

American Petroleum Institute
2101 L Street, Northwest
Washington, D.C. 20037
202-457-7000



Medicine and Biological
Science Department

API Medical Research
Publication: 27-32610

EVALUATION OF THE THREE MONTH
INHALATION TOXICITY OF TWO MOTOR FUELS

Study performed by:

Huntingdon Research Center
New York

Under API Contract PS-6

April 1976

Subchronic Inhalation Toxicity of Two Motor Fuels

R.A. KUNA¹ and C.E. ULRICH²

ABSTRACT

Rats and monkeys were exposed to 1552 and 384 ppm unleaded gasoline vapor or 374 and 103 ppm leaded gasoline vapor 6 hours per day, 5 days per week, for 90 days. No remarkable changes were found during the experimental period. Specific parameters investigated included body weight, hematology, CNS response, pulmonary function tests, urinalysis, deposition of IgG in the renal glomerulus, lead levels in blood, urine, and tissue, organ weights, organ to body weight ratios, and histopathology. Despite sporadic statistically significant changes in some of the above parameters, no patterns suggesting biological significance were established. However, upon subsequent reevaluation of the kidney histopathology, male rats exposed to 1552 ppm unleaded gasoline displayed regenerative epithelium and dilated tubules in the kidneys. The significance of this observation is not yet understood.

INTRODUCTION

GASOLINES are exceedingly complex mixtures of aromatic hydrocarbons, alkanes, cycloalkanes, and olefins boiling in a range of 80–345°F. The exact composition of gasolines varies depending on the crude oil source, the refining conditions, and so on. Organometallic compounds are added in relatively small amounts in order to produce desired handling, storage, and combustion properties.

The literature is relatively sparse with respect to gasoline toxicity studies. The earliest animal study, by Haggard (1921), defined the acute anesthetic and toxic effects of gasoline vapors in dogs. Central nervous system effects were observed at about 10,000 ppm, and death was observed at about 25,000 ppm. Lykke and Stewart (1978) exposed rats for 6–12 weeks to 100 ppm of a commercial 98 octane leaded gasoline. They found progressive focal interstitial fibrosis of the lung that was associated with irregular alveolar collapse. Gasoline vapor exposures in humans were investigated by Drinker et al. (1943), who observed signs of eye irritation at 700 ppm and slight anesthesia at 2600 ppm.

Much of our knowledge regarding the toxicity of gasoline derives from cases involving gasoline sniffers who deliberately inhale gasoline at exposures well in excess of those noted above. Clinical observations include signs of central and peripheral nervous system abnormalities, muscle atrophy, and hepatic and renal damage (Robinson, 1978). Irreversible encephalopathy has been attributed to added organic lead and not to the gasoline hydrocarbon itself (Valpey et al., 1978).

¹Exxon Corporation Medicine & Environmental Health Department, Research & Environmental Health Division, East Millstone, New Jersey.

²Huntingdon Research Center, New City, N.Y. Current address: International Research and Development Corporation, Mattawan, Michigan.

KUNA AND ULRICH

In order to examine the subchronic effects of gasoline exposures and to set exposure levels for a chronic inhalation study, a comprehensive 90-day subchronic study using rats and monkeys was initiated with leaded and unleaded gasoline. The results of the investigation are presented in this paper.

MATERIALS AND METHODS

Test Material

An unleaded EPA reference fuel (Fuel A) and a commercially available leaded gasoline (Fuel B) were used. The compositional information is presented in Table 1.

Animals

One hundred male and one hundred female Sprague-Dawley albino rats, weighing between 100 and 150 g were obtained from Charles River Breeding Laboratories. The rats were randomly divided and caged into 5 groups of 20 males and 20 females each in stainless steel wire mesh cages. Purina Rodent Diet and tap water were available ad libitum. Rats were acclimatized to the laboratory environment for 2 weeks prior to the commencement of exposures.

Thirty male and thirty female squirrel monkeys (*Saimiri sciureus*), weighing between 0.5 and 1.0 kg, were obtained from Prime Labs (Monmouth County Airport, Farmingdale, New Jersey). Each monkey was weighed and marked with an identifying number (chest tattoo) upon receipt. All monkeys were observed for an 8-week period prior to the commencement of exposure. During this time, they were tuberculin tested twice (250 TU in the palpebral fold), treated with an antihelminthic

TABLE 1. COMPOSITIONAL PROPERTIES OF GASOLINES

<i>Calculated Data</i>	<i>Fuel A Unleaded EPA Reference Fuel</i>	<i>Fuel B Leaded Commercial</i>
Research octane no.	93	87
Motor octane no.	88	86
Reid vapor pressure, PSIA	6.9	6.3
Distillation, ASTM D-86, °F		
Initial boiling point	80	80
10%	135	160
50%	210	217
90%	275	295
100%	345	340
FIA analysis		
% aromatics	30.1	27.4
% olefins	8.2	7.8
% Saturates	61.7	64.8
<i>Experimental Data</i>		
API gravity at 60°F	57.0	58.4
Sulfur, ppm	240.0	75.0
Lead, g/gallon	<0.005	1.94
Benzene, LV%	0.2	0.4
Toluene, LV%	16.7	11.4
n-Butane, LV%	1.0	0.4
Isopentane, LV%	5.4	5.5
n-Pentane, LV%	4.8	4.0

GASOLINE SUBCHRONIC TOXICITY

(thiabendazole 100 mg/kg), and given 2 general physical examinations. Unhealthy monkeys were removed from consideration for the study.

Forty healthy monkeys were then selected from this pool and randomly divided into 5 groups, each containing 4 males and 4 females. The monkeys were caged in pairs of the same sex in stainless steel cages with food and tap water available ad libitum.

Exposure Methods

Target exposure levels for both rats and monkeys were: control (group I); unleaded gasoline 1500 ppm (group II) and 400 ppm (group III); and leaded gasoline 400 ppm (group IV) and 100 ppm (group V).

The selection of the high-dose leaded gasoline group (400 ppm) was made on the basis of the 1974 ACGIH Threshold Limit Value for exposure to airborne lead. A 7-day probe study indicated that this level (400 ppm) should be tolerated during a 90-day exposure period. The selection of the high-dose unleaded gasoline group (1500 ppm) was based on a maximum level to minimize aerosol formation and potential CNS effects (Drinker et al., 1943).

The 5 groups of rats and monkeys were each housed in 1 m³ glass and stainless steel exposure chambers 24 hours a day and only removed for daily feeding and cleaning. The duration of the exposures was 6 hours per day, 5 days per week, for 13 weeks.

Vapor Generation and Analysis

Each chamber was equipped with a positive displacement Roots Whispair blower that provided approximately 250 L/min of chamber ventilation. This ventilation rate resulted in the chambers operating under a negative pressure of approximately 0.3 inches of water. Each chamber was provided with an absolute filter, such that inlet air was particulate free (99.9% at 0.3 μ). Air flow through the chamber was continuously monitored for a pressure drop across the inlet filter.

Design of an adequate vapor generation system for gasoline was complicated by the wide range of boiling points for the various constituents (80–345°F) and the explosion hazard posed by gasoline vapors. Gasoline was contained in a 1-gallon stainless steel safety can, which had been modified by the addition of a 1/4 inch Swagelok fitting. Gasoline was withdrawn from this container with a constant displacement metering pump (Fluid Metering, Inc.) and piped to the atomizer (Spraying Systems 1/4 J Body with a No. 1650 fluid nozzle and a No. 64 air nozzle). Nitrogen, a nonvolatile carrier gas, was heated to 105°C by passing it over a 100 W quartz immersion heater and then supplied to the atomizer at 10 psig.

The gasoline was atomized into a small (ca. 2 ft³) stainless steel chamber connected to the exposure chamber inlet. The exposure chamber ventilation air was drawn through a mixing pipe, which passed through the small atomization chamber. A pressure differential between the vaporization chamber and the mixing pipe caused a mass transfer of gasoline vapors to the exposure chamber airflow.

Due to the broad boiling range of gasoline, incomplete vaporization and subsequent introduction of aerosolized material into the animal exposure chambers was an experimental concern. The presence of aerosolized materials was analyzed using a modified Air Techniques Incorporated Model 727 forward light-scattering aerosol photometer. The amount of aerosol generated with a chamber atmosphere of 1500 ppm gasoline was 10 μ g/L.

Exposure chamber atmospheres were analyzed for gasoline vapor concentration with a total hydrocarbon analyzer (Scott Model 116) that was connected to an automatic sampling device. These determinations were recorded twice per day, once midmorning and once midafternoon.

Vapor phase alkyl lead was measured by the method of Purdue et al. (1973).

Hematological Methods

Blood for hematological evaluation of animals in the control, 1500 ppm unleaded, and 400 ppm leaded groups was taken from 10 rats and 4 monkeys per sex per group. Rats were bled only at the

KUNA AND ULRICH

termination of the study, while monkeys were bled prior to exposure and after 1.5 and 3 months of exposure. Unanesthetized animals were bled by tail clip for rats or by fingerprick for monkeys. Erythrocyte and leukocyte counts were determined with a Model ZBI Coulter Counter. Hemoglobin was determined by the Drabkin cyanmethemoglobin method utilizing a Fisher Model 55 Hemophotometer. Differential leukocyte count was recorded by direct count on Wright-stained smears. The reticulocyte count was determined by a microscopic observation of brilliant cresyl violet-stained smears.

CNS Evaluations

All monkeys in the control and high level exposure groups were implanted with permanent electrodes in the visual cortex. Access to these electrodes was via a connector permanently affixed to the animal's head. Simultaneous exposure and evoked response recordings were obtained by placing the animal in a restraining chair and connecting an amplifier to the implanted connector. The experiment was begun by placing a monkey in a small (ca. 150 L) stainless steel exposure chamber that had a 12-inch diameter window. The flash lamp of a Grass photostimulator was positioned in front of the chamber window and was set for paired flashes. Time between these flashes was set for 135 msec. The photostimulator was controlled by a minicomputer (PDP 8/e). At time *A*, the computer started accepting EEG data; at times *B* and *C*, the computer triggered the photostimulator and light flashes were produced, and at time *D*, the computer stopped accepting EEG data. After 10 seconds, the sequence was repeated once. Responses were recorded before the start of the study and after the final exposure (day 90).

Pulmonary Function

Evaluation of the mechanical properties of the lung was conducted by real time computerized techniques similar to those reported by Alarie et al. (1971). These procedures were carried out on each monkey in all groups prior to exposure and on each monkey in the control and high-level unleaded groups at 1.5 and 3 months.

Urinalysis

Urine samples were analyzed on all animals at 1.5 and 3 months using Ames Bili-Labstix (pH, protein, glucose, ketones, bilirubin, and blood). The urine was also used for lead analysis by adjusting the pH of the urine to 7.0 and then complexing with ammonium pyrrolidinedithiocarbamate, extracting into methylisobutyl ketone, and analyzing by atomic absorption spectrophotometry.

Termination

All animals that died spontaneously or were sacrificed were necropsied. Animals were sacrificed by sodium pentobarbital overdose and exsanguinated via the abdominal aorta. At the time of necropsy, organ weights were taken and tissues saved in 10% neutral formalin. Lungs, kidneys, spleen, heart, brain, and bone marrow from the control and high-dose groups were evaluated for histopathology.

All male and female animals from the control and high-exposure level groups were evaluated for the presence and deposition of IgG in the renal glomerulus and lungs (Muftuoglu et al., 1974; Dixon, 1979).

Samples of rat brain, kidney, liver, urine, and blood from both leaded groups as well as control animals were sent to the Ethyl Corporation for lead analysis.

RESULTS

The mean exposure concentrations for both unleaded and leaded fuels are presented in Table 2. Three rats (one each in groups II, IV, and V) and three monkeys (one each in groups I, II, and IV)

GASOLINE SUBCHRONIC TOXICITY

TABLE 2. MEAN EXPOSURE CONCENTRATIONS FOR UNLEADED AND LEADED FUELS

<i>Animal Group</i>	<i>Gasoline Vapor Exposure Concentrations</i>		
	<i>Mg/Liter ± SD^a</i>	<i>ppm</i>	<i>Alkyl Lead µg Pb/L ± SD</i>
Control (I)	—	—	—
Unleaded gas (II)	6.35 ± 0.44	1552	—
Unleaded gas (III)	1.57 ± 0.15	384	—
Leaded gas (IV)	1.53 ± 0.23	374	0.72 ± 0.10
Leaded gas (V)	0.42 ± 0.04	103	0.19 ± 0.04

^aSD, standard deviation.

died during the course of the study. These deaths were not considered to be treatment related. Two female monkeys in group II exhibited emesis after 13 days of exposure. Two other female monkeys in group IV showed the same sign after 17 days of exposure. No statistically significant differences were observed in body weight changes between controls and any treated group of either species with the exception of those noted in male rats in groups III and V. The latter displayed increased mean body weights at the end of the study.

Table 3 presents hematology data obtained at the final sacrifice for male and female Sprague-Dawley rats. Males in the high-exposure level unleaded group (1552 ppm) had a statistically significant increase in thrombocytes. Females in the same group had a statistically significant increase in reticulocyte counts. Males exposed in the high-leaded fuel group (374 ppm) had a statistically significant decrease in mean corpuscular hemoglobin concentration, while females of the same group showed statistically significant increases in hematocrit and mean corpuscular volume and a decrease in the white blood cell count.

No statistically significant changes in hematological parameters were noted in male and female squirrel monkeys (Table 4).

Mean flash-evoked response time for squirrel monkeys prior to the start of the test was 37.89 ± 0.88 msec. No statistically significant differences were noted at the end of the study in any of the dosing regimens.

Pulmonary function data are presented in Table 5 and 6. Increased minute volumes, which were statistically significant, were recorded after 90 days of the exposure regimen for male monkeys in both high-level fuel groups. Females exhibited statistically significant decreases in respiratory rate at preexposure and 42 days in the high-level unleaded group (1552 ppm). The females exposed to high-dose leaded gasoline (374 ppm) had a statistically significant decrease in tidal volume at the 90-day testing interval.

Urinalysis data for rats and monkeys after 1.5 and 3 months of exposure revealed no exposure-related differences in any of the groups.

Renal immunofluorescent evaluations of all male and female rats and monkeys displayed no evidence of IgG deposition in the kidneys following the 90-day experimental period.

Lead levels in blood, urine, and selected tissues of rats exposed to leaded gasoline vapors are presented in Table 7. Tissue samples from rats that were pooled by group and sex showed in descending order increased lead levels in liver, kidney, brain, and blood. Urine lead values were similar in all groups.

Statistically significant changes in organ weights after the 90-day exposure period were as follows: male rats—increased liver weights in groups III and V; female rats—decreased liver weights in group IV and increased kidney weights in group V; male monkeys—decreased kidney weights in group IV and increased thyroid weights in groups II and III. When organ weight as a percentage of body weight was examined, statistically significant differences were seen in the following: male

TABLE 3. TERMINAL HEMATOLOGY FOR MALE AND FEMALE SPRAGUE-DAWLEY RATS EXPOSED TO LEADED AND UNLEADED GASOLINE VAPORS OVER A 90-DAY PERIOD

	HTC ^a %	Hgb ^b g/dl	RBC ^c 10 ⁶ /mm ³	MCH ^d pg	MCHC ^e %	MCV ^f cu	Reticulo- cytes %	Thrombo- cytes 10 ³ /mm ³	WBC ^g 10 ³ /mm ³
Males									
Controls	45 ± 0.8 ^h	15.6 ± 0.28	8.06 ± 0.133	19.4 ± 0.28	34.9 ± 0.47	55.3 ± 1.12	0.2 ± 0.04	436 ± 54.2	4.5 ± 0.48
1552 ppm unleaded	44 ± 0.7	16.4 ± 0.48	8.25 ± 0.155	19.3 ± 0.18	35.5 ± 0.29	54.1 ± 0.62	0.3 ± 0.04	712 ± 32.2 ⁱ	5.0 ± 0.43
374 ppm leaded	45 ± 0.7	15.5 ± 0.21	7.92 ± 0.133	19.6 ± 0.21	33.4 ± 0.32 ⁱ	57.9 ± 0.92	0.2 ± 0.04	408 ± 76.3	4.2 ± 0.45
Females									
Controls	42 ± 0.5	14.6 ± 0.20	7.47 ± 0.170	19.7 ± 0.39	35.1 ± 0.58	56.1 ± 1.34	0.2 ± 0.03	420 ± 22.9	4.4 ± 0.29
1552 ppm unleaded	42 ± 0.3	14.9 ± 0.09	7.48 ± 0.129	19.9 ± 0.32	35.4 ± 0.25	55.6 ± 0.68	0.5 ± 0.07 ⁱ	696 ± 63.5	4.1 ± 0.40
374 ppm leaded	46 ± 0.6 ⁱ	14.9 ± 0.22	7.41 ± 0.160	20.1 ± 0.17	32.2 ± 0.49	62.0 ± 1.45 ⁱ	0.2 ± 0.03	495 ± 61.4	3.3 ± 0.24 ⁱ

^aHematocrit count.^bHemoglobin.^cRed blood cell.^dMean corpuscular hemoglobin.^eMean corpuscular hemoglobin concentration.^fMean corpuscular volume.^gWhite blood cell.^hStandard error of the mean.ⁱStatistically significant from control, $P \leq 0.05$, Student's t = test.

GASOLINE SUBCHRONIC TOXICITY

TABLE 4. TERMINAL HEMATOLOGY FOR MALE AND FEMALE MONKEYS EXPOSED TO LEADED AND UNLEADED GASOLINE VAPORS OVER A 90-DAY PERIOD

	HTC ^a %	Hgb ^b g/dl	RBC ^c 10 ⁶ /mm ³	MCH ^d pg	MCHC ^e %	MCV ^f cu	Reticulo- cytes %	Thrombo- cytes 10 ³ /mm ³	WBC ^g 10 ³ /mm ³
Males									
Controls	42 ± 1.6 ^h	12.9 ± 0.53	5.85 ± 0.389	22.3 ± 1.23	31.2 ± 0.13	71.6 ± 3.87	1.0 ± 0.29	338 ± 41.4	4.8 ± 1.13
1552 ppm unleaded	42 ± 1.7	13.7 ± 0.54	6.73 ± 0.357	20.4 ± 0.38	32.7 ± 0.55	62.5 ± 0.84	1.0 ± 0.22	425 ± 115.5	5.7 ± 0.35
374 ppm leaded	44 ± 1.0	13.9 ± 0.36	6.70 ± 0.295	20.7 ± 0.67	31.4 ± 0.98	66.2 ± 1.82	1.0 ± 0.09	355 ± 85.8	7.0 ± 0.93
Females									
Controls	40 ± 1.2	13.0 ± 0.15	6.63 ± 0.086	19.6 ± 0.29	32.6 ± 0.58	60.3 ± 1.77	0.6 ± 0.03	344 ± 56.8	7.7 ± 1.40
1552 ppm unleaded	40 ± 0.6	13.1 ± 0.15	6.20 ± 0.177	21.2 ± 0.81	32.2 ± 0.56	65.9 ± 1.52	1.4 ± 0.26	429 ± 25.6	6.1 ± 1.25
374 ppm leaded	39 ± 1.8	12.8 ± 0.64	6.58 ± 0.250	19.5 ± 1.39	32.7 ± 2.05	59.8 ± 1.31	1.0 ± 0.32	301 ± 16.2	7.7 ± 0.75

^aHematocrit count.

^bHemoglobin.

^cRed blood cell.

^dMean corpuscular hemoglobin.

^eMean corpuscular hemoglobin concentration.

^fMean corpuscular volume.

^gWhite blood cell.

^hStandard error of the mean.

TABLE 5. MEAN PULMONARY FUNCTION DATA (PREEXPOSURE 42 AND 90 DAYS) FOR MALE MONKEYS EXPOSED TO LEADED AND UNLEADED GASOLINE

	Respiratory Rate (breaths/min)	Tidal Volume (ml)	Minute Volume (ml)	Airway Resistance (cm H ₂ O/ml/sec)	Dynamic Compliance Data (ml/cm H ₂ O)	Breaths to 1% Nitrogen
Preexposure						
Control	52 ± 2.8 ^a	9.1 ± 2.10	450 ± 77.2	0.050 ± 0.0179	2.40 ± 0.738	56 ± 8.3
1552 ppm unleaded	46 ± 2.8	8.4 ± 0.64	388 ± 33.7	0.055 ± 0.0170	1.40 ± 0.264	57 ± 3.7
374 ppm leaded	49 ± 5.7	9.4 ± 0.41	452 ± 38.3	0.066 ± 0.0197	2.37 ± 0.222	33 ± 5.9
42 Days						
Control	52 ± 3.3	10.5 ± 0.89	540 ± 50.3	0.032 ± 0.0059	2.42 ± 0.581	40 ± 4.2
1552 ppm unleaded	59 ± 5.2	9.8 ± 1.92	613 ± 160.9	0.040 ± 0.0132	2.04 ± 0.311	42 ± 1.0
374 ppm leaded	56 ± 5.8	7.5 ± 0.46	423 ± 47.2	0.062 ± 0.0204	1.95 ± 0.442	54 ± 5.8
90 Days						
Control	43 ± 5.5	7.7 ± 0.84	318 ± 24.7	0.072 ± 0.0276	2.39 ± 0.630	44 ± 3.2
1552 ppm unleaded	57 ± 2.8	7.5 ± 0.44	431 ± 32.8 ^b	0.046 ± 0.0175	1.39 ± 0.231	57 ± 3.0
374 ppm leaded	54 ± 3.4	9.1 ± 0.82	486 ± 42.2 ^b	0.055 ± 0.0076	1.34 ± 0.203	47 ± 7.0

^aStandard error of the mean.^bStatistically significant from control $P \leq 0.05$ Student's t -test.

GASOLINE SUBCHRONIC TOXICITY

TABLE 6. MEAN PULMONARY FUNCTION DATA (PREEXPOSURE 42 AND 90 DAYS) FOR FEMALE MONKEYS EXPOSED TO LEADED AND UNLEADED GASOLINE

	Respiratory Rate (breaths/min)	Tidal Volume (ml)	Minute Volume (ml)	Airway Resistance (cm H ₂ O/ml/sec)	Dynamic Compliance Data (ml/cm H ₂ O)	Breaths to 1% Nitrogen
Preexposure						
Control	66 ± 4.2 ^a	7.6 ± 0.29	501 ± 47.0	0.065 ± 0.0203	1.74 ± 0.429	49 ± 4.5
1552 ppm unleaded	46 ± 1.5 ^b	9.7 ± 0.66	447 ± 43.8	0.128 ± 0.0473	2.11 ± 0.651	36 ± 6.2
374 ppm leaded	63 ± 3.8	9.1 ± 0.71	580 ± 77.9	0.061 ± 0.0186	1.85 ± 0.137	31 ± 3.9
42 Days						
Control	70 ± 2.7	7.3 ± 0.12	519 ± 25.5	0.111 ± 0.0369	2.01 ± 0.299	48 ± 4.9
1552 ppm unleaded	55 ± 2.2 ^b	6.4 ± 0.75	355 ± 45.6	0.065 ± 0.0111	1.24 ± 0.221	56 ± 8.7
374 ppm leaded	61 ± 5.5	7.4 ± 0.86	468 ± 85.6	0.119 ± 0.0663	2.30 ± 0.870	51 ± 6.6
90 Days						
Control	60 ± 7.2	8.7 ± 0.15	530 ± 68.3	0.024 ± 0.0013	2.09 ± 0.369	50 ± 5.8
1552 ppm unleaded	54 ± 2.9	7.2 ± 0.54	391 ± 12.4	0.065 ± 0.0224	1.91 ± 0.569	47 ± 7.6
374 ppm leaded	64 ± 15.4	6.8 ± 0.54 ^b	448 ± 148.0	0.086 ± 0.0060	3.33 ± 1.184	48 ± 8.0

^aStandard error of the mean.

^bStatistically significant from control $P \leq 0.05$ Student's *t*-test.

TABLE 7. AVERAGE LEAD LEVELS IN BLOOD, URINE, AND SELECTED TISSUES OF SPRAGUE-DAWLEY RATS FOLLOWING 90-DAY EXPOSURE TO LEADED GASOLINE VAPORS^a

Exposure Concentrations ppm	Tissue Lead Levels							
	Brain $\mu\text{g/g}$		Kidney $\mu\text{g/g}$		Liver $\mu\text{g/g}$		Blood $\mu\text{g/g}$	
	Male	Female	Male	Female	Male	Female	Male	Female
Rats								
Controls	1.26	1.44	1.71	2.97	0.71	1.21	0.61	0.24
374 leaded	9.49	5.39	12.40	9.57	17.90	19.70	6.10	1.32
103 leaded	7.23	2.32	7.06	13.00	6.51	8.41	0.77	0.46

^aSamples were pooled by group and by sex, thus the data represent an average lead concentration.

GASOLINE SUBCHRONIC TOXICITY

rats—decreased heart weights in groups IV and V and decreased brain weights in groups II and III; female rats—decreased liver weights in group IV and decreased adrenal weight in group II; and male monkeys—decreased kidney weights in group III.

Examination of tissues from rats and squirrel monkeys showed no evidence of treatment-related histopathology, with the exception of lesions noted in the kidneys of all male rats. The lesions were characterized by subtle but discernible increases in the incidence and severity of regenerative epithelium and dilated tubules. The latter were seen to contain protein in their lumens.

DISCUSSION

Gasoline vapors of 1552 ppm (unleaded) and 384 ppm (leaded) had no significant toxic effect on rats and monkeys exposed for 90 days as evidenced by body weight changes. The cause of death for the few animals that died during the study were not considered treatment related.

Sterner (1941) published the results of an extensive survey of workers in a spray-painting operation where gasoline was used as a diluent. The exposure-related effects included decreases in hemoglobin concentration, erythrocyte count, and cell volume, as well as increases in mean corpuscular hemoglobin, mean corpuscular volume, and reticulocyte counts. Although some of the hematological changes reported by Sterner were observed in the study reported in this paper, they were only seen in rats and were distributed in a random fashion between the various exposure groups and sexes. The patterns of hematological responses noted in Sterner's study, as well as in this report, are similar to those reported by Carpenter et al. (1975a,b, 1977) and are all within normal limits.

Eye irritation has been implicated with a number of petroleum hydrocarbons (Gage, 1970; Heuss and Glasson, 1968). These studies were both acute and subchronic in duration, with exposures ranging from 2 to 6000 ppm. Further investigations of petroleum hydrocarbon-induced eye irritation were conducted by Gamberale and Hulfengren (1972). Using humans as test subjects, these investigators demonstrated a statistically significant impairment in reaction time (visual flash evoked responses) and perceptual speed after 4 20-minute exposures to 300 and 700 ppm toluene. Abdel-Rahman et al. (1976) and Schaumburg and Spencer (1978) have reported that 1000 ppm of methyl n-butyl ketone, a metabolite of n-hexane, produces an increase in the latency of visual evoked response (VER). Fox et al. (1977) showed similar findings in rat pups receiving lead in milk. Since toluene, n-hexane, and lead are typical components in gasoline, the study of VER in squirrel monkeys exposed to leaded and unleaded fuel was considered appropriate.

Although the use of VER in the study of neurotoxicity in the central nervous system is still being developed, it is a relatively sensitive noninvasive test to assess the functional capacity of the optic nerve and the cerebral cortex. Results of the VER tests in this study are negative, suggesting that the fuels as tested are devoid of neurotoxicity.

Both Swann et al. (1974) and Gage (1970) have demonstrated a variety of petroleum hydrocarbons to be respiratory irritants following acute exposures. Lykke and Stewart (1978) showed that subchronic exposure to leaded gasoline produce interstitial fibrosis of the lung. In order to investigate the respiratory effects of exposing monkeys subchronically to leaded and unleaded gasoline, pulmonary function data were obtained after the 90-day exposure period. The data showed increased minute volumes in male monkeys in both high-level fuel groups. A decrease in tidal volume for female monkeys in the high-level leaded group was also noted. As with the hematological values, the lack of clear and/or consistent effects within or between species does not support a conclusion of treatment-related effects in the lungs of the animals. The large variability observed in the control group across time tends to obscure any meaningful comparison between the control and treated animals.

Beirne and Brennan (1972) have implicated some petroleum products (solvents) in antibody-mediated glomerulonephritis (Goodpasture's syndrome). No evidence of IgG deposition was found in the renal glomerulus in either rats or monkeys exposed to gasoline vapors in this study. Since the lung has also been reported (Beirne and Brennan, 1972) as a possible target organ for Goodpasture's syndrome, lung tissues were reviewed. There was no evidence of IgG deposition.

KUNA AND ULRICH

These study results would suggest that hydrocarbons as found in the tested fuels are devoid of any immunotoxic effects in kidney and lung tissue.

Rats exposed to gasoline gave no evidence of any abnormal histopathological findings, with the exception of the lesions noted in the male rat kidneys. These findings have been noted previously by Gaworski et al. (1980, 1982) and Phillips and Egan (1984) and were also noted in the Carpenter study series (1975a,b, 1977).

The renal lesions in the gasoline study are mild and do not suggest any significant compromise in kidney function. The impact of these changes for man, however, are still to be determined.

The study presented in this paper was designed to evaluate the effects of a 90-day exposure of rats and monkeys to leaded and unleaded gasoline vapor. A variety of parameters were monitored, and there appeared to be a few sporadic incidences of statistically significant physiological changes. No clear patterns emerged, which might suggest significant biological effects in any measured parameters due to exposures to gasoline.

ACKNOWLEDGMENT

This investigation was sponsored by the American Petroleum Institute, 1220 L Street NW, Washington, DC. 20005. The authors wish to acknowledge the efforts of the secretaries and staff of the Toxicology Section, Research and Environmental Health Division, Exxon Corporation Medicine and Environmental Health Department for their support in the preparation of this manuscript.

REFERENCES

- ABDEL-RAHMAN, M.S., SALADIN, J.J., BOHMAN, C.E., and COURI, D. (1978). The effect of 2-hexanone and 2-hexanone metabolites on pupillomotor activity and growth, *Am. Ind. Hygiene Assoc. J.* **39**, 94-99.
- ALARIE, Y., KRUMM, A.A., JENNINGS, H.J., and HADDOCK, R.H. (1971). Distribution of ventilation in *Cynomolgus* monkeys. *Arch. Environ. Health* **22**, 633-642.
- BEIRNE, G.J., and BRENNAN, J.T. (1972). Glomerulonephritis associated with hydrocarbon solvents. *Arch. Environ. Health* **25**, 365-369.
- CARPENTER, C.P., KINKEAD, E.R., GEARY, Jr. D.L., SULLIVAN, L.J., and KING, J.M. (1975a). Petroleum hydrocarbon toxicity studies. III. Animal and human response to vapors of Stoddard solvent. *Toxicol. Appl. Pharmacol.* **32**, 282-297.
- CARPENTER, C.P., KINKEAD, E.R., GEARY, Jr. D.L., SULLIVAN, L.J., and KING, J.M. (1975b). Petroleum hydrocarbon toxicity studies. VI. Animal and human responses to vapors of "60 Solvent." *Toxicol. Appl. Pharmacol.* **34**, 374-394.
- CARPENTER, C.P., GEARY, D.L., Jr., MYERS, R.C., NACHREINER, D.J., SULLIVAN, L.J., and KING, J.M. (1977). Petroleum hydrocarbon toxicity studies. XV. Animal response to vapors of "high naphthenic solvent." *Toxicol. Appl. Pharmacol.* **41**, 251-260.
- CROSSON, J.T., LUBOWITZ, H., MAZUMDAR, D.C., WEISSEN, F., LANG, S., ROLF, D., and GERMUTH, F.G. (1974). The production of experimental glomerulonephritis in the rat. *Arch. Pathol.* **98**, 344-350.
- DIXON, F.J. (1979). Scripps Clinic and Research Foundation, La Jolla, California, Personal communication.
- DRINKER, P., YAGLOU, C.P., and WARREN, M.F. (1943). The threshold of toxicity of gasoline vapor. *J. Ind. Hyg. Toxicol.* **25**, 225-232.
- ETHYL CORPORATION: Publication 72-9, *Analysis for Trace Quantities of Lead*.
- FOX, D.A., LEWKOWSKI, J.P., and COOPER, G.P. (1977). Acute and chronic effects of neonatal lead exposure on development of the visual evoked response in rats. *Toxicol. Appl. Pharmacol.* **40**, 449-461.
- GAGE, J.C. (1970). The subacute inhalation toxicity of 109 industrial chemicals. *Br. J. Ind. Med.* **27**, 1-18.
- GAMBERALE, F., and HULFENGREN, M. (1972). Toluene exposure. II. Physiological functions. *Work Environ. Health* **9**, 131-139.
- GAWORSKI, C.L., LEAHY, H.F., and BRUNER, R.H. (1980). Subchronic inhalation toxicity of decalin. *Proceedings of the Tenth Conference on Environmental Toxicology*. pp. 226-237.
- GAWORSKI, C.L., MacEWEN, J.D., VERNOT, E.H., BRUNER, R.H., and COWAN, M.J. (1982). Comparison of the subchronic inhalation toxicity of petroleum and oil shale JP-5 jet fuels. In: *The Toxicology of*

GASOLINE SUBCHRONIC TOXICITY

- Petroleum Hydrocarbons*, H.N. MacFarland, C.E. Holdsworth, J.A. MacGregor, R.W. Call, and M.L. Kane, (eds). pp. 67-75.
- HAGGARD, H.W. (1921). The anesthetic and convulsant effects of gasoline vapor. *J. Pharmacol. Exp. Ther.* **16**, 401-404.
- HEUSS, J.M., and GLASSON, W.A. (1968). Hydrocarbon reactivity and eye irritation. *Environ. Sci. Technol.* **2**, 1109-1116.
- LYKKE, A.W.J., and STEWART, B.W. (1978). Fibrosing alveolitis (pulmonary interstitial fibrosis) evoked by experimental inhalation of gasoline vapors. *Experimentia* **34**, 498.
- MACHLE, W. (1941). Gasoline intoxication. *JAMA* **117**, 1965-1971.
- MUFTUOGLU, A.U., ERBENZI, T., KASMANCI, M., KARAZEL, T., GURSOY, E., and TAHSINOGLU, M. (1975). Acute glomerulonephritis. *Am. J. Clin. Pathol.* **63**, 300-309.
- PHILLIPS, R.D., and EGAN, G.F. (1984). Effect of C₁₀-C₁₁ isoparaffinic solvent on kidney function in Fischer 344 rats during eight weeks of inhalation. *Toxicol. Appl. Pharmacol.* **73**, in press.
- PURDUE, L.J., ENRIONE, R.E., THOMPSON, R.J., and BONFIELD, B.A. (1973). Determination of organic and total lead in the atmosphere by atomic absorption spectrometry. *Anal. Chem.* **45**, 527-530.
- ROBINSON, R.O. (1978). Tetraethyl lead poisoning from gasoline sniffing. *JAMA* **240**, 1373-1374.
- SCHAUMBERG, H.H., and SPENCER, P.S. (1978). Environmental hydrocarbons produce degeneration in cat hypothalamus and optic tract. *Science* **199**, 199-200.
- STERNER, J.H. (1941). Study of hazards in spray painting with gasoline as a diluent. *J. Ind. Hyg. Toxicol.* **23**, 437-448.
- SWANN H.E., Jr., KWON, B.K., HOGAN, G.K., and SNELLINGS, W.M. (1974). Acute inhalation toxicology of volatile hydrocarbons. *Am. Ind. Hygiene Assoc. J.* **35**, 511-518.
- VALPEY, R., SUMI, S.M., COPASS, M.K., and GOBLE, G.J. (1978). Acute and chronic progressive encephalopathy due to gasoline sniffing. *Neurology* **28**, 507-510.
- WILSON, C.B., and DIXON, F.J. (1973). Anti-glomerular basement membrane antibody-induced glomerulonephritis. *Kidney Int.* **3**, 74-89.

Address reprint requests to:

R.A. Kuna
Exxon Corporation Medicine & Environmental Health Department
Research & Environmental Health Division
PO Box 235, Mettlers Road
East Millstone, NJ 08873

Submitted January 18, 1983

Accepted May 29, 1984

FINAL REPORT

PROJECT NUMBER 745-096

EVALUATION OF THE THREE MONTH
INHALATION TOXICITY OF TWO MOTOR FUELS

SUBMITTED TO:

James M. McNerney, M.P.H.
Staff Toxicologist
Environmental Affairs - Medical
American Petroleum Institute
2101 L Street, N.W.
Washington, D.C. 20006

SUBMITTED BY:

Huntingdon Research Center
216 Congers Road
New City, New York 10956

PRINCIPAL INVESTIGATOR:

Charles E. Ulrich
Manager
Inhalation Toxicology Department
April 14, 1976

APPROVED BY:

Charles O. Ward, Ph.D.
Director, Toxicology

TABLE OF CONTENTS

	<u>Pages</u>
I. INTRODUCTION	1 - 5
II. PURPOSE	6
III. EXPERIMENTAL DESIGN	6
IV. MATERIALS	6
A. Experimental Compounds	6 - 8
B. Animals	8 - 9
V. EXPERIMENTAL METHODS	9
A. Exposure Methods	9 - 10
B. Vapor Generation Methods	10 - 12
C. Analytical Methods	12
1. Methods for total gasoline vapors	12 - 16
2. Determination of alkyl lead in gasoline	16 - 18
3. Determination of vapor phase alkyl lead	18 - 20
4. Methods for gas chromatographic analysis of gasoline	20 - 21
D. Hematological Methods	21 - 22
E. Pulmonary Physiology Methods	22
F. Flash Evoked Response Methods	22 - 24
G. Urinalysis Methods	24
H. Renal Immunofluorescent Methods	24 - 25
I. Necropsy and Histopathology Methods	25 - 26

FIGURES

Figure 1 - Diagram of Exposure System

Figure 2 - Schematic of Gasoline Vapor Generation System

Figure 3 - Temperature Calibration For Gasoline Vapor Generation System

TABLE OF CONTENTS

Pages

FIGURES

- Figure 4 - Calibration of FMI Model G6 Pump
- Figure 5 - Calibration of FMI Model G20 Fluid Pump
- Figure 6 - Calibration of FMI Model G50 Fluid Pump
- Figure 7 - N₂ Flowrate Vs. Pressure for Spraying Systems Atomizer
- Figure 8 - Chamber Concentration Equilibration Profile
- Figure 9 - Schematic Diagram of Gasoline Vapor Calibration System
- Figure 10 - Temperature Calibration for Vaporization System Variac
Setting @ 70V From 0 to 12.5 min. Variac Off After 12.5 min.
- Figure 11 - Calibration Curve for Fuels A and B as Measured On Total
Hydrocarbon Analyzer
- Figure 12 - Typical Daily Record Of Chamber Concentrations
- Figure 13 - Gas Chromatogram of Fuel A
- Figure 14 - Gas Chromatogram of Fuel B
- Figure 15 - Gas Chromatogram of Reference Gasoline
- Figure 16 - Example of Computer Print-Out From Pulmonary Function Testing System
- Figure 17 - Flash Evoked Response Data From Monkey No. 118 Exposed to 1.8 mg/L
Fuel B For Six Hours
- Figure 18 - Immunoelectrophoresis To Demonstrate That Serum Conjugate Reacts
With Monkey Serum

VI. RESULTS	27
A. Exposure Concentrations	27 - 28
B. Pharmacotoxic Signs	29
C. Body Weights	29
1. Rats	29 - 30
2. Monkeys	30

TABLE OF CONTENTS

	<u>Pages</u>
D. Food Consumption	30
1. Rats	30
E. Hematologic Evaluations	31
1. Rats	31
2. Monkeys	31
F. CNS Evaluations	32
G. Pulmonary Function Evaluations	32
1. Monkeys	32 - 33
H. Urine Lead Evaluations	33
1. Rats	33
2. Monkeys	34
I. Tissue Lead Evaluations	34 - 35
J. Renal Immunofluorescent Evaluations	35
K. Urinalysis Evaluations	35
1. Rats	35
2. Monkeys	35
L. Organ Weight Data	35
1. Rats	35 - 36
2. Monkeys	36
M. Histopathology	36
VII. SUMMARY	37 - 38
REFERENCES	

TABLE OF CONTENTS

Pages

FIGURES

- Figure 1 - Mean Body Weight Data For Control Group and Group II
Exposed To 6.35 mg/L Fuel A - Rats
- Figure 2 - Mean Body Weight Data For Control Group and Group III
Exposed to 1.57 mg/L Fuel A - Rats
- Figure 3 - Mean Body Weight Data For Control Group and Group IV
Exposed to 1.53 mg/L Fuel B - Rats
- Figure 4 - Mean Body Weight Data For Control Group and Group V
Exposed to 0.42 mg/L Fuel B - Rats
- Figure 5 - Mean Body Weight Data For Control Group and Group II
Exposed to 6.35 mg/L Fuel A - Monkeys
- Figure 6 - Mean Body Weight Data For Control Group and Group III
Exposed to 1.57 mg/L Fuel A - Monkeys
- Figure 7 - Mean Body Weight Data For Control Group and Group IV
Exposed to 1.53 mg/L Fuel B - Monkeys
- Figure 8 - Mean Body Weight Data For Control Group and Group V
Exposed to 0.42 mg/L Fuel B - Monkeys
- Figure 9 - Daily Group Mean Food Consumption Data For Control Group and Group II
Exposed to 6.35 mg/L Fuel A - Rats
- Figure 10 - Daily Group Mean Food Consumption Data For Control Group and Group III
Exposed to 1.57 mg/L Fuel A - Rats
- Figure 11 - Daily Group Mean Food Consumption Data For Control Group and Group IV
Exposed to 1.53 mg/L Fuel B - Rats
- Figure 12 - Daily Group Mean Food Consumption Data For Control Group and Group V
Exposed to 0.42 mg/L Fuel B - Rats
- Figure 13 - Mean Hematocrit Data For Male and Female Squirrel Monkeys
- Figure 14 - Mean Hemoglobin Concentration Data For Male and Female Squirrel Monkeys
- Figure 15 - Mean Erythrocyte Counts For Male and Female Squirrel Monkeys
- Figure 16 - Average Mean Corpuscular Hemoglobin Data For Male and Female Squirrel
Monkeys



Documents

Document Sharing Hub

TABLE OF CONTENTS

Pages

FIGURES

- Figure 17 - Average Mean Corpuscular Hemoglobin Concentration Data for Male and Female Squirrel Monkeys
- Figure 18 - Average Mean Corpuscular Volume Data for Male and Female Squirrel Monkeys
- Figure 19 - Mean Reticulocyte Counts for Male and Female Squirrel Monkeys
- Figure 20 - Mean Platelet Counts for Male and Female Squirrel Monkeys
- Figure 21 - Mean Leukocyte Counts for Male and Female Squirrel Monkeys
- Figure 22 - Mean Respiratory Rate Data for Male and Female Squirrel Monkeys
- Figure 23 - Mean Tidal Volume Data for Male and Female Squirrel Monkeys
- Figure 24 - Mean Minute Volume Data for Male and Female Squirrel Monkeys
- Figure 25 - Mean Airway Resistance Data for Male and Female Squirrel Monkeys
- Figure 26 - Mean Dynamic Compliance Data for Male and Female Squirrel Monkeys
- Figure 27 - Mean Breaths to 1% Nitrogen Data For Male and Female Squirrel Monkeys

TABLES

- Table 1 - Weekly Mean Exposure Concentrations for Each Group
- Table 2 - Individual and Mean Hematology Data for Male Rats after 3 Months of Exposure
- Table 3 - Individual and Mean Hematology Data for Female Rats after 3 Months of Exposure
- Table 4, 4A - Statistical Summary of Hematology Data for Male Rats after 3 Months of Exposure
- Table 5, 5A - Statistical Summary of Hematology Data for Female Rats after 3 Months of Exposure
- Table 6 - Individual and Mean Hematology Data for Male Monkeys Prior To Exposure
- Table 7, 7A, 7B - Statistical Summary of Hematology Data For Male Monkeys Prior To Exposure



Documents

Document Sharing Hub

TABLE OF CONTENTS

Pages

TABLES

Table 8 - Individual and Mean Hematology Data for Female Monkeys Prior To Exposure	
Table 9, 9A, 9B - Statistical Summary of Hematology Data For Female Monkeys Prior To Exposure	
Table 10 - Individual and Mean Hematology Data for Male Monkeys after 1½ Months of Exposure	
Table 11, 11A - Statistical Summary of Hematology Data for Male Monkeys after 1½ Months of Exposure	
Table 12 - Individual and Mean Hematology Data for Female Monkeys after 1½ Months of Exposure	
Table 13, 13A - Statistical Summary of Hematology Data for Female Monkeys after 1½ Months of Exposure	
Table 14 - Individual and Mean Hematology Data for Male Monkeys after 3 Months of Exposure	
Table 15, 15A - Statistical Summary of Hematology Data for Male Monkeys after 3 Months of Exposure	
Table 16 - Individual and Mean Hematology Data for Female Monkeys after 3 Months of Exposure	
Table 17, 17A - Statistical Summary of Hematology Data for Female Monkeys after 3 Months of Exposure	
Table 18 - Flash Evoked Response Data for Monkeys in Group I - Control	
Table 19 - Flash Evoked Response Data for Monkeys in Group II Exposed to Approximately 6.5 mg/L Fuel A	
Table 20 - Flash Evoked Response Data For Monkeys in Group IV Exposed to Approximately 1.6 mg/L Fuel B	
Table 21 - Individual and Mean Pulmonary Function Data for Male Monkeys Prior to Exposure	
Table 22 - Individual and Mean Pulmonary Function Data for Female Monkeys Prior to Exposure	
Table 23, 23A - Statistical Summary of Pulmonary Function Data for Male Monkeys Prior To Exposure	

TABLE OF CONTENTS

Pages

TABLES

- Table 24, 24A - Statistical Summary of Pulmonary Function Data For Female Monkeys Prior to Exposure
- Table 25 - Individual and Mean Pulmonary Function Data for Male Monkeys After 1½ Months of Exposure
- Table 26 - Individual and Mean Pulmonary Function Data for Female Monkeys After 1½ Months of Exposure
- Table 27 - Statistical Summary of Pulmonary Function Data for Male Monkeys After 1½ Months of Exposure
- Table 28 - Statistical Summary of Pulmonary Function Data for Female Monkeys After 1½ Months of Exposure
- Table 29 - Individual and Mean Pulmonary Function Data for Male Monkeys After 3 Months of Exposure
- Table 30 - Individual and Mean Pulmonary Function Data for Female Monkeys After 3 Months of Exposure
- Table 31 - Statistical Summary of Pulmonary Function Data for Male Monkeys After 3 Months of Exposure
- Table 32 - Statistical Summary of Pulmonary Function Data for Female Monkeys After 3 Months of Exposure
- Table 33 - Urine Lead Data for Rats in the Control and Exposed Groups After & 33A 1½ Months of Exposure
- Table 34, 34A - Urine Lead Data for Rats in the Control and Exposed Groups After 3 Months of Exposure
- Table 35 - Urine Lead Data for Monkeys in the Control and Exposed Groups at the Pre-Exposure Interval
- Table 36 - Urine Lead Data for Monkeys in the Control and Exposed Groups after 1½ Months of Exposure
- Table 37 - Urine Lead Data for Monkeys in the Control and Exposed Groups after 3 Months of Exposure



Documents
Document Sharing Hub

TABLE OF CONTENTS

Pages

TABLES

- Table 38, 39, 40 - Immunofluorescent Staining Results on Rat and Squirrel Monkey Renal Tissue for IgG Deposition
- Table 41 - Urinalysis Data for Male and Female Rats in Group I after Exposure to Filtered Air for 1½ Months
- Table 42 - Urinalysis Data for Male and Female Rats in Group II after Exposure to 6.35 mg/L Fuel A for 1½ Months
- Table 43 - Urinalysis Data for Male and Female Rats in Group III after Exposure to 1.57 mg/L Fuel A After 1½ Months
- Table 44 - Urinalysis Data for Male and Female Rats in Group IV after Exposure to 1.53 mg/L Fuel B for 1½ Months
- Table 45 - Urinalysis Data for Male and Female Rats in Group V after Exposure to 0.42 mg/L Fuel B for 1½ Months
- Table 46 - Urinalysis Data for Male and Female Rats in Group I after Exposure to Filtered Air for 3 Months
- Table 47 - Urinalysis Data for Male and Female Rats in Group II after Exposure to 6.35 mg/L Fuel A for 3 Months
- Table 48 - Urinalysis Data for Male and Female Rats in Group III after Exposure to 1.57 mg/L Fuel A for 3 Months
- Table 49 - Urinalysis Data for Male and Female Rats in Group IV after Exposure to 1.53 mg/L Fuel B for 3 Months
- Table 50 - Urinalysis Data for Male and Female Rats in Group V after Exposure to 0.42 mg/L Fuel B for 3 Months
- Table 51 - Urinalysis Data For Male Squirrel Monkeys - Pre-Exposure
- Table 52 - Urinalysis Data for Female Squirrel Monkeys - Pre-Exposure
- Table 53 - Urinalysis Data for Male Squirrel Monkeys after 1½ Months of Exposure
- Table 54 - Urinalysis Data for Female Squirrel Monkeys after 1½ Months of Exposure
- Table 55 - Urinalysis Data for Male Squirrel Monkeys after 3 Months of Exposure
- Table 56 - Urinalysis Data for Female Squirrel Monkeys after 3 Months of Exposure



TABLE OF CONTENTS

Pages

TABLES

Table 57 - Individual and Mean Organ Weight Data for Male Rats after 3 Months of Exposure

Table 58 - Individual and Mean Organ Weight Data for Female Rats after 3 Months of Exposure

Table 59, 59A, 59B - Statistical Summary of Organ Weight Data for Male Rats After 3 Months of Exposure

Table 60, 60A, 60B - Statistical Summary of Organ Weight Data for Female Rats After 3 Months of Exposure

Table 61, 61A, 61B - Statistical Summary of Organ Weight as a Percentage of Body Weight for Male Rats after 3 Months of Exposure

Table 62, 62A, 62B - Statistical Summary of Organ Weight as a Percentage of Body Weight for Female Rats after 3 Months of Exposure

Table 63 - Individual and Mean Organ Weight Data for Male Monkeys after 3 Months of Exposure

Table 64 - Individual and Mean Organ Weight Data for Female Monkeys after 3 Months of Exposure

Table 65, 65A, 65B - Statistical Summary of Organ Weight Data for Male Monkeys After 3 Months of Exposure

Table 66, 66A, 66B - Statistical Summary of Organ Weight Data for Female Monkeys After 3 Months of Exposure

Table 67, 67A, 67B - Statistical Summary of Organ Weight As a Percentage of Body Weight for Male Monkeys after 3 Months of Exposure

Table 68, 68A, 68B - Statistical Summary of Organ Weight As a Percentage of Body Weight for Female Monkeys after 3 Months of Exposure



Documents
Document Sharing Hub

I. INTRODUCTION

Gasolines are exceedingly complex mixtures of aromatic hydrocarbons, cycloparaffins, and alkanes distributed over the C_4 to C_{11} range. The exact composition of gasolines varies widely depending on the crude oil source, the refining method, and the intended ultimate use - among other variables. In addition to the basic refined composition, other organic and organo-metallic compounds are added to produce desired handling, storage, and combustion results. Thus even brief review of the composition of gasoline produces a number of compounds which may have toxic properties individually or in combination with some other constituents.

A brief review of the literature reveals very few in-depth evaluations of the chronic toxicity of gasoline vapors, particularly in recent years. This may, in part, be due to the fact that except for acute high level exposures, gasoline vapors have been considered to be rather nontoxic. The work of Haggard ⁽¹⁾ served to define the very acute anesthetic and toxic effects of gasoline vapors in dogs. Central nervous system (CNS) effects were observed at about 10,000 ppm (1%), and death was observed at about 25,000 ppm (2.5%). Sterner ⁽²⁾ published the results from an extensive survey of workers in a spray painting operation where gasoline was used as a diluent. A review of the routine physical examinations of the employees of the plant over approximately 10 years did not reveal any exposure-related effects, except sporadic CNS and gastrointestinal complaints associated with unusual exposure conditions. However, an in-depth analysis of the hematology data for the exposed workers compared to a suitable control group did reveal

exposure-related effects. Specifically, a decrease in hemoglobin concentration; a decrease in erythrocyte count (RBC) a decrease in cell volume; an increase in mean corpuscular hemoglobin (MCH); an increase in mean corpuscular volume (MCV); and an increase in reticulocytes were evident in the exposed workers as a group. Typical concentrations of total aromatics, to which these workers were exposed, ranged from 200 to 800 ppm as reported from simulation experiments. An excellent and extensive review of gasoline intoxication has been published by Machle ⁽³⁾. Machle pointed out the confusion in earlier literature between the toxic manifestations of benzene (benzol) and gasoline, as have others ⁽⁴⁾. The chronic exposure to low concentrations of gasoline vapor was described as producing the following symptoms: anorexia, nausea, nervousness, headaches, conjunctival irritation, vertigo, drowsiness, muscular weakness, and muscle tremor. The study of Drinker, et al. ⁽⁵⁾ defined the lower limit of gasoline vapor exposure which would elicit symptoms and signs of irritation or toxicity in human subjects. One of the significant attributes of this study was the information of composition and concentration of the gasoline and the resulting vapors. The results of this study indicated that symptoms - i.e., eye irritation - were evident at exposure concentrations of 700 ppm (0.07%); and that at about 2,500 ppm (0.25%) CNS effects were evident. Much of the early literature on the toxicity of gasoline involves the hazards associated with aspiration. Zucker ⁽⁶⁾ et al. has reviewed much of this literature and concluded that respiratory symptoms develop within a few hours with a predilection for the lower lobe of the right lung; also, that fever and leukocytosis are commonly observed. Amorati ⁽⁷⁾ et al. reported on the examination of women employed for a number of years in an occupation where exposure to relatively low concentrations of gasoline vapors was probable.

Approximately 1/3 of the 39 workers revealed hypochromic anemia; however, all the workers exhibited granulocytes with Mosen granules. Davis (8) has reported on the subjective responses of volunteers exposed for a short period, i.e. 30 minutes, to concentrations of 200, 500, or 1,000 ppm (0.02, 0.05, and 0.1%) of gasoline vapor. The only significant effect observed was eye irritation, which was related on a quantal basis to the exposure concentrations.

Summarizing then, the probable effects of chronic low level exposure to gasoline vapors are: eye irritation; CNS effects; gastrointestinal system involvement, possibly secondary to the CNS effects; and effects on the hematopoietic system.

The effects on the hematopoietic system, as outlined above, are very similar to those observed from the aromatic hydrocarbons, such as benzene and the xylenes (9). Due to benzene's exceptional activity in this regard, it is important to thoroughly evaluate the hematopoietic system in any chronic gasoline exposures - even though modern gasolines may contain a relatively small amount of benzene (0.5 to 1.0%).

Although CNS involvement is unanimously described as one of the major toxic effects of exposure to gasoline vapors in humans and animals alike, this cursory literature review did not reveal any quantitative information on this subject. There is, however, considerable information - from both behavioral and electrophysiologic standpoints - on individual hydrocarbons and particularly on those used as anesthetic agents (10,11,12,13). Therefore,

it is imperative that a comprehensive chronic inhalation study, oriented toward occupational exposures, should include a quantitative evaluation of CNS effects.

Eye irritation is also almost unanimously reported to be associated with exposure to gasoline vapors. Since eye irritation is not a serious "toxic" response, an in-depth evaluation of the parameter is not considered necessary for the evaluation of the chronic inhalation toxicity of gasoline.

Contact dermatitis, allergic sensitization, and photosensitizing effects have been reported as occurring from contact with gasoline ^(4,18). However, the contact dermatitis resulting from defatting of the skin is probably the most significant problem.

There are a number of less well-documented toxic effects which are attributed to gasoline or hydrocarbon solvents in general. Chenoweth ⁽¹⁴⁾ has reported that gasoline vapors at 80,000 ppm (8%) or less will sensitize the dog heart to exogenous epinephrine, resulting in ventricular fibrillation; and in some cases, death. Heptane and benzene, at approximately 50,000 ppm (5%), also induced cardiac sensitization. Bierne ⁽¹⁵⁾ has reported an immunologically mediated glomerulonephritis associated with hydrocarbon solvents. He referred to the work of Klavis and Drommer ⁽¹⁶⁾ who evidently induced glomerular damage in rats by chronic exposure to gasoline vapors. Therefore, the possibility that gasoline exposure may induce Goodpasture's syndrome should not be overlooked in any chronic exposure study. Although effects on the

respiratory system are not commonly encountered with inhalation of gasoline vapors, they are routinely observed with Goodpasture's syndrome (17). In addition, because gasoline is an excellent lipid solvent, it is not unreasonable to hypothesize an effect on the surfactant layer of the alveoli. Therefore, a chronic study of gasoline vapors should include an evaluation of the respiratory system.

II. PURPOSE

The purpose of this experiment was to evaluate the subacute inhalation toxicity of two different gasolines in a rodent and non-rodent species; with the intention of obtaining information relating to the occupational situation.

III. EXPERIMENTAL DESIGN

Five (5) groups of animals were used for this experiment. Four (4) groups were exposed to the gasoline vapors, while the fifth group served as an unexposed control. Two of the exposure groups were exposed to vapors of one type gasoline at two different concentrations, while the other two exposure groups were exposed to vapors of a second type gasoline at two different concentrations. Each group consisted of twenty (20) male and twenty (20) female rats and four (4) male and four (4) female monkeys. The duration of the exposures was six (6) hours per day, five (5) days per week for thirteen (13) weeks. Prior to the exposures and after one and a half (1½) and three (3) months of exposure, various observations were conducted on the experimental and control animals. Immediately after the three-month exposure, all monkeys were sacrificed; while only half of the rats from each group were sacrificed. The remaining rats in each group were sacrificed either five (5) or ten (10) months post-exposure. Table No. 1 summarizes the experimental design.

IV. MATERIALS

A. Experimental Compounds

The experimental compounds were supplied by the sponsor as two fifty-five (55) gallon drums of each gasoline. The drums were identified as follows:

HRC I.D.

Fuel A

Fuel B

Drum Identification

Gasoline NDI (Class 65)
Octane 93½

0342 Fire Chief Gas

Fuel A was a non-leaded EPA reference fuel while Fuel B was a commercial leaded fuel. Analytical data, as provided by the sponsor, is presented below:

Fuel A

Research Octane Number	93.2
Motor Octane Number	84.7
Ron-Mon	8.5
Reid Vapor Pressure, psia	10.2
Distillation, ASTM D-86 °F	
10%	123
50%	199
95%	325
100%	383
API Gravity @ 60°F	61.6
FIA Analysis	
Aromatics, %	24.0
Olefins, %	8.3
Paraffins, %	67.7

ASTM Gum, mg/100 ml	0.57
Stability, hrs.	244
Sulfur, ppm	127
Phosphorous, ppm	1
Lead, gm/gallon	0.00004
Diene Number, meq./liter	0.0
Fuel Composition, LV%	
Benzene	0.1
Toluene	8.1
n-Butane	8.0
Isopentane	8.3
n-pentane	5.4

Fuel was inhibited with 5 lbs/1000 bbls of Dupont 22 oxidation inhibitor.

Fuel B

Data not available as of March 20, 1976.

The fuels were stored in a covered outside enclosure. Dry nitrogen was used to replace fuel as it was drawn from the drums. A positive pressure of approximately twenty-five (25) inches of water was maintained at all times. Samples were transferred to the laboratory in one (1) gallon stainless steel solvent storage cans.

B. Animals

One hundred (100) male and one hundred (100) female albino rats of the Sprague-Dawley strain, weighing between 100 and 150 grams, were obtained from Charles River Breeding Laboratories. The rats were randomly divided into five (5) groups of twenty (20) males and twenty (20) females. They were caged in groups of twenty in stainless steel

wire mesh cages with food and tap water available *ad libitum*. The rats were permitted two (2) weeks to acclimatize to the laboratory environment prior to the commencement of exposures.

Thirty (30) male and thirty (30) female squirrel monkeys (*Saimiri sciureus*), weighing between 0.5 and 1.0 kg, were obtained from Prim Labs (Monmouth County Airport, Farmingdale, New Jersey). Upon receipt, each monkey was weighed and marked with an identifying number. All monkeys were observed for an eight-week period. During this time, they were tuberculin tested twice (250 T.U. in the palpebral fold); treated with an antihelminthic (thiabendazole 100 mg/kg); and given two general physical examinations. Unhealthy monkeys were removed from consideration for the study.

Forty (40) healthy monkeys were selected and randomly divided into five (5) groups, each containing four (4) males and four (4) females. The monkeys were caged in pairs in stainless steel cages with food and tap water available *ad libitum*. Purina monkey chow was provided twice daily.

V. EXPERIMENTAL METHODS

A. Exposure Methods

The five groups of rats and monkeys were each housed in one-cubic meter glass and stainless steel exposure chambers. Each of the five chambers contained eight (8) monkeys and forty (40) rats. Figure No. 1 illustrates the distribution of animals in each chamber. The animals were housed in the chambers twenty-four (24) hours a day and only removed for daily feeding and cleaning.

Each chamber was provided with a positive displacement Roots Whispair® blower which provided approximately 250 L/min of chamber ventilation. This ventilation rate results in the chambers operating under a negative pressure of approximately 0.3 in. of water. Each chamber is provided with an absolute filter such that inlet air is particulate free (99.9% at 0.3 μ). Air flow through the chamber was continuously monitored as the pressure drop across the inlet filter, which was connected to an alarm system as protection against power failure or pump breakdown.

B. Vapor Generation Methods

Design of an adequate vapor generation system for gasoline is complicated by the wide range of boiling points for the various constituents (32⁰ to 250⁰C) and the obvious explosion hazard. After considerable evaluation the following design approach was decided upon:

- Atomization was utilized to increase the surface area and thus reduce the temperature required to achieve adequate vaporization.
- Nitrogen was utilized in the pneumatic side of the atomizer in order to reduce explosion hazards.
- The nitrogen was heated to a temperature of 105⁰C in order to assist vaporization and to compensate for the cooling inherent with vaporization.
- Transition through the explosive range should occur as rapidly as possible and in as small a volume as possible.

Figure No. 2 presents a diagram of the gasoline vaporization system used for this study. The system operates as follows: Gasoline was contained in a one-gallon stainless steel safety can which had been modified by addition of an 1/8 inch swag-elok[®] fitting. Gasoline was withdrawn from this container with a constant displacement metering pump (Fluid Metering, Inc.) and fed to the atomizer (Spraying Systems $\frac{1}{4}$ J Body, with a #1650 fluid nozzle and a #64 air nozzle). Nitrogen was heated by passing over a 100 watt quartz immersion heater operated from a variac. All parts of this generation system were calibrated such that the operational characteristics of the complete system were well defined. Figure No. 3 presents temperature calibration data for the nitrogen heater. Figures 4, 5, and 6 present flow rate calibrations for the FMI pumps. These calibrations were made while the pumps were operating into the atomizers. A Roots pump, on the exhaust side of each chamber, pulled room air through the absolute filter and the mixing pipe in each generator chamber. Due to a pressure differential between the vaporization chamber and the mixing pipe, a mass transfer of gasoline vapors to exposure chamber airflow was accomplished.

Although this system operated very well with respect to transferring the maximum amount of gasoline from the liquid phase to the vapor phase it had one significant drawback.

Exposure chamber equilibration time was not a function of chamber flow rate and volume as usual, but a function of vaporization chamber equilibration rate. This is illustrated in Figure No. 8 which shows exposure chamber

concentration versus time. The chamber was operated at 360 L/min. which would normally produce a 99% equilibration time of twelve (12) minutes. As can be seen from the graph actual equilibration time was about sixty (60) minutes. Therefore it was necessary to conduct exposures for an additional fifteen (15) minutes every day in order to compensate for this equilibration time.

C. Analytical Methods

1. Methods for total gasoline vapors

Exposure chamber atmospheres were analyzed for gasoline vapor concentration with a total hydrocarbon analyzer (Scott Model 116). The instrument was a typical flame-ionization type operated with compressed air and a hydrogen-helium mixture (40% - 60%).

This instrument was calibrated with mixtures of gasoline vapors of known concentration prepared in the laboratory. The system utilized for preparing known concentrations of gasoline vapors is illustrated in Figure No. 9. The system operates as follows: An Ace Glass midget bubbler (Cat. No. 9513) was modified by removing the frit and providing an injection port tube. This vaporization tube was placed in the center of a coiled copper tube, which was fitted in a small electrically heated oven. An evacuated fifty (50) liter mylar bag was attached to the exit port on the vaporization tube. A known volume of gasoline was taken up in a pre-weighed 1 ml gas tight syringe (Pressure-Lok, Precision Sampling Corp.), the syringe was then re-weighed and the weight of gasoline calculated.

The wet test meter was set to zero and a N_2 flowrate of approximately three (3) liters per minute was started. Gasoline in the syringe was then injected into the vaporization tube via a serum stopper over the injection port. Immediately thereafter seventy (70) volts was applied to the heating element. Temperature in the vaporization tube gradually increased over the next twelve and one-half ($12\frac{1}{2}$) minutes, at which time voltage was removed from the heating element. Figure No. 10 shows the temperature profile as measured at the bottom of the vaporization tube. Depending on the vapor concentration desired the N_2 flow rate was maintained until a pre-determined volume had been added to the bag. The contents of the bag were then thoroughly mixed and used to calibrate the hydrocarbon analyzer.

Prior to actual use for instrument calibration the technique was checked for the stability of gasoline vapors in mylar, and for reproducibility. Reproducibility was evaluated by preparing eight (8) different calibration bags over a period of five (5) days. All bags contained the same calculated concentration, 3.5 mg/L. These vapor mixtures were analyzed by the hydrocarbon analyzer set to produce an approximately correct response. The following table presents this data:

<u>Bag No.</u>	<u>Analyzer Response Arbitrary Units</u>
1	3.2
2	2.8
3	3.2
4	3.3
5	3.8
6	3.9
7	3.8
8	3.5
$\bar{X} = \frac{3.4}{8} \sigma = 0.38$	

The coefficient of variation for this data was 11.1% which was considered adequate in view of the technique.

The next step was to evaluate the stability of these standards such that we would know how rapidly they had to be used. Three (3) different concentrations were prepared in triplicate and analyzed at various times. The following table presents this data:

		<u>Analyzer response - Arbitrary units</u>			
<u>Concentration</u> mg/L	<u>Bag</u> <u>No.</u>	<u>Immediately</u>	<u>24 hrs.</u>	<u>%Δ</u>	<u>48 hrs.</u>
3.5	1	3.8	3.4	10.5	2.9
	2	3.6	3.1	13.9	3.1
	3	3.5	3.1	<u>11.4</u>	3.0
	$\bar{X}$			<u>11.9</u>	
5.0	1	5.0	4.5	10.0	4.2
	2	4.8	4.4	8.3	4.0
	3	5.0	4.4	<u>12.0</u>	3.8
	$\bar{X}$			<u>10.1</u>	
7.0	1	6.0	6.4	7.2	6.0
	2	6.7	6.4	4.5	6.1
	3	7.1	6.8	<u>4.2</u>	6.8
	$\bar{X}$			<u>5.3</u>	

From this data we calculated a linear decay rate of approximately 0.5% per hour. Thus we elected to only use bags on the day they were prepared.

Having verified the utility of the methods we next prepared a calibration curve for the analyzer. Figure No. 11 presents the calibration curve. The plotted points are means of a number of calibration bags (four for 1.75 mg/L, nine for 3.5 mg/L, and six for 7.0 mg/L) while the bars represent the standard deviations.

There was no difference in calibration between fuel A and fuel B. At bi-weekly intervals throughout the study instrument calibration was checked with additional points. These points are shown on Figure No. 11 as encircled points.

During animal exposures the analyzer was connected to an automatic sampling device such that each exposure chamber was automatically sampled for approximately twelve (12) minutes out of every hour. Figure No. 12 presents an example of the strip chart recorder output. This type of record was utilized for quality control of chamber concentrations and not for reporting actual chamber concentrations. Actual chamber concentrations were recorded twice per day, once mid-morning, and once mid-afternoon.

2. Determination of alkyl lead in gasoline

The analytical method utilized for determining alkyl lead concentration in the leaded fuel involved reacting the gasoline with an iodine-octanol solution, extracting the lead with dilute nitric acid and measuring lead via atomic absorption spectrophotometry. A standard alkyl lead solution was provided by Ethyl Corporation, and a standard curve was prepared utilizing this standard. The lead content of fuel B was then determined on a number of aliquots. The data is presented in the following table:

<u>Sample No.</u>	<u>Analyzed μgPb/ml</u>	<u>Calculated gmPb/gal</u>
1	4.0	1.50
2	4.53	1.70
3	5.01	1.88
4	4.48	1.68
5	4.48	1.68
6	3.68	<u>1.38</u>

$$\bar{X} = 1.65$$

A sample of fuel B was submitted to Ethyl Corporation for lead analysis, with the following results: Total lead was found to be 1.87 gm/gal. while distribution among the fire lead alkyls was approximately as follows:

<u>Alkyl Lead</u>	<u>Mole %</u>
tetramethyl lead	6
trimethylethyl lead	25
dimethyldiethyl lead	37
methyltriethyl lead	25
tetraethyl lead	6

3. Determination of vapor phase alkyl lead

Vapor phase alkyl lead was measured with a method reported by Purdue, *et. al.*⁽³⁰⁾ Briefly, the method involved collecting alkyl lead vapors in iodine monochloride (ICl) solution, adjusting pH, complexing with ammonium pyrrolidinedithiocarbamate, extraction with methyl isobutyl ketone, and Pb determination by atomic absorption spectrophotometry.

Alkyl lead samples were collected in 10 ml ICl contained in gas bubblers (Ace Glass, cat. no. 7529-16). Sampling flowrate was 0.56 liters per minute. Prior to collecting data for the animal experiments we evaluated collection efficiency for this sampling system. An exposure chamber was set up and fuel B vapors were generated and analyzed at an approximate concentration of 1 mg/L. Two sampling bubblers were set up in series and three (3) paired samples were collected and analyzed for lead. The following table presents this data:

<u>Sample No.</u>	<u>Pb conc. $\mu\text{g/ml}$</u>	
	<u>First bubbler</u>	<u>Second Bubbler</u>
1	0.10	none detected
2	0.14	none detected
3	0.10	none detected

From the above we concluded that collection efficiency with one bubbler was adequate.

The next experiment we conducted consisted of generating fuel B vapors and measuring simultaneously the fuel concentration and the vapor phase lead concentration. Since the lead content of the fuel was known (1.76 mg/gal) and the vapor phase fuel concentration was known then a theoretical lead concentration could be calculated. This data is provided in the following table:

Sample No.	Fuel conc. mg/L	Theoretical Pb conc. µg/L	Measured Pb conc. µg/L	% of Theoretical conc.
1	1.90	1.23	1.07	87
2	2.20	1.42	1.32	93
3	2.10	1.36	1.00	73
4	2.10	1.36	1.22	90
5	1.56	1.01	0.75	74
6	1.56	1.01	0.80	79
7	1.61	1.05	0.87	83
				$\bar{X} = 83$

From this data it appeared that 83% of the lead in the fuel was being transferred to the vapor phase.

4. Methods for gas chromatographic analysis of gasoline

A Tracor gas chromatograph was set up with the following conditions for analysis of both liquid and gas phase fuel samples:

Column:	20 foot, 1/8 in. stainless steel
Column temp:	Programmed at 4°C/min from ambient to 210°C
Packing:	5% OV-101 on 80/100 mesh Gas Chrom A
Injection port temp:	230°C
Detector temp.:	300°C
Type of detector:	Flame ionization
FID flows:	H ₂ -52 ml/min; air - 540 ml/min.
Carrier gas:	N ₂ - 45 ml/min.
Sample size:	0.5 µl for pure fuel A and B 2.0 µl for fuels diluted 1:100 in CS ₂ 500 - 1000 µl for vapor samples from the chambers

Figures No. 13, 14, and 15 present typical chromatograms for Fuel A, Fuel B, and a reference fuel respectively. The reference fuel was provided by Mr. F.M. Roberts along with a very detailed GLC chromatogram of the material. This chromatogram was obtained with a high resolution capillary column, and was not directly comparable to those generated by HRC. Repeated attempts to obtain chromatograms of vapor mixtures from exposure chambers were unsuccessful with the equipment available. Thus we were unable to provide compositional information on the exposure atmospheres.

5. Methods for urine lead analysis

Twenty-four (24) hour urine specimens were collected utilizing standard metabolism cages for rats and monkeys.

The rat units were of all stainless steel construction, while the monkey units consisted of stainless steel cages with polyethylene funnels under each cage. Urine was collected in acid washed glass bottles.

The average 24 hour urine output for rats was approximately twenty-five (25) milliliters, with a range of twelve (12) to forty-five (45) milliliters, and only on rare occasions was it necessary to collect for more than 24 hours. Collection of primate urine was much more difficult.

Due to the large floor area in the monkey cages a rather large collection funnel (24 in. diam.) was required. This together with a normally low urine output, 10 ml per 24 hours, created some difficulties. The average output was increased to approximately twenty (20) milliliters by providing a sweetened fruit juice in place of water. However, it was also necessary to collect for 48 or 72 hours in some cases, such that at least twenty-five (25) milliliters was available for analysis. Urine samples were frozen until analysis could be conducted.

Briefly, the analysis consisted of a pH adjustment, complexing with ammonium pyrrolidinedithiocarbamate, extraction into methyl isobutyl ketone, and analysis by atomic absorption spectrophotometry.

D. Hematological Methods

Blood for all hematological evaluations was collected for unanesthetized animals, either by tail clip for rats or by finger prick for monkeys. Erythrocyte and leukocyte count were determined with a Model ZB1 Coulter Counter. Hemoglobin was determined by the Drabkins cyanmethemoglobin method utilizing a Fisher Model 55 Hemophotometer. Differential leukocyte count and platelet count were determined by direct count on Wright

stained smears. The reticulocyte count was determined by microscopic observation of brilliant cresyl violet stained smears. The differential count was reported as follows:

N = neutrophils

S = stabs

L = lymphocytes

E = eosinophils

B = basophils

M = monocytes

E. Pulmonary Physiology Methods

Evaluation of the mechanical properties of the lung were conducted by real time computerized techniques very similar to those reported by Alarie (28, 29). Basically the technique consisted of measuring intrapleural pressure, and air flow rate at the mouth. Air flow was electronically integrated to yield tidal volume.

The computer then digitized these analog signals and performed data reduction. A typical set of computer reduced data is presented in Figure No. 16.

Evaluation for distribution of ventilation was conducted by determining the number of breaths required to reduce expired N_2 to 1% while the animal breathed pure oxygen.

F. Flash Evoked Response Methods

All monkeys in the control and high level exposure groups (Group I, II, and IV) were implanted with chronic electrodes in the visual cortex. Access to these electrodes was via a connector permanently

affixed to the animal's head. Simultaneous exposure and evoked response recording was obtained by placing the animal in a restraining chair, and connecting an amplifier to the implanted connector. The monkey was then placed in a small (ca. 150 liter) exposure chamber. This chamber was all stainless steel except for a 12 in. diameter window.

The flash lamp of a Grass photostimulator was positioned in front of the chamber window. The photostimulator was set for paired flashes. Time between these flashes was set for 135 ms. The photostimulator was controlled by a minicomputer (PDP 8/e) programmed as follows: Refer to figure 17. At time "A" the computer starts accepting EEG data, at time "B" the computer triggers the photostimulator and a light flash is produced, at time "C" a second light flash is produced under control of the photostimulator, at time "D" the computer stops accepting EEG data. After 10 seconds, which is under computer control, the sequence is repeated. This occurs 100 times which results in one of the four evoked responses shown in figure 17.

Each monkey was tested as follows: After allowing thirty (30) minutes for the animal to accommodate to the testing environment a control evoked response was recorded. Exposure was then started to a fuel type and concentration approximately equal to what the animal would normally receive. Additional evoked responses were recorded after two (2), four (4), and six (6) hours of exposure. The important data obtained from these evoked responses was the latency. Within the

context of this study, latency is defined as the time from a light flash (points B and C in figure 17) to the peak of the first surface negative wave (points E and F which refer to the control trace in figure 17).

In actuality the data is obtained numerically from the computer and does not have to be read from the plotted evoked responses.

Figure 17 shows data obtained from one animal, No. 118♀ from group IV. This figure is a photocopy of the evoked responses plotted by the computer. The calibration data and the two vertical lines were added for illustrative purposes. These lines help to show the shift in latency toward the right, i.e., increased time from the light flash to the evoked response, as this animal was exposed to 1.8 mg/L of fuel B.

G. Urinalysis Methods

Urine samples were collected as described above. All data was collected utilizing the Ames Bili-Labstix®.

H. Renal Immunofluorescent Methods

Numerous publications attest to the validity of immunofluorescent staining as an appropriate means of detecting the deposition of IgG (31-33) in the renal glomerulus. Additionally, deposition of IgG in the renal glomerulus has been shown to be indicative of various forms of glomerulonephritis.

Renal tissues obtained fresh at necropsy were mounted in Tissue Tech II O.C.T. mounting medium and were sectioned (4 μ) in a cryostat. Tissue sections were mounted on a glass slide and fixed in acetone for 10 minutes at room temperature.