic studies of automobile engines with and without catalytic converters, the biochemistry group, in collaboration with Dr. Finelli of the Department of Environmental Health at the University of Cincinnati, has studied early biochemical alterations in rats exposed to auto exhaust emissions.

Materials and Methods

Experimental Animals - Each exposure experiment consisted of thirty female Sprague-Dawley rats, each group weighing approximately 200 grams, divided into three groups of ten animals Clean Air (CA), non-irradiated (N-I), and irradiated (I).

Exposure Conditions - The exposure system has been described by Hinner et. al., earlier in this report. The concentrations of major exhaust components in the exposure chambers were also described in a earlier report by Malanchuck, et. al. Temperature and humidity in the exposure chambers were kept constant throughout the experiment at 22°C and 50 percent relative humidity respectively. The exposures were conducted 24 hours a day, for 7 consecutive days. Two animal exposure experiments were conducted using the exhaust from the same engine with and without the catalytic converter. In addition, an experiment was performed by exposing a group of animals to carbon monoxide alone (experiment CO) at a concentration of 575 mg/m^3 (500 ppm) which approximately reflects the carbon monoxide level observed in exposure chambers when emissions from the engine without the catalytic converter was tested.

The following parameters were determined: hematocrits, serum latate dehydrogenase (LDH), serum glutanic oxaloacetate transaminase (SGOT), and serum lysozyme. Serum LDH and GOT were determined by using DADE reagent sets (American Hospital Supply Corp., Miami, Florida, white lysozyme was assayed with Worthington kit (Worthington Biochemical Corp., Freehold, N. J.). Blood samples were obtained from animals by tail vein puncture.

Results and Discussion

Figure 1 shows the drastic effects of the exposure to emissions from the engine without the catalytic converters on the hematocrit. At the end of the 7-day exposure, very high hematocrit levels were observed in the experimental animals, 62.3 $\pm$ 1.5 percent for N-I and 66.2 $\pm$ 0.5 percent for I, as compared to a normal value of 43.2 $\pm$ 0.9 percent for the clean air group. During a recovery period of 3 weeks, the hematocrit values were obtained weekly and a gradual return to normality was seen in the animals of both N-I and I groups. The animals exposed to carbon monoxide showed a average hematocrit of 62.5 $\pm$ 0.9 which is equal to the value found for N-I group in the experiment without converter.

The hematocrit in the animals expsoed to emissions from the engine when equipped with the catalytic converter did not differ from control values. From the above data it seems that the elevation of the hematocrit is due to the carbon monoxide concentration in the exposure chambers. The levels of carbon monoxide in N-I and I groups in the experiment without the catalytic converter were 551 and 559 ppm, respectively, while for N-I and I with the catalytic converter the carbon monoxide levels were reduced to 46 and 41 ppm. The increased hematocrit may be due to polycythemia and/or dehydration. Total serum protein or albumin analyses

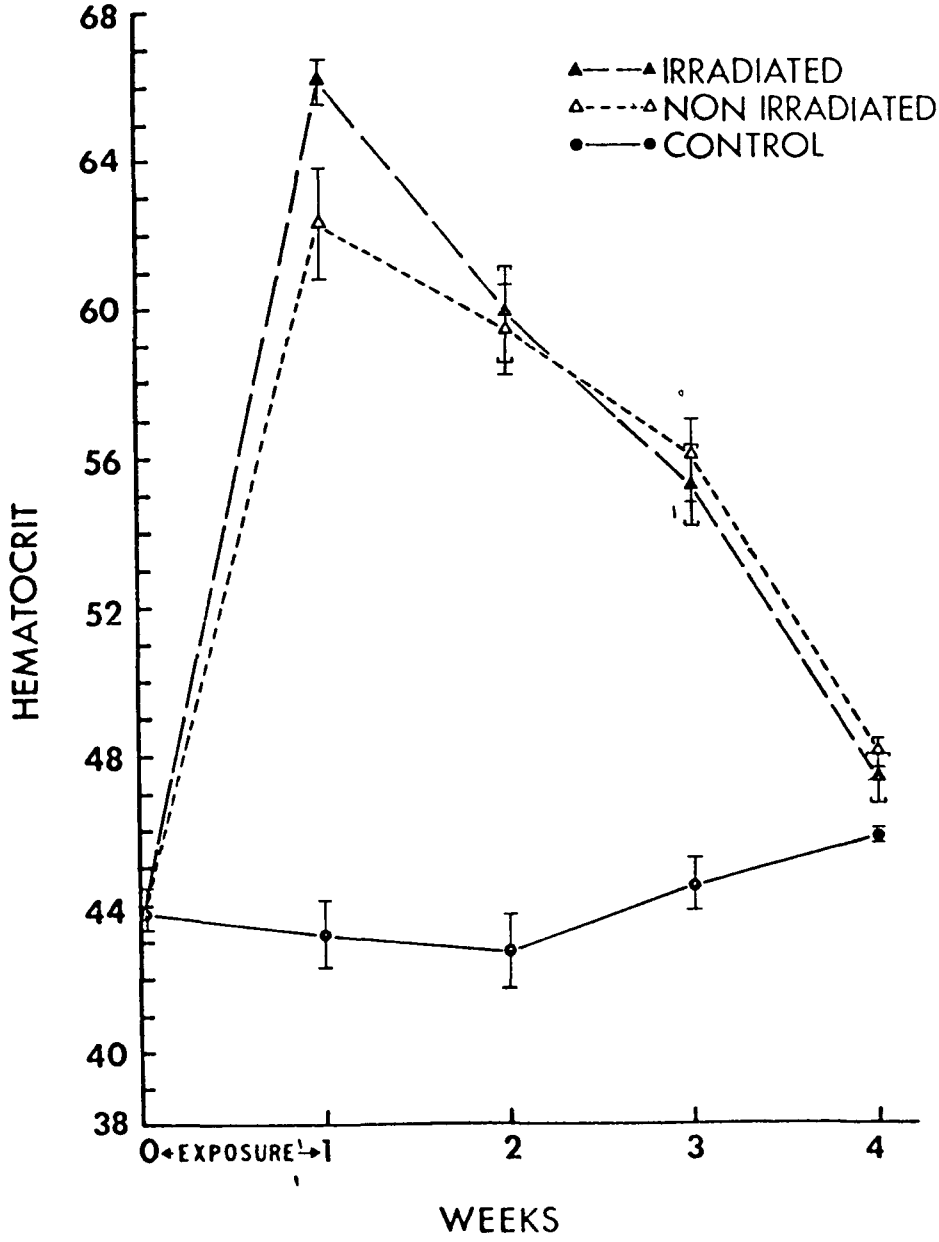

Fig. 1 Hematocrit from animals exposed to automobile emissions without catalytic converter.

were not obtained and therefore the occurence of dehydration cannot be confirmed; however the data collected from histological examination of the experimental animals, presented in a report by Hysell et. al., revealed the presence of a large numbers of reptured red blood cells which may indicate a polycythemic response.

In order to assess organ damage in exposed animals, the activity of LDH, GOT, and lysozyme in serum was assayed. These intracellular enzymes are characteristic of appropriate organs and an increase of enzymatic activity in serum would indicate presumably a leakage of enzymes from injured cells. Serum GOT was not significantly elevated in any of the exposed animals, this would indicate that neither liver nor heart were damaged by exposure to various types of emissions and to carbon monoxide. Serum LDH was elevated in the animals exposed to emissions from engine without catalytic converter. Figure 2 shows that, at the end of the exposure period, the animals from both N-I and I groups presented approximately 200% increase in serum LDH activity. In the recovery period, while the N-I group values tended to return to normal, the I group values presented an unexplained erratic behavior, moreover we cannot explain the low value obtained in the third week for the CA group. No significant changes in LDH activity were observed in the experimental animals when the converter was used and in the animals exposed to CO. Serum Lysozyme activity was not assayed in the experiment conducted without catalytic converter, however, in the experiment with converter, the exposed animals did not show any statistically significant elevation. From the above preliminary results it appears that the target organs of the toxic components present in the emissions from engine without catalytic converter are probably the lungs and/or kidneys.

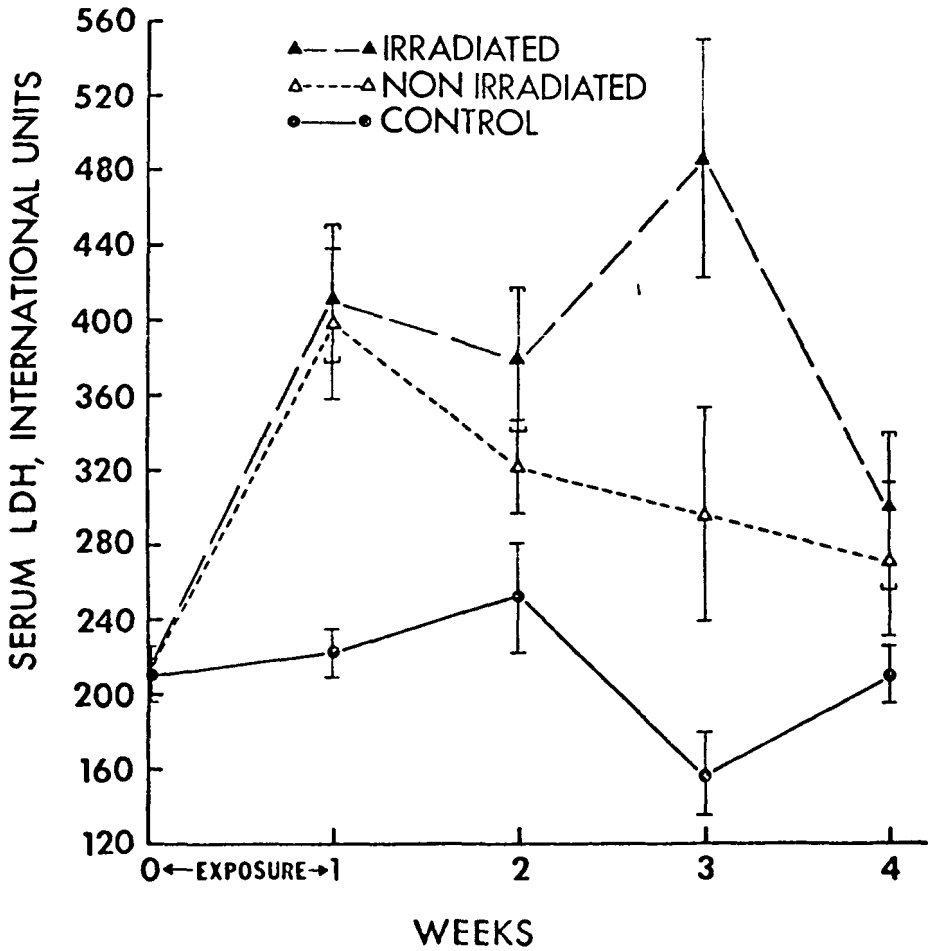

Fig. 2 Serum LDH values from animals exposed to automobile emissions without catalytic converter.

It can be concluded that the introduction of catalytic converters into the automobile exhaust system, not only has reduced the levels of certain exhaust constituents but has effectively decreased or eliminated biological effects studied.

Reference

1. Stara, J. F. and R. Hinners Toxicology of Atmospheric Pollutants Associated with the Use of Automobile Catalytic Converters, Annual Report, Environmental Research in 1973, U S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio

B.10. EXPOSURE OF MATERNAL, PREGNANT, AND NEWBORN RATS TO EXHAUST FROM MODERN AUTOMOBILE ENGINE WITH CATALYTIC CONVERTER AND OTHER EMISSION CONTROLS (TAME J)

K. Campbell, E. George, L. Hall and J. Stara

Toxicity to newborn and pregnant rats of diluted exhaust from a prototype automotive engine system including oxidative catalytic converter was evaluated by two experiments in the TAME J study. Details as to test atmosphere generation and characterization are provided in preceding reports (See Hinners et al., Malanchuk et al.). Similar studies were performed in TAME K (high sulfur fuel used) but data are not sufficiently complete to permit a comparative evaluation of the sulfur effect.

Experiment A

Ten litters of day-old Charles River COBS suckling rats and their dams were exposed for six days in each of three atmospheres purified air (controls), nonirradiated (NI) exhaust, and irradiated (I) exhaust. Dams and litters were weighed and pups were counted when placed in exposure (Day 0), when removed to clean air (Day 6), and subsequently while housed in purified air (on Days 11, 15 and 19). On Day 19 the young were 20 days of age and this was designated as "weaning date." Maternal body weight and infant survival and growth of treated groups were compared to control values.

Results, which are summarized in Table 1, are preliminary in the sense that the data has not as yet been subjected to statistical analysis Nevertheless, the largest contrasts between treated and control means are 7 per cent for maternal weight gain deficit (for Day 6), 3 per cent for infant growth deficit, and 8 per cent for infant survival to weaning, all in NI. Without

Table 1. Maternal Body Weight and Infant Growth and Survival in Rats Exposed to Catalytic Converter-Treated Automotive Emissions in TAME J, Experiment A.

Bioeffect Criterion	Treatment		
	Clean Air (Control)	Non-Irradiated	Irradiated
Maternal Lactating Rats:			
Pre-treatment body weight, gms (mean)	275.1	292.0	310.0
Mean fraction of pre-treatment body weight Day 0	1.00	1.00	1.00
Day 6 (End of Expos.)	1.163	1.085	1.105
Day 11	1.270	1.252	1.208
Day 15	1.245	1.172	1.212
Day 19 (Weanling)	1.283	1.262	1.226
Infant Growth:			
Pre-treatment body weight, gms (mean)	8.76	9.09	8.74
Mean fraction of above			
Day 0	1.00	1.00	1.00
Day 6	2.139	2.138	2.191
Day 11	3.337	3.285	3.310
Day 15	4.257	4.112	4.252
Day 19	5.708	5.666	5.707
Infant Mortality & Survival:			
Original No.	100	99	98
No lost, % survival to age 7 Days	0, 100	1, 99	0, 100
No lost, % survival to weaning	1, 99	8, 91.9	0, 100

additional data by which a consistent pattern might be discerned as a basis for interpretation and validation, these relatively small differences are suspected as being neither statistically* nor toxicologically significant Most infant deaths in NI occurred between 16 and 20 days of age. The cause of these was not clear, and they are not as yet considered as clearly treatment-related It would thus appear, at least tentatively, that despite the low exhaust dilution ratio, the test atmospheres from this engine and its emission controls were not sufficiently harmful to be detected by the methods used It should be noted that in the previous study (TAME I) in which the catalyst was not used, there were obvious body weight deficits and morbidity in exposed animals, suggesting a beneficial effect of the catalytic converter in regard to the latter criteria.

Experiment B

Twelve Charles River COBS female rats pregnant 15 days were exposed for 6 days to each of 3 atmospheres purified control air, nonirradiated (NI) exhaust, and irradiated (I) exhaust, as with Experiment A After exposure, all were transferred to brood cages and housed in clean room air. Body weight of the maternal animals were recorded when they were placed into exposure (Day 0), when removed from exposure to room air (Day 6), and subsequently on days 11, 15, 19, 25 and 29. Offspring were counted at parturition following exposure (Day 8), and weighed and counted on days 11, 15, 19, 25 and 29. On Day 29 the offspring were 20 days of age, this date was designated as the weaning date Maternal body weights and infant survival and growth in exhaust-exposed groups were compared to those in the control group.

*Subsequent statistical analyses indicated that the infant survival in NI was significantly less than in CA and IR, however, this suspicious pattern was not observed in a subsequent experiment (TAME K) The weight-data analyses suggested no significant treatment effects

The resulting data, which are summarized in Table 2, and which have not yet been statistically analyzed, are tentatively interpreted as indicating no significant effect by either treatment (NI or I) on maternal body weight, litter size, or infant survival or growth. The largest contrast noted was a mean 9% smaller litter size in NI, but litter size varied considerably in all groups, and the mean I litter size was 3.5% larger than control. The largest single contrast in maternal body weights was -4.5% in I, and in infant body weight was +8.2% in NI. Without additional data to establish a clear pattern it is doubtful that the pattern and magnitudes of mean differences observed in this experiment are statistically or toxicologically meaningful.[+] Since this experiment was not performed in TAME I, a comparative evaluation of converter-no converter emission toxicity on these criteria is not yet possible, but it is speculated from other observations in TAME I that effects might well have been demonstrable with this system.

+ Subsequently completed statistical analyses indicated no significant treatment effects on maternal or infant body weights or on infant survival in this experiment

Table 2. Maternal Body Weight and Infant Growth and Survival in Rats After In Utero Exposure to Catalytic Converter-Treated Automotive Emissions in TAME J, Experiment B

Bioeffect Criterion	Treatment					
	Clean Air (Control)		Non-irradiated		Irradiated	
Maternal rats -- Body Weight						
Mean body weight (gm) and fraction of initial (pre-expos) weight. N=12						
Day 0 (Pre-exposure)	227.5	1.00	221.0	1.00	234.6	1.00
Day 6 (Post-exposure)	303.3	1.333	299.4	1.355	297.4	1.268
Day 11 (3 day after partur)	278.6	1.225	262.8	1.189	280.1	1.194
Day 15	301.5	1.325	285.9	1.294	297.0	1.266
Day 19	318.9	1.402	308.9	1.398	322.5	1.375
Day 25	331.2	1.456	327.0	1.480	326.7	1.392
Day 29 (weaning)	321.8	1.415	310.9	1.407	316.9	1.351
Living offspring --						
Mean body weight (gm) and fraction of 1st weight (age 3 days)						
Day 11 (3 days of age)	9.08	1.00	8.71	1.00	8.46	1.00
Day 15	16.25	1.790	16.03	1 840	15.68	1.853
Day 19	24.26	2.672	24.24	2.783	23.70	2.801
Day 25	36.78	4.051	37.96	4.358	36.37	4.299
Day 29 (weaning, age 20 days)	46.63	5.135	48.38	5.555	46.29	5.472
Infant mortality and survival						
Original No. and mean litter size	115	9.58	105	8.75	119	9.92
No. lost and % survival 1st 3 days	2	98.3	1	99.0	0	100.0
No. lost and % survival to weaning	3	97.4	2	98.1	1	99.2

B.11. GROSS MORPHOLOGIC AND FUNCTIONAL DAMAGE TO PLANT SPECIES BY DILUTED EXHAUST OF AUTOMOTIVE ENGINES OPERATED WITH AND WITHOUT A CATALYTIC EMISSION CONTROL DEVICE

K. Campbell, R. Miller and J Enright

Effects on vegetation of automotive exhaust from engines with and without an oxidative catalytic converter, and operated on low-sulfur Indolene fuel, were assessed by exposing multiple plant species to clean air (control), irradiated and nonirradiated atmospheres in each of the two sequential "TAME" experiments 'I' (without converter) and 'J' (with converter) Details concerning the experimental atmosphere generation and characterization are provided in preceding reports (see Hinners et al. and Malanchuk et al). In TAME 'K', in which the atmospheres were produced by the same engine fitted with converter, but using high-sulfur Indolene, the time-related aspects of vegative damage by exhaust to a single plant specie were investigated These studies were conducted collaboratively with faculty and student representatives of the Department of Biology (Botany), University of Cincinnati Results reported are based on preliminary data evaluation.

Experiment A Damage to vegetation by automotive exhaust from an engine operated on low-sulfur fuel with (TAME J) and without (TAME I) an oxidative catalytic converter.

Phytotoxicity in exposed plants was evaluated on the basis of morphologic alterations (gross visible alterations of leaf and stem character) in all species and functional changes (C^{14}-determined photosynthetic activity) in some. Post-exposure recovery was also observed Effects of irradiated (I) and nonirradiated (NI) atmospheres were compared in each experiment and

between experiments Nine species in varying young stages of development were used in duplicate in each experiment. Of these, five were the same in both and four were different, and developmental stages were comparable between exposures. The species used in each experiment are shown in Table 1. Duration of exposure was 127 hours for TAME 'I', and 151 hours for TAME 'J'

Table 1. Plant Species Used in TAME 'I' and 'J'

TAME 'I' - Without Catalytic Converter

Taxas cuspidata	Japanese yew
Picea abies	Norway spruce
Ilex hetzi	Japanese hollyberry
Cotoneaster lofast	Cotoneaster
Euonymus coloratus	
Alianthus altissima	Tree of heaven
Episcia cupreata (Silver Sheen)	Episcia
Pinus strobus	White pine
Basilico	

TAME 'J' - With Catalytic Converter

Dancus carota	Red-cored chantenay carrot
Phaseolus mungo	Bean
Oxalis corniculata	Oxalis
Coleus blumei	Coleus
Cotoneaster lofast	Cotoneaster
Euonymus coloratus	
Episcia cupreata (Silver Sheen)	Episcia
Alianthus altissima	Tree of heaven
Basilico	

In both experiments, exposure to both NI and I atmospheres damaged all species with varying degrees of severity. Although modes of damage were also variable, the most generally common were wilting, bleaching and loss of leaves. In some species, younger leaves were damaged first and in others the older leaves showed earliest damage Generally, even though exposure duration was a little longer in TAME 'J' than in 'I', damage was somewhat less in TAME 'J' (with converter) than in TAME 'I' (without converter). However, this difference in severity was less remarkable than the difference in gross toxic severity shown by animals, suggesting that the plants were fundamentally more susceptible subjects than animals and that their damage "thresholds" were greatly exceeded in both studies Three of the five species used in both studies showed less damage in the converter-treated emissions than in the non-converter emissions TAME 'I' atmospheres killed Cotoneaster, Ailanthus and Episcia with no post-exposure recovery, in TAME 'J' atmospheres Cotoneaster showed slightly less damage even though it was wilted badly, and Ailanthus and Episcia showed post-exposure recovery, i.e., formed new buds after apparent death with loss of original leaves. Basilico seedlings, however, recovered after the 'I' exposure but died from the 'J' and Euonymus damage was similar in both exposures.

In TAME 'I' damage produced by the NI atmospheres was generally more severe than that by I Five of nine species showed greater visible damage by NI than by I while the reaminder were about equally damaged by NI and I. There was a drastic depression of C^{14}-measured photosynthetic rate and, in Euonymus, photosynthesis was depressed more by NI than by I, while the reverse was true for Picea. In three species visible damage was delayed

until about one week post-exposure, at which time needle burn was observed in Pinus and Picea, and bleach in Taxus. In Ilex there was direct damage to petioles.

In TAME J, also, all nine species were damaged by both NI and I but the visible damage severity by NI and I was generally not as distinguishable as in TAME I. Photosynthetic depression by I was greater than by NI in Euonymus, while the reverse was true for Cotoneaster The species showing delayed effects in I were not used in J

Within the context of the vegetation damage from the severe exposure conditions in TAME studies I and J, it is tentatively concluded that, in general, there is a detectable but minor advantage to the use of the catalytic emission control system.

Experiment B The time course of severe damage to Episcea Cupreata by diluted automotive exhaust in TAME K

Since plant species exposed for several days to the TAME I and J atmospheres exhibited severe to fatal damage, specimens of one of the most susceptible species were exposed for varying durations in this experiment in order to better understand the temporal "dose-response" relationships of the damage at similar pollutant levels In each of the control (clean air), nonirradiated (NI) and irradiated (I) atmosphere chambers duplicate Episcia Cupreata (Silver Sheen) plants were exposed for periods of six days, one day, six hours and one hour. The six-day plants were examined daily. Degree of visible damage was subjectively rated on a scale of 0-12, Degree of visible damage was subjectively rated on a scale of 0-12, essentially as described previously (Campbell, 1973) After exposure, photosynthetic rate and recovery were also assessed

As noted in previous studies, foliar damage was distinctly visible as early as six hours of exposure, and progressively became severe as exposure continued. In this study, generally, the I plants were damaged more than the NI plants. There was a visually distinguishable difference in the pattern of damage shown in the two types of atmosphere, as well, in terms of spacial pattern of discoloration and of degree of wilt and turgor. There appeared to be a somewhat steeper slope to the degree of damage x duration of exposure curve than in the previous studies (TAME I and J). Preliminary results are illustrated in Figure 1.

A subsequent study of duration - and concentration - related effects, using the new Ford engine (with catalyst), shorter exposure periods and multiple lower exposure levels (greater dilution), was conducted recently. Preliminary data evaluation suggested grossly visible effects at 3 hours of exposure or less in the high-level atmosphere (corresponding to those reported above), and duration and concentration relationships in both gross and functional effects. CO and total HC levels were substantially lower, and NO_x levels higher, than in TAME K

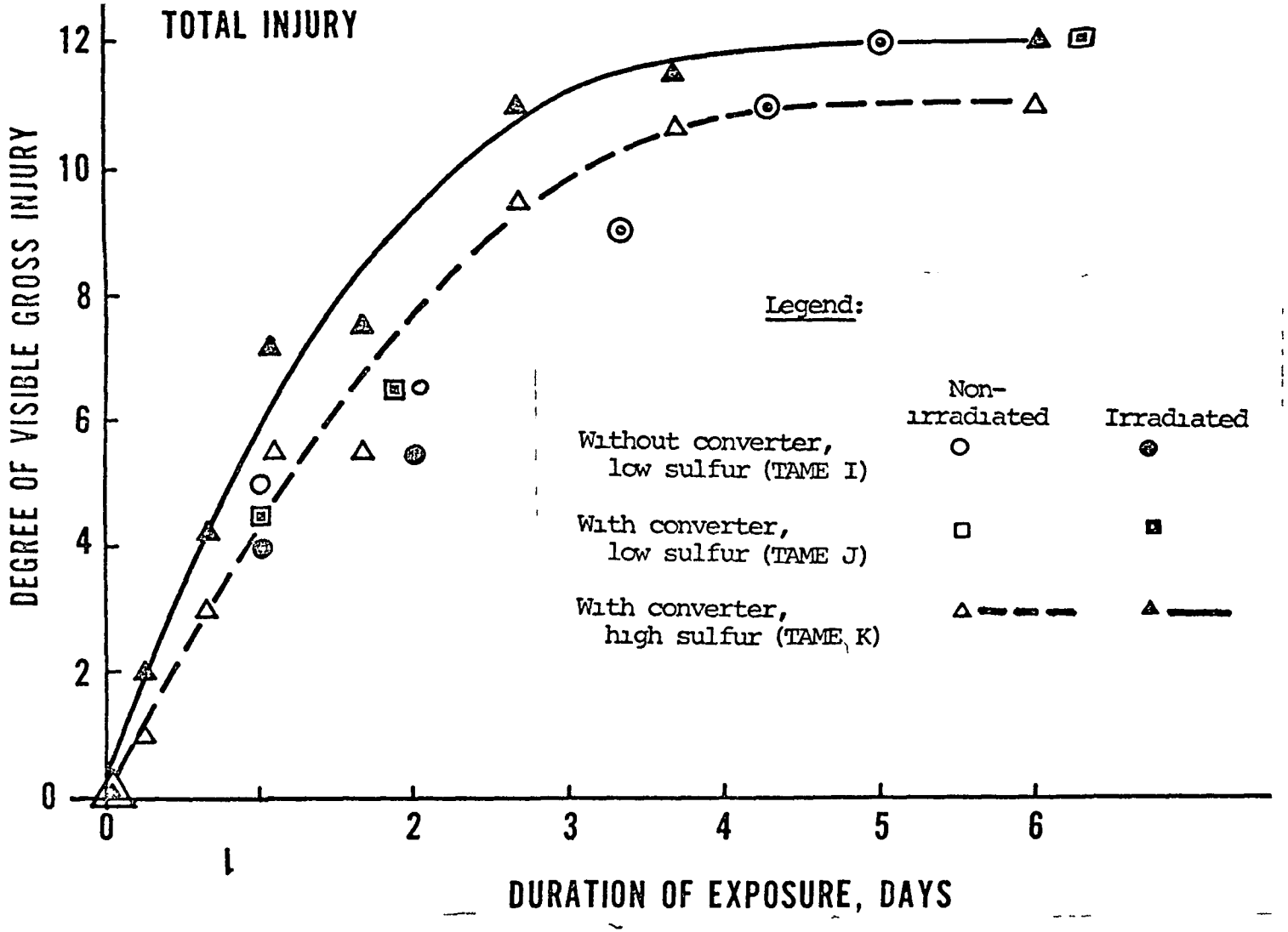

FIGURE 1. DAMAGE TO FOLIAGE OF EPISCIA PLANTS EXPOSED TO AUTOMOTIVE EXHAUST