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ESTIMATION OF THE PUBLIC HEALTH RISK FROM EXPOSURE TO GASOLINE VAPOR VIA THE GASOLINE MARKETING SYSTEM

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to the Science Advisory Board

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Office of Health and Environmental Assessment
Office of Air Quality Planning and Standards
Environmental Protection Agency

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1.0 INTRODUCTION

The Environmental Protection Agency is conducting a regulatory analysis of the gasoline marketing system to determine whether the emissions of gasoline vapor or specific constituents of gasoline vapor warrant control to protect public health. EPA's analysis also evaluates available options for the control of emissions from the gasoline marketing system, and the impacts of such controls. The background for the gasoline marketing industry and the regulatory history of emission standards that currently control gasoline vapor emissions are discussed in Section 2 of this paper. The decisions facing the Agency and the analytical approach used in this analysis are described in Sections 3 and 4, respectively.

To aid in determining the extent to which regulation may be warranted, EPA has estimated the potential public health risks posed by gasoline vapors emitted from bulk storage facilities, delivery trucks, service station storage tanks, and from motor vehicle tanks during refueling. The risk assessment focuses on the potential cancer risks associated with gasoline vapor and three gasoline components: benzene, ethylene dibromide (EDB), and ethylene dichloride (EDC), substances for which EPA's Carcinogen Assessment Group (CAG) has derived carcinogenic risk factors. The assessment estimates cancer risks for exposed populations residing near gasoline storage facilities and service stations, and as a result of exposure during self-service refueling of motor vehicles. The results of this analysis are expected to be released in July 1984.

Based on the preliminary results of this analysis, the cancer risks estimated from use of the gasoline vapor risk factor dominate the risks attributable to the three constitutent substances (benzene, EDB, and EDC). The gasoline vapor risk factor is derived from a chronic inhalation study

in two rodent species, sponsored by the American Petroleum Institute (API). This study was forwarded to EPA as a "draft final report" in March 1982 and as a final report in early 1984. At the request of EPA's Office of Mobile Sources, CAG evaluated the results of the API study and developed a human carcinogenic risk factor. The review by CAG of the health literature pertinent to the issue of the carcinogenicity of gasoline vapor and the derivation of unit risk factors by CAG are presented in Section 5.

The final report of the inhalation study performed by International Research and Development Corporation for API consists of six volumes. One copy of the complete six volume set has been forwarded to the chairman of the Science Advisory Board. In addition, a paper summarizing the results of the API inhalation study are included within this paper (Appendix A). Because of the importance of the API study in EPA's gasoline marketing analysis, EPA requests that the Science Advisory board provide peer review of this unpublished work as well as the CAG's evaluation of the implications of this research for human exposure to gasoline vapor. In addition, EPA requests that the Science Advisory Board address the issues listed in Section 6 of this paper.

2.0 BACKGROUND

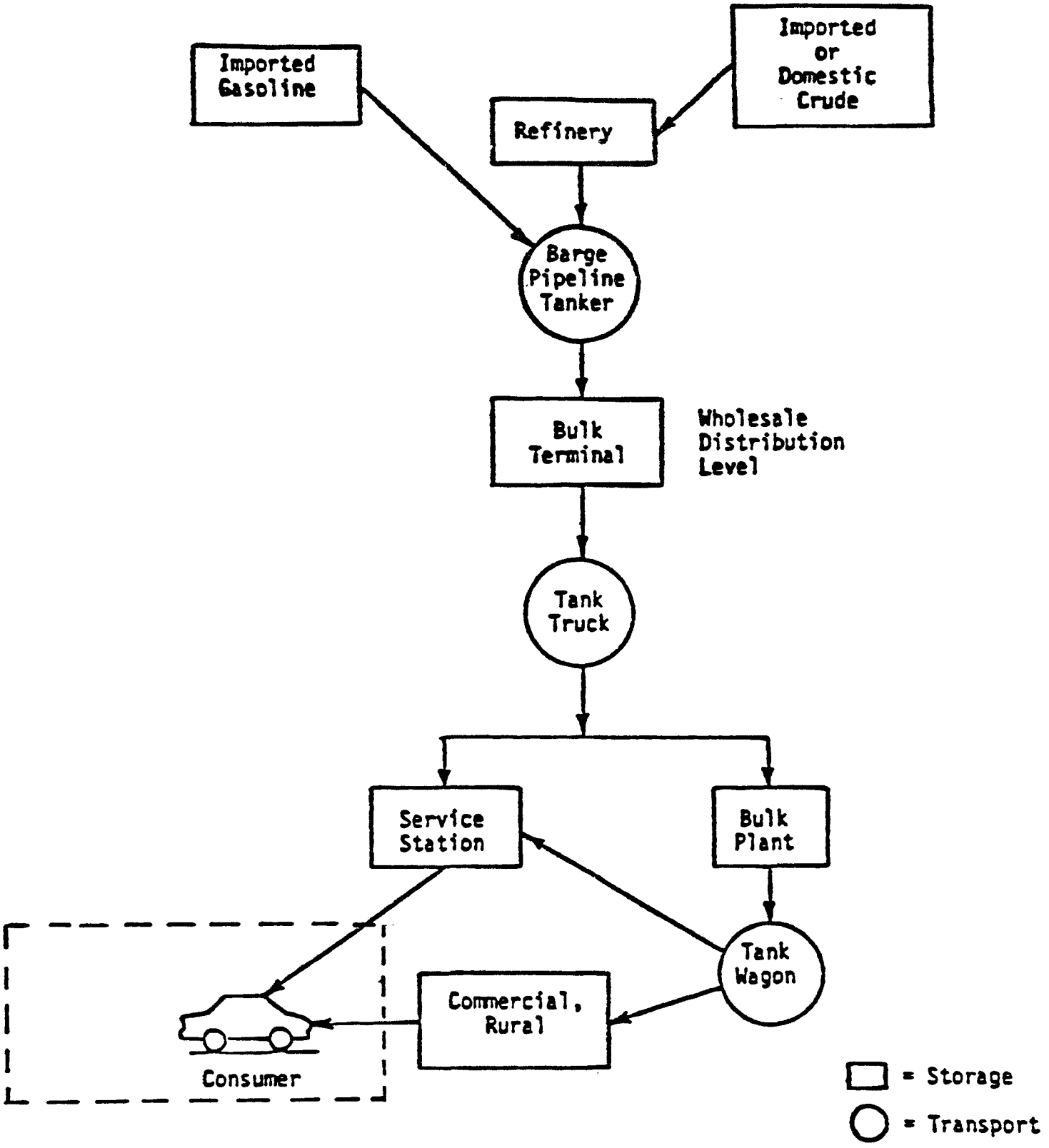
2.1 The Gasoline Marketing Industry

In 1982, about 103 billion gallons of motor vehicle gasoline were distributed in the U.S. An extensive network of storage, transportation, and dispensing facilities were used by refiners, marketers, distributors and dealers to deliver an average 280 million gallons of gasoline each day to ultimate consumers. Gasoline produced at domestic refineries or that is imported (about 3%) is distributed by ship, barge, or pipeline to the gasoline distribution system of bulk terminals, bulk plants, tanker trucks, and service stations. The diagram in Figure 2-1 shows the path of motor vehicle gasoline distribution through these facilities.

2.1.1 Industry Structure

Bulk terminals typically receive most of the motor vehicle gasoline delivered by ships, barges, or pipelines. About 1500 bulk terminals in current use store gasoline in large above-ground storage tanks. Separate tanks are used for each type of petroleum product distributed (e.g., three grades of gasoline). Typical bulk terminals have 4 to 5 tanks and larger terminals have more tanks that may be spaced over several acres. From these bulk terminals, gasoline is loaded into large tank trucks that make deliveries to local distributors operating bulk plants or directly to service stations. Gasoline is delivered to a national network of 15,000 bulk plants operating smaller above-ground storage tanks. The gasoline is distributed from these plants by smaller trucks to businesses, institutions, or dealers operating retail outlets.

FIGURE 2-1 GASOLINE MARKETING IN THE U.S.



About 210,000 retail outlets such as service stations or convenience stores are currently dispensing gasoline to the public. A roughly equal number of outlets exist for dispensing gasoline in a wide range of governmental and private business uses. Government owned outlets fuel Federal, State and local passenger vehicles, trucks, buses, and military vehicles. Business outlets include auto rental, utility, taxi, delivery, and commercial trucking operations.

2.1.2 Emission Sources

Emission of gasoline vapor occurs at each distribution facility in the gasoline marketing chain. From the time gasoline is received by a bulk terminal (e.g., from a pipeline) until delivered to the ultimate consumer through a network of bulk storage tanks, gasoline delivery trucks, and service stations, gasoline vapor is expelled to the atmosphere each time a transfer occurs. Displacement of saturated gasoline vapor from tanks during these gasoline liquid transfers is the primary source of gasoline vapor emissions. However, the evaporation of gasoline through tank pressure equalization vents is also a significant contributor to gasoline vapor emissions from very large storage tanks at bulk terminals.

2.1.3 Options for Emission Control

Control methods for containment of gasoline vapors are available and are currently being used at many facilities. Equipment designed to contain and recover gasoline vapors as they are expelled from storage tanks or tank trucks being filled are designated by EPA as "Stage I" control systems. Stage I systems provide for the recovery of gasoline vapors from the vessel being filled into the vessel from which the liquid gasoline is being discharged (i.e., from the service station underground storage tanks to the gasoline tank delivery truck, from the truck to the bulk plant, and via

tank truck, to the bulk terminal where they are liquified by refrigeration and compression equipment and returned to storage). Stage II control systems complete the chain of custody of gasoline vapor emissions by recovering gasoline vapor displaced from an automobile's fuel tank during filling and returning these vapors to the service stations' underground storage tanks. In addition to Stage I and Stage II controls, gasoline vapor emissions from very large storage tanks at bulk terminals can be controlled by use of a floating roof tank system. With this tank design, the tank roof is not fixed to the walls at the top of the vessel, but is supported by pontoons floating on the surface of the gasoline pool. Unlike a fixed-roof tank where a large amount of gasoline vapor may exist above the level of liquid gasoline in the tank and may be expelled as the tank is filled, the floating roof moves with the liquid level and does not create a large vapor space as the tank is emptied.

2.1.3.1 Stage I Controls

Stage I control systems at bulk terminals and bulk plants collect and recover gasoline vapors from empty, returning tank trucks as they are refilled with gasoline from the storage tanks (Figure 2-2). These systems are now in place at about 2/3 of the bulk terminals and at about half of the bulk plants in operation in the U.S. Floating roof storage tanks are also generally in use at those bulk terminals where Stage I controls have been applied.

Stage I controls are also in use at roughly half of U.S. service stations (Figure 2-3). Stage I controls at service stations contain the gasoline vapors within the station's underground storage tanks for transfer to empty gasoline tank trucks returning to the bulk terminal or bulk plant.

Figure 2-2. Bulk Plant Vapor Balance System

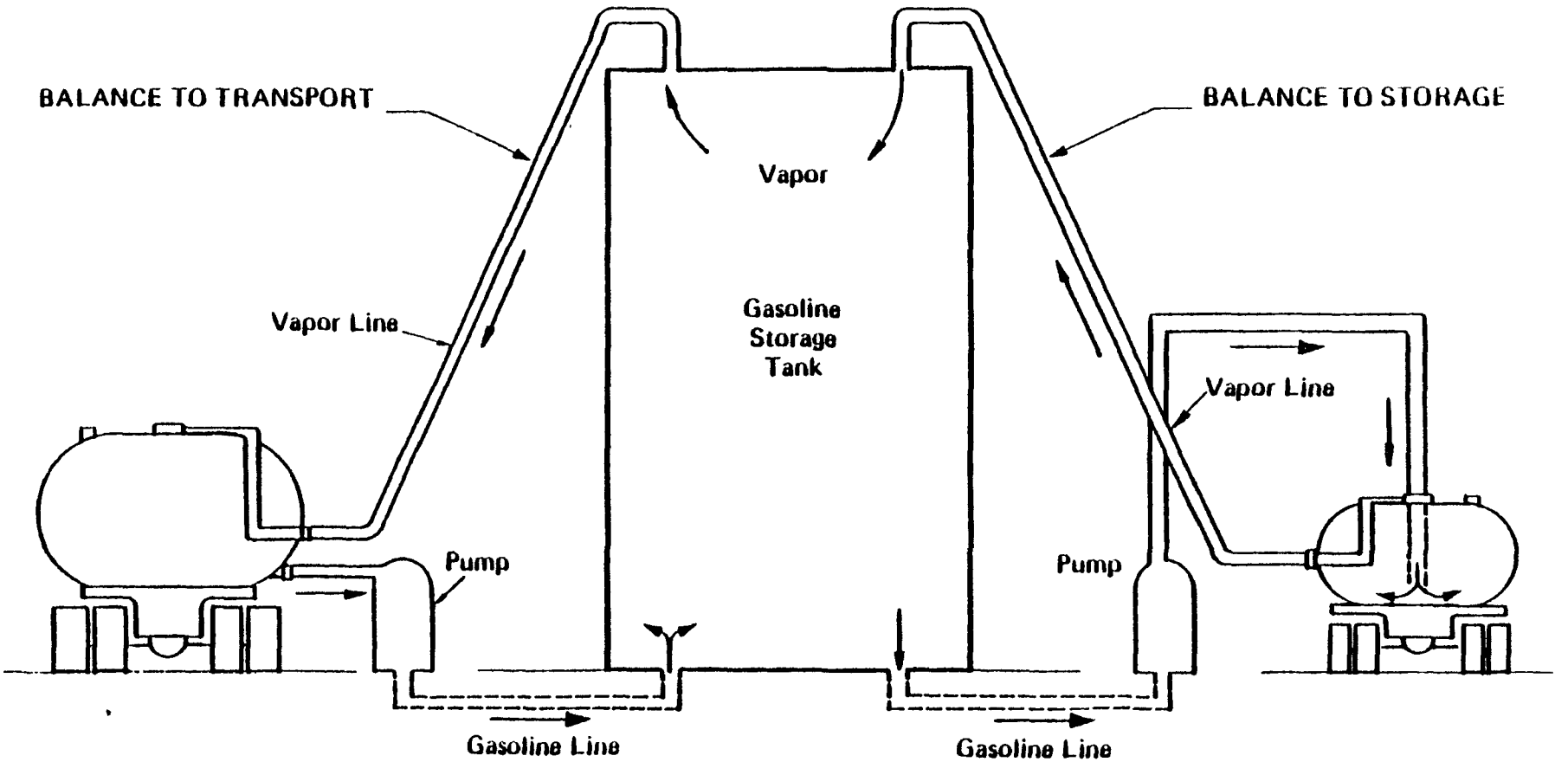
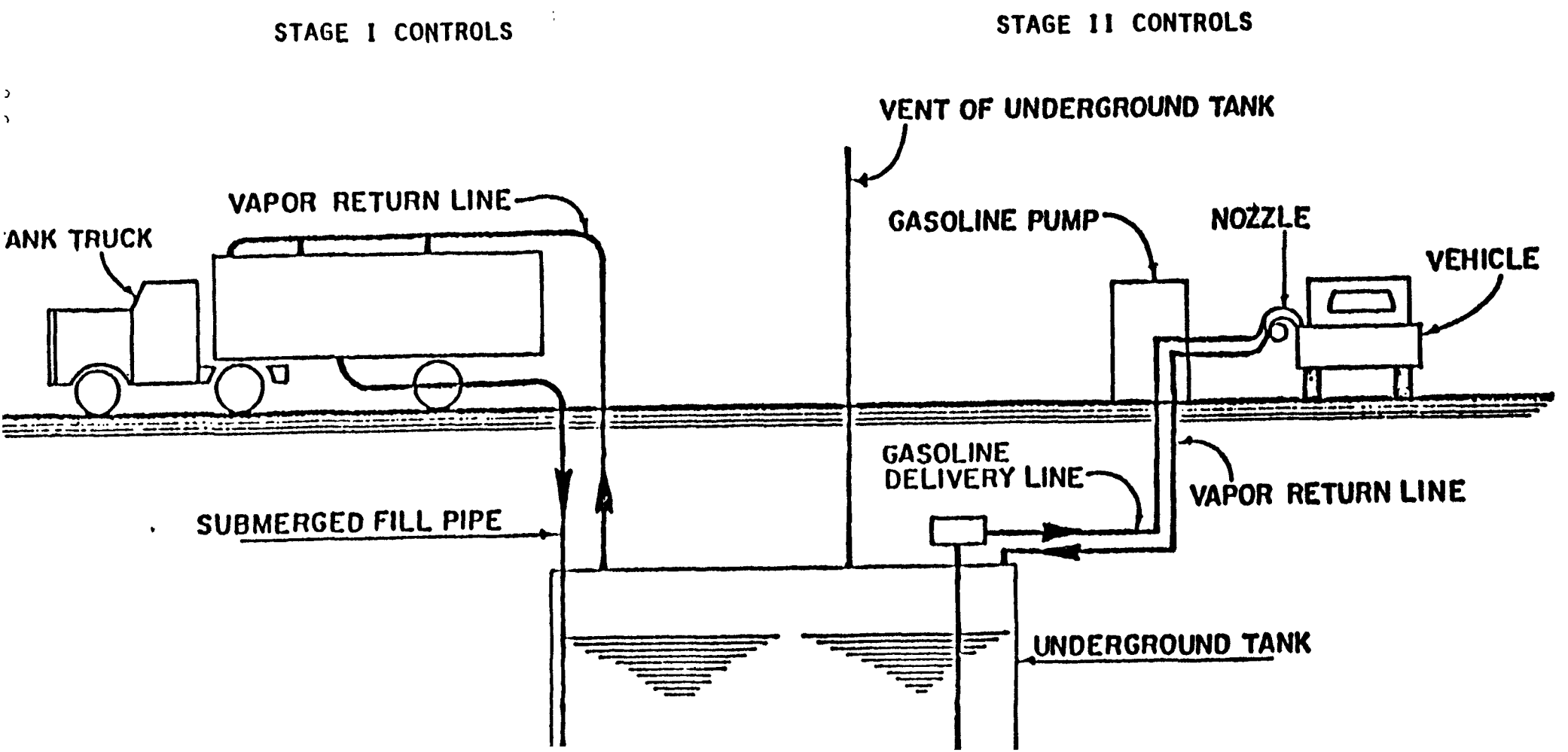


FIGURE 2-3 SERVICE STATION VAPOR BALANCE SYSTEM



2.1.3.2 Stage II Controls

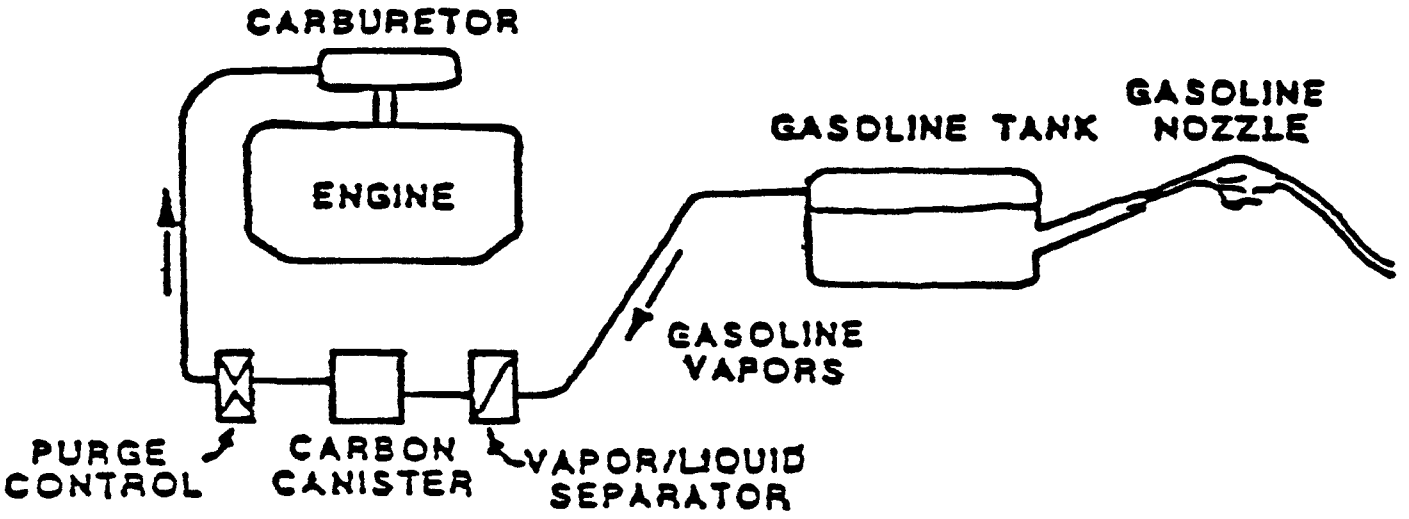
In addition to underground storage tanks, the other source of gasoline vapor emissions at service stations (420,000 in use nationally) results from the uncontrolled venting of displaced vapors from the motor vehicles fuel tank during filling. With the prevalent use of self-service pumps, relatively high exposures of a large segment of the population to gasoline vapors results from motor vehicle refueling operations. About 70 percent of all gasoline used by the public is dispensed by self-service pumps. Stage II controls which recover the vapors from vehicle tanks to service station storage tanks (Figure 2-3) are currently used at about 38,000 service stations nationally (less than 10 percent) located primarily in California and in the District of Columbia.

2.1.3.3 Onboard Controls

A feasible alternative for recovery of Stage II emissions displaced from motor vehicle fuel tanks during refueling consists of the use of vapor control system designed into new model automobile and light duty trucks. The onboard system includes a sealed fill pipe and carbon canister that adsorbs displaced vapors during filling and purges them to the carburetor for combustion during operation (Figure 2-4). Use of onboard carbon canisters for control of evaporative emissions of gasoline vapors has been required on all new automobile production since 1971. Enlargement of this canister would be necessary to accomodate an increased recovery of gasoline vapor during motor vehicle refueling.

A summary of the sources and amounts of annual gasoline vapor emissions are shown in Table 2-1. These emission estimates reflect the influence of Stage I and Stage II controls that are currently being applied by EPA and State regulations.

FIGURE 2-4 ONBOARD CONTROLS



FILL PIPE MODIFICATIONS

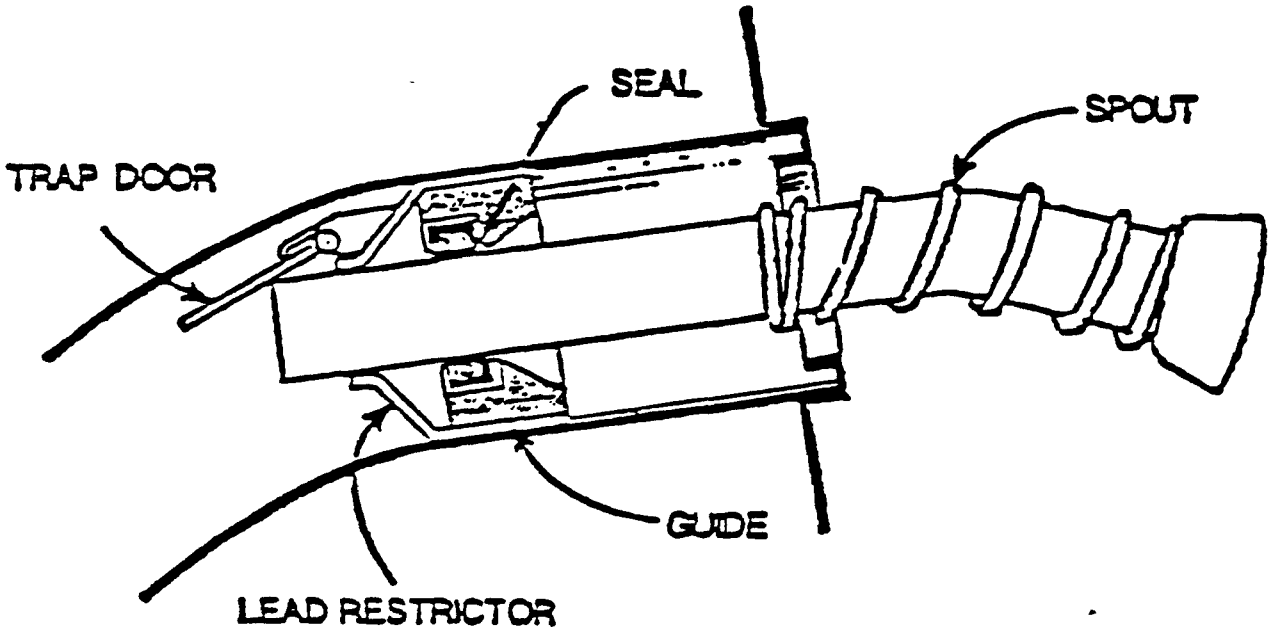


Table 2-1. ESTIMATED GASOLINE VAPOR EMISSIONS IN 1982

Facility	Annual Emissions[a] (Mg/year)
Bulk Terminals	
- Storage tanks	52,000
- Truck loading	140,000
Bulk Plants	208,000
Service Stations	
- Storage tanks	222,000
- Motor vehicle refueling	407,000
Total National Emissions	1,029,000

(a) Emission estimates assume controls required by current EPA and State standards are in place.

2.2 Regulatory History

The gasoline marketing industry has come under regulatory scrutiny by EPA in several contexts. Volatile organic compound (VOC) emissions, including gasoline vapors, participate in atmospheric photochemical reactions that produce ozone and other constituents of "smog." Emissions of gasoline vapor are also of concern to EPA due to the potential health risks of exposure to certain gasoline constituents (benzene, ethylene dibromide and ethylene dichloride) and due to exposure to gasoline vapor itself. EPA listed benzene as a hazardous air pollutant in June 1977 and uses of EDB as a citrus and grain fumigant have been curtailed. In addition, a number of regulatory actions have been taken by EPA and the States to control gasoline vapors for the purpose of reducing atmospheric ozone.

Because National Ambient Air Quality Standards (NAAQS) for ozone have not been attained in all air quality control regions of the U.S., States are developing additional regulations to control volatile organic compounds to attain these standards. Control of gasoline vapors has been incorporated in some State plans since 1974 in ozone non-attainment areas.

In addition to State plans for attainment of the ozone NAAQS, EPA has issued Federal New Source Performance Standards (NSPS) that require controls for new, modified, or reconstructed storage tanks. EPA issued these standards in June of 1973 for bulk gasoline storage tanks with a capacity over 40,000 gallons. Bulk terminal or bulk plant storage tanks affected by these standards are required to have floating roofs or a vapor recovery system.

EPA has acted to require additional control of gasoline vapor emissions by bulk terminals. NSPS were proposed in December of 1980 and were promulgated in August 1983. This action required Stage I controls for all new, modified or reconstructed storage tanks nationally regardless of ozone NAAQS attainment

status. The standards also require control of tank truck gasoline vapor displacement emissions when filled from loading racks servicing these storage tanks.

Although the gasoline marketing industry has frequently been considered by EPA as a candidate for regulation on the basis of its photochemical reactivity and ozone formation contribution, and on the basis of its benzene content, the Agency has not addressed the need for the regulation of this industry on the basis of the carcinogenic potential of gasoline vapor itself. The questions raised by the recent API study concerning the carcinogenicity of unleaded gasoline vapor and the known health effects of gasoline constituents, including benzene, have stimulated the public debate on this issue and led advocacy groups to press EPA for a regulatory determination.

On July 14, 1983, The Environmental Defense Fund (EDF) and the National Resources Defense Council (NRDC) filed a citizen suit to compel EPA to either take final action on benzene emission source categories or find that benzene clearly is not a hazardous pollutant pursuant to section 112 of the Clean Air Act. NRDC v. EPA (D.D.C). The plaintiffs requested that the court require EPA to promulgate standards on the categories for which proposals had been made, propose an emission standard for coke oven by-product plants, and propose standards for benzene emissions from the gasoline marketing system and "chemical manufacturing plants". On January 27, 1984, the Court ordered EPA to publish in the Federal Register, by May 23, 1984, its final action on the source categories for which proposals had been made (maleic anhydride and EB/S process vents, benzene storage vessels, and benzene fugitive sources), and to propose action for coke oven by-product recovery plants. The court did not issue an order concerning chemical manufacturing or gasoline marketing. In response to this suit, EPA announced

on May 23, 1984 final standards for benzene fugitive emissions, proposed standards for coke oven by-product recovery plants, and also acted to withdraw previously proposed standards for benzene storage vessels, and maleic anhydride and ethylbenzene/styrene process vents.

A regulatory analysis of the gasoline marketing industry cateogry is now being completed and a notice of availability of the background document will soon be published in the Federal Register. No decision has been made at this time to regulate the gasoline marketing industry under section 112 (hazardous air pollutants) of the Clean Air Act.

In addition to risk to human health resulting from the benzene content of gasoline, additional animal studies provided to EPA by the American Petroleum Institute in 1982 seem to indicate that constituents other than benzene alone in gasoline vapor may have health related impacts. Thus there are three environmental impacts that may contibute to a decision to further regulate gasoline marketing sources: gasoline vapor contributions to photochemical smog (ozone), benzene related health risks, and additional gasoline constituent health risks. All of these potential environmental impacts and other factors relating to the implementation and costs of controls must be evaluated by EPA to reach a decision on an appropriate regulatory strategy.

3.0 DECISIONS FACING EPA

3.1 Health Basis for Regulation

The impact on public health will be a key factor in EPA's decision on what further controls (if any) may be warranted for the gasoline marketing industry. Before a decision can be reached with respect to additional control of gasoline marketing emissions, the health basis for any action selected must be determined.

Gasoline vapor emissions are precursors to ozone formation and on this basis alone EPA could consider action to control gasoline marketing operations. However, actions under these authorities may affect only ozone non-attainment areas and exposure to gasoline marketing emissions would not be reduced for a significant portion of the U. S. population. Thus, controls implemented by EPA to control ozone formation in the atmosphere may not be an adequate strategy if a significant health risk from exposure to gasoline marketing emissions is found to exist.

EPA has prepared an analysis of the health risks of exposure to gasoline vapor from the gasoline marketing system, the regulatory control alternatives, and associated costs. The document containing this analysis is expected to be released in July 1984. A description of the risk analysis methodology is presented in Section 4 of this paper.

3.2 Control Strategy Decisions

In considering the impacts of emissions from the gasoline marketing system and reaching a decision on the additional controls (if any) that may be warranted, EPA has authority under several sections of the Clean Air Act (CAA) by which gasoline vapor emissions could potentially be further regulated. Each authority was granted to address specific risks or categories of sources. Pollutants for which National Ambient Air Quality Standards

(NAAQS) have been established (criteria pollutants) may be regulated through several mechanisms. Authority under CAA section 110 provides for attainment of NAAQS through a program of State standards development for new and existing stationary sources. CAA section 111 provides authority for establishment of Federal standards for new stationary sources, and Title II of the CAA provides authority for EPA to require installation of controls by manufacturers on new motor vehicles. For pollutants to which no NAAQS applies and for which EPA determines a health risk of mortality or serious illness would result (a hazardous pollutant) CAA section 112 provides for Federal regulation of new and existing sources of emissions.

Thus, to a degree, the magnitude of the public health risk posed by gasoline marketing emissions may influence the selection of control strategies for gasoline marketing emission sources.

3.2.1 Actions to Reduce Ozone Precursors

With NAAQS attainment programs, control is applied only in specific areas of the U.S. to provide an effective air quality improvement strategy at minimum cost. Previous actions by EPA to control gasoline marketing emissions from existing sources have focused on specific regions of the U.S. where attainment of the ozone NAAQS has been and continues to be a problem. To assist States in attainment of the ozone NAAQS, EPA has prepared and published Control Technique Guideline (CTG) documents for every sector of the gasoline marketing system with the exception of automobile refueling. EPA has also taken action to control new bulk terminals under CAA section 111 as part of an overall strategy to reduce VOC emissions. Thus, many existing bulk terminals, bulk plants, and service station storage tanks primarily in ozone non-attainment areas and all storage tanks and loading racks for newly constructed bulk terminals in all

areas of the U.S. are currently required to install Stage I gasoline vapor recovery systems. However, gasoline vapor recovery during automobile refueling remains largely uncontrolled. Stage II recovery systems have been applied only in California and the District of Columbia.

Should the potential risk to human health resulting from exposure to gasoline vapor or its constituents be determined to be a significant hazard, a more widespread system of controls for the gasoline marketing industry would be a logical consideration. Since many of the current regulations in effect are based on a strategy for ozone attainment, many areas of the U.S. remain uncontrolled with respect to existing gasoline marketing sources because they either do not have an ozone attainment problem or because control of VOC emissions from stationary sources other than gasoline marketing have been adequate to attain the ozone NAAQS.

3.2.2 Actions to Reduce Risks from Gasoline Vapor Exposure

A hazardous pollutant designation for gasoline vapor or a decision to pursue regulation of the gasoline marketing system on the basis of a gasoline constituent such as benzene would impose a requirement upon EPA to reduce exposure for a broader spectrum of the U.S. population than would be provided by a program to control ozone. The outcome of listing under section 112 would be regulations for those source categories found to pose significant health risks.

3.2.2.1 Regulation of Automobile Refueling

EPA could decide that a national program of gasoline marketing vapor emission reductions should include the automobile refueling operation since these emissions are currently uncontrolled in all but two areas of the U.S., and a large segment of the population is exposed to relatively high concentrations of vapor during self-service gasoline pump use. Two methods

(Stage II and Onboard) are available for control of gasoline vapor emissions from fuel tanks. Either of these methods would greatly reduce gasoline vapor emissions during refueling. However, these approaches would produce different costs and emission results over time.

Stage II control systems could be retrofitted at the nations's service stations within a relatively short time span (e.g., in 3 years). This action would provide more immediate reductions in gasoline vapor emissions. However, the Stage II nozzles and hoses for gasoline pumps would receive constant use and would be subject to being torn, crushed, or otherwise degraded. The experience in California has been that a continuing program of inspection is needed to maintain the effectiveness of Stage II control systems. As a result, the annual average control efficiency of Stage II is estimated to be less than with vehicles using on-board controls.

On-board control systems could be designed into new automobiles and other light-duty vehicles. Even though the controls would be installed only on new vehicles and would not provide as much control as Stage II during the first several years of implementation, the efficiency of the controls is likely to be better over time than Stage II. In comparision to Stage II, on-board controls would provide about one-half as much emission reduction after the fourth model year, about the same amount by the ninth model year, and superior emission reductions in subsequent years.

3.2.2.2 Regulation of Bulk Terminals, Bulk Plants, and Service Stations

If gasoline vapors or its constituents are determined to impose a significant health risk on the public, a national program for implementation of Stage I vapor recovery systems at bulk terminals, bulk plants, and service stations would also be evaluated. Regulatory requirement for

Stage I control systems now in effect in ozone non-attainment areas could be extended by section 112 standards to all gasoline marketing facilities in the U.S. Currently, about one-third of bulk terminals and about one-half of bulk plants and service stations remain uncontrolled. These uncontrolled facilities could attain a 90 percent or greater reduction in their losses of gasoline vapor emissions by implementing Stage I controls.

4.0 ANALYSIS OF THE GASOLINE MARKETING INDUSTRY

To provide a basis for any regulatory action that may be warranted and to evaluate potential control strategies, EPA has performed an analysis of the gasoline marketing industry. The analysis includes an estimate of the national incidence of cancer expected to result from the public's exposure to gasoline vapors from all segments of the gasoline marketing industry. These national incidence estimates were developed for the current level of gasoline vapor emission control applied by the industry and at alternative levels of control that could be applied. The analysis evaluates the emission control efficiency, costs, and cost-effectiveness of alternative gasoline vapor emission control strategies.

4.1 Exposure/Risk Analysis

Several animal studies of cancer risk resulting from exposure to gasoline vapor and three of its constituents, benzene, ethylene dibromide (EDB), and ethylene dichloride (EDC) have been reported. Human epidemiological studies of benzene exposure are also available. From these studies, EPA has derived unit risk factors for gasoline vapor and those constituents currently identified as potential cancer risks (i.e., benzene, EDB, and EDC). Together with other information on the exposed population and exposure concentration levels for each emission source, EPA has estimated national cancer incidence resulting from gasoline vapor emissions from the gasoline marketing system. Incidence estimates were developed assuming no change in the current level of emission control in all segments of the gasoline marketing distribution system, and as a result of application of additional controls on specific segments of the industry. An explanation of the approach taken to derive these estimates are contained in the following sections. The results of the risk and incidence analyses are described in a document scheduled for release by EPA in July 1984.

4.1.1 Selection of Suspect Agents for Evaluation

Gasoline is a complex mixture of over 200 hydrocarbons with the paraffinic and aromatic compounds constituting the largest fraction. Aromatics including benzene and toluene are about 20 to 35 percent of the total gasoline mixture by volume. The majority of these aromatics are alkyl-benzene compounds; pure benzene accounts for 0.2 to 4.0 percent of the total gasoline mixture based on 1977 analyses of gasoline produced by several refiners. The average benzene concentration was found to be 1.3 percent.

In addition to benzene, leaded gasoline contains ethylene dibromide (EDB) and ethylene dichloride (EDC) which are used as lead scavengers. To improve octane ratings, gasoline contains a large number of hydrocarbons that have been cracked, reformed, or otherwise chemically altered. At present, quantitative cancer risk factors are available for only three consistituents of gasoline vapor (i.e., benzene, EDB, and EDC). A more recent study, conducted for the American Petroleum Institute, suggests that gasoline vapor may be a potential human carcinogen. The maximum lifetime risks associated with gasoline vapor exposure, based on a preliminary analysis, were much greater than those attributable to the three constituents for which unit risk factors were available. For this reason, EPA selected gasoline vapor in addition to benzene, EDB, and EDC for the risk assessment analysis. The basis for these selections is described for each substance in the following sections and a summation of the unit risks values are shown in Table 4-1.

4.1.1.1 Benzene. An association between benzene exposure and leukemia has been documented in several human studies of occupationally exposed populations. Benzene has also been found to be carcinogenic in both rats and mice by gavage and inhalation routes of exposure. The benzene unit risk factor (the risk of cancer resulting from a 70 year lifetime of

TABLE 4-1. UNIT RISK FACTOR SUMMARY

Pollutant	Unit Risk (probability of cancer given lifetime exposure to 1 ppm)	Health Effects Summary	Comments
Gasoline Vapor		Kidney tumors in rats, liver tumors in mice.	Gasoline test samples in the animal studies were completely volatilized, therefore may not be completely representative of ambient gasoline vapor exposures.
Plausible Upper Limit:[b]			
Rat studies	3.5×10^{-3}		
Mice studies	2.1×10^{-3}		
Maximum Likelihood Estimates:			
Rat studies	2.0×10^{-3}		
Mice studies	1.4×10^{-3}		
Benzene[c]	2.2×10^{-2}	Human evidence of leukemoginicity Zymbal gland tumor in rats; lymphoid and other cancers in mice.	EPA: listed as a hazardous air pollutant, emission standards proposed. IARC[b]: sufficient evidence to support a causal association between exposure and cancer.
Ethylene Dibromide	4.2×10^{-1}	Evidence of carcinogenicity in animals by inhalation and gavage. Rats: nasal tumors; Mice: liver tumors.	EPA: suspect human carcinogen; recent restrictions on pesticidal uses.
Ethylene Dichloride	2.8×10^{-2}	Evidence of carcinogenicity in animals. Rats: Circulatory system, forestomach, and glands; Mice: liver, lung, glands, and uterus.	EPA: suspect human carcinogen. Draft health assessment document released for review March 1984.

[a] Unit risk factor is in terms of the probability of a cancer incidence (occurrence) in a single individual for a 70-year lifetime of exposure to 1 ppm of pollutant.

[b] 95% confidence interval.

[c] Derived from human epidemological data; not the same factor shown in Table 5-27 based on animal data.

exposure to a unit concentration) was derived from the average of three occupational studies, assuming a linear dose-response function. A unit factor risk derived from the animal data is very close to the value derived from the human studies thereby indicating a similar dose-response relationship.

4.1.1.2 Unleaded Gasoline. The evidence of carcinogenicity comes primarily from the American Petroleum Institute chronic inhalation study of unleaded gasoline vapor in rats and mice. The unit risk estimates for each species based on a linear non-threshold dose extrapolation were derived from this study. Although API studied unleaded gasoline, other gasoline grades (e.g., leaded gasoline) are expected to have as much carcinogenic potency. A detailed discussion of the study results and the risk factor derivation is presented in Section 5 of this paper.

4.1.1.3 Ethylene Dichloride (EDC). No human evidence of carcinogenicity is available. The animal evidence consists of positive responses at several sites in male rats and mice via gavage. The unit risk for EDC inhalation was estimated by two separate methods: (1) a direct estimation based on the EDC gavage study, assuming that the absorption rate by inhalation is one-third of that by the oral route; and (2) an indirect estimation from the EDB inhalation study. The potencies calculated from both approaches are similar.

4.1.1.4 Ethylene Dibromide (EDB). No human evidence of carcinogenicity is available. The animal evidence consists of positive reponses in mice, in both inhalation and gavage bioassays, as well as nasal cavity tumors in rats following inhalation exposure. The unit risk was obtained from the rat inhalation experiment using the linear dose-response extrapolation procedure.

4.1.2 Assessment of Exposure and Estimated Cancer Incidence

This section briefly outlines the methodology and assumptions used to estimate the concentrations of benzene, EDB, EDC, and gasoline vapors from each source category to which the nation's population as a whole (and to which selected individuals subject to high exposures) would be expected to be exposed as well as their associated health risks. Estimates of gasoline vapor exposure were made for each of the source categories of emissions in the gasoline marketing industry (see Table 4-2). These estimates were developed for uncontrolled and controlled emission rates. A national mix of uncontrolled and controlled rates were used to determine current exposure levels according to the number of emission sources that had controls in operation in 1982. Estimates of incidence due to EDB, EDC, and gasoline vapor were projected for the years 1986 through 2020 in proportion to the total or leaded (for EDB and EDC) gasoline throughput for the source category.

4.1.2.1 Location and Distribution of Plants

Since there are about 1,500 bulk terminals, 15,000 bulk plants, and 420,000 service stations in the United States handling gasoline, limited resources would not allow modeling each plant individually, even if data were available regarding exact location and throughput. Model plants (four for bulk terminals, four for bulk plants, and five for service stations) for a range of representative gasoline throughputs were used to estimate exposures nationwide.

In order to calculate exposure to emissions in specific locations (and the resultant risk) from bulk terminals and plants, assumptions were made concerning their geographical distribution. The fundamental assumption was that facilities were located in proportion to the gasoline throughput for an area. For example, the largest model plants would be located in large

TABLE 4-2. LIFETIME EXPOSURE ESTIMATES FOR EMISSION SOURCES CONSIDERED IN RISK ANALYSIS (a)

SOURCE CATEGORY	UNCONTROLLED (ppm)	CONTROLLED (ppm)
Bulk Terminals	1.41	0.18
- loading racks (b)		
- storage tanks		
- vapor processors		
Bulk Plants	0.073	0.015
- loading racks (b)		
- storage tanks		
Service Stations (c)	0.026	0.003
- underground storage tanks		
- automobile refueling (d)		
Self-Service (e)	0.029	0.003 (f)
- automobile refueling		

(a) The HEM model was used to estimate community exposure for bulk terminals, bulk plants, and service stations for the highest exposed population. Actual measurements of exposure by service station attendants were used for self-service estimates. The self-service estimates are based on the average of all the attendant exposure measurements; not the maximum exposures.

(b) The exposure estimates are based on emissions from displacement of gasoline vapors from the tank trucks. Loading racks are used to fill the tank trucks.

(c) Exposure is estimated for communities nearby service stations.

(d) Exposure estimates include vehicle fuel tank emissions displaced from fill pipe and emissions from spillage in the vicinity of the gasoline pumps.

(e) Exposure estimates for individuals engaged in self-service from emissions displaced from the vehicle's fuel tank through the fill pipe.

(f) Exposure varies from 0.001 to 0.004 ppm depending on the control system applied.

urban areas where throughput (and population density) were highest. Further, each model plant type in each source category (bulk terminals and bulk plants) was distributed over a range of ten urban area sizes. The largest terminals, for instance, were assumed to be located in cities ranging in size from New York City to Des Moines, Iowa; the smallest terminals were assumed to be located in cities ranging in size from Spokane to Effingham, Illinois. Estimates were also made of the extent of existing control at these terminals. Most of those in the large cities (likely to be ozone nonattainment areas) are currently controlled with proportionately fewer facilities controlled in the less densely populated areas.

In a similar fashion, model service stations were allocated to 35 localities (multi-county metropolitan areas or single counties), grouped by seven population size ranges. The model plants were selected to be representative of the total national service station distribution. The localities and seven population size ranges were selected to be representative of the total national population distribution.

Amient concentrations, exposure, and incidence for bulk terminals, bulk plants, and service stations were calculated using the SHEAR version of the EPA Human Exposure Model (HEM). The HEM is a model capable of estimating ambient concentrations and population exposure due to emissions from sources located at any specific point in the continguous United States.

4.1.2.2 Self-Service Exposure. As with calculation of incidence due to community exposure, calculation of incidence due to self-service exposure involves estimates for the unit risk factor, the concentrations to which people are exposed, the length of time they are exposed to the concentrations, and the number of people exposed. The same unit risk factors were used for self service exposure as for commmmunity exposure. The concentrations to which people are exposed were estimated based on a

study conducted by API in which benzene and gasoline vapor concentrations in the region of the faces of persons filling tanks were measured.

Concentrations for the other pollutants (i.e., EDB and EDC) were calculated using the ratio of the emissions of those pollutants to benzene emissions. The length of exposure during filling was calculated using a pumping rate of 8 gallons per minute, or 1.25 minutes per 10 gallons. It was assumed that 70 percent of gasoline consumption is purchased through self-service. Calculation of self-service user exposure assumes that someone is exposed to the concentrations measured for benzene and gasoline vapors (and prorated for the other pollutants) for 1.25 minutes for each 10 gallons purchased. Since the linear dose-response model is the basis for the unit risk factor, any exposure (no matter how small) is assumed to result in some risk of cancer. The risks across the exposed population is summed to determine the total cancer incidence expected. For self service, wherein some person is always pumping fuel, the total annual incidence is directly proportional to annual self service gasoline throughput. Thus, knowing the throughput, pumping rate, and pollutant concentration, total annual incidence was calculated.

"Lifetime risk due to high exposure" was calculated using the same assumptions for exposure as were used for the incidence calculations. These include the assumptions for the concentration in the person's face during tank filling (based on API measurements) and the length of time for a tank filling (1.25 minutes per 10 gallons) However, total gasoline consumption is not a relevant variable for this calculation. Rather it is important to know how much of their lifetime individuals experiencing high exposures may spend filling their tank at self-service stations. The EPA predicted that people with high exposures (e.g., outside salesmen) could purchase 40 gallons

of gasoline per week from such stations (i.e., 5 minutes of exposure per week while the vehicle is refueled) for 50 years of their life.

The estimates of risk, in terms of individual lifetime risk from high exposure and aggregate incidence, are applicable to the public in the vicintiy of gasoline marketing sources and those persons that refuel their vehicles at self-service pumps. This analysis did not examine the risk to workers from occupational exposure (e.g., terminal workers and service station attendants). The lifetime risk from high exposure for these workers is probably substantially higher than for the general public. In addition, the estimates of aggregate incidence would be higher if such worker populations were included in the analysis. Of course, any controls to reduce gasoline marketing emissions would reduce exposure for workers as well as for the general public.

5.0 EVALUATION OF THE CARCINOGENICITY OF UNLEADED GASOLINE

5.1 Introduction

The International Research and Development Corporation (IRDC) has recently conducted an inhalation study of unleaded gasoline vapor in Fischer 344 rats and B6C3F1 mice. The study was conducted at the request of the American Petroleum Institute (API) to determine the carcinogenicity of inhaled gasoline vapor. Preliminary results of this study were forwarded to EPA on March 3, 1982 and a draft report (Appendix A) was forwarded later on February 24, 1983.

Results of the study have been accepted for publication in the Journal of the American College of Toxicology. This Section contains a review by EPA's Carcinogen Assessment Group (CAG) of the draft IRDC report and other studies related to the health impact of gasoline vapor or its constituents.

5.2 Animal Studies

An evaluation of the likelihood that unleaded gasoline is a human carcinogen and a basis for estimating its possible public health impact, including a potency evaluation in relation to other carcinogens, is presented in this section. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, other factors, including mutagenicity, metabolism (particularly in relation to interaction with DNA), and pharmacokinetic behavior have an important bearing on both the qualitative and the quantitative assessment of carcinogenicity. This chapter presents an evaluation of the animal bioassays and relevant toxicity studies, the human epidemiologic evidence, the quantitative aspects of assessment, and finally, a summary and conclusions dealing with all of the relevant aspects of the carcinogenicity of unleaded gasoline.

5.2.1 Lifetime Inhalation Bioassay in Rats and Mice (International Research and Development Corporation 1983)

The following is a review of a final report of an unpublished study on the carcinogenicity of unleaded gasoline vapor in Fischer 344 rats and B6C3F1 mice. The study was completed by the International Research and Development Corporation (IRDC) for the American Petroleum Institute (API) in 1983. A report of this study has been accepted for publication (MacFarland et al. 1984).

The physicochemical properties and the formulation of unleaded gasoline test sample, as described by the sponsor, are presented in Tables 5-1 and 5-2. The unleaded gasoline used in the API inhalation study was blended specifically for the experiment. The test gasoline contained no EDB or EDC (as does leaded gasoline). The benzene content of the test gasoline was 2.0 percent. In comparison to commercial unleaded gasoline the test gasoline contained a higher proportion of benzene (average percentage in commercial gasoline was 1.3 in 1977) and of heavy catalytic cracked naptha (HCCN). Six fractions of the gasoline blend (HCCN) has been evaluated seperately in animal inhalation tests. Five of the gasoline fractions induced renal lesions. The HCCN fraction was the only fraction for which no renal effects were noted (see section 5.2.3).

Exposures in the API animal inhalation study of the total gasoline vapor were conducted in 16-m^3 glass and stainless steel chambers. Humidity and temperature within the chambers were approximately 55% and 25°C, respectively. Gasoline vapor was generated by metering liquid through a heated vaporization column; the vapor was carried by dry nitrogen to the inlet port of the chamber, where the vapor was diluted with filtered air at a flow rate of 910 to 1,900 L/min to achieve the desired atmospheric concentrations.

Exposure concentrations are given in Table 5-3. Actual concentrations, measured by gas chromatography, and nominal concentrations approximated desired concentrations rather closely.

The same protocol was used for the study in rats and mice. Rats and mice were about 6 weeks old when the study began. Initial body weights were: male rats, 95 to 129 g; female rats, 79 to 105 g; male mice, 14 to 26 g; female mice, 12 to 20 g. Animals were randomly assigned to exposure groups according to body weight. Three treatment groups, each composed of 100 males and 100 females, were exposed to measured levels of 67, 292, or 2,056 ppm of gasoline vapor. An untreated group of 100 males and 100 females was exposed to filtered chamber air only. Animals were exposed 6 hours/day, 5 days/week until final sacrifice at 107 weeks (male rats and male mice), 109 weeks (female rats), and 113 weeks

TABLE 5-1. PHYSICOCHEMICAL CHARACTERISTICS[a] OF THE TEST MATERIAL (IRDC 1982)

Research octane no.	92.0
Motor octane no.	84.1
(R+M)/2	88.1
Reid vapor pressure, lbs.	9.5
Distillation, ASTM D-86	
IBP, °F	93
10% evap., °F	116
50% evap., °F	216
90% evap., °F	340
End point, °F	428
API gravity	60.6
Gum, ASTM D381, mg/gal	1
Sulfur, ppm	97
Phosphorus, g/gal	<0.005
Lead, g/gal	<0.05
Stability, hours	24+
HC analysis, ASTM D1319	
Aromatics, vol. %	26.1
Olefins, vol. %	8.4
Saturates, vol. %	66.5
Benzene content, vol. %	2.0

[a]All of the above information was supplied by the sponsor, the American Petroleum Institute.

TABLE 5-2. FORMULATION OF UNLEADED GASOLINE

Generic stream[a]	CAS number	Volume %
Light catalytic cracked naphtha	64741-55-5	7.6
Heavy catalytic cracked naphtha	64741-54-5	44.5
Light catalytic reformed naphtha	64741-63-5	21.3
Light alkylate naphtha	64751-66-8	22.0
Benzene added to bring to 2%		0.8
Butane added to increase Reid vapor pressure		3.8
Plus: Antioxidant 5 lbs/1,000 bbl		
Metal Deactivator 5 lbs/1,000 bbl		

[a]Toxic Substance Control Act (TSCA) PL 94-469: Candidate List of Chemical Substances, Addendum 1, Generic Terms Covering Petroleum Refinery Processed Streams, January 1978.

(female mice). Ten males and 10 females per group were sacrificed at 3, 6, 12, and 18 months.

Animals were observed daily, and body weights were recorded monthly for the first 17 months and biweekly thereafter. Hematology was evaluated for seven males and seven females per group at 18 and 24 months. Serum from seven males and seven females per group was biochemically analyzed at 3, 6, 12, 18, and 24 months. Ten animals from each dose/sex group were killed after 3, 6, 12, and 18 months of exposure to provide for periodic histopathologic evaluation.

Survivors, interim sacrificed animals, and decedents were necropsied, and tissues, organs, and tumors were examined microscopically. Major organs were weighed.

TABLE 5-3. INHALATION EXPOSURE CONCENTRATIONS FOR A CARCINOGENICITY STUDY ON UNLEADED GASOLINE VAPOR IN FISCHER 344 RATS AND B6C3F1 MICE (IRDC 1982)

Exposure group	Desired concentration (ppm)	Nominal concentration[a] (ppm)	Actual concentration[a] (ppm)
Low	50	129	49.7
Mid	275	596	273
High	1,500	2,963	1,501

[a]The actual concentration data have not been corrected for the "nitrogen effect" on instrument calibration. Furthermore, an error in chamber airflow rate calibrations was reported which increased the actual airflow rate to approximately twice the assumed flow rate. If the corrections discussed in the study report are applied, the most probable nominal and actual concentrations were as follows:

Exposure group	Nominal concentration (ppm)	Actual concentration (ppm)
Low	72	67
Mid	310	292
High	1,713	2,056

Exposure to unleaded gasoline vapor did not affect survival. All groups of rats and female mice had greater than 50% survival for the entire study, and survival for all groups of male mice was greater than 50% for at least 95 weeks.

Body weight trends are given in Tables 5-4 and 5-5. Modest reduction of weight gain was found in male and female rats and male mice in the high-dose groups. No effect of gasoline vapor on weight gain in female mice was observed.

Organ weights (absolute and organ/body) did not appear to be affected by treatment with gasoline vapor, with the exception of significant ($P < 0.05$) increases in kidney weights and kidney/body weight ratios in high-dose male rats, as shown in Table 5-6.

At the 3-month interim sacrifice, dose-related nonneoplastic histopathologic changes were observed in the male rats. These consisted of cortical multifocal renal tubular basophilia, protein casts, and chronic interstitial inflammation. The basophilia was present in epithelial cells of renal tubules. The proteinaceous tubular casts occurred within dilated renal tubules and were commonly located at the corticomedullary junction. The incidence was 70 and 100% in mid- and high-dose males, respectively. Chronic interstitial inflammatory foci with a predominantly lymphoid cell type were observed at 20 and 70% incidence in mid- and high-dose males, respectively. In addition, renal congestion and very small foci of renal cortical mineralization were noted in several rats.

In animals dying in the 3- to 6-month interval or sacrificed at 6 months, the nonneoplastic renal changes in male rats described above were again evident. The incidence of tubular basophilia was 0, 40, 100, and 100% in control, low-, mid-, and high-dose male rats, respectively. Proteinaceous casts were observed in 27% of the control male rats, 80% of the mid-dose male rats, and 100% of the high-dose male rats. The incidence of chronic interstitial inflammation was 18, 20, 100, and 100% in control, low-, mid-, and high-dose male rats, respectively. Mineralization in a radial pattern within the renal pelvis, with

TABLE 5-4. BODY WEIGHT TRENDS IN A CARCINOGENICITY STUDY OF UNLEADED GASOLINE VAPOR IN FISCHER 344 RATS (adapted from IRDC 1982)

	Mean body weight ± S.D. (grams)			
Study week	Control	67 ppm	292 ppm	2,056 ppm
Males				
0	112 ± 8	113 ± 8	113 ± 9	112 ± 8
13	306 ± 18	316 ± 15[b]	312 ± 16[a]	290 ± 18[b]
26	348 ± 19	361 ± 19[b]	350 ± 20	340 ± 16[b]
52	409 ± 27	412 ± 27	398 ± 24[a]	376 ± 20[b]
78	401 ± 31	406 ± 41	393 ± 20	376 ± 25[b]
106	416 ± 29	403 ± 44	388 ± 33	364 ± 32
Females				
0	93 ± 6	93 ± 6	92 ± 6	92 ± 6
13	173 ± 11	186 ± 11[b]	177 ± 12[a]	173 ± 9
26	209 ± 12	210 ± 11	201 ± 12[b]	192 ± 10[b]
52	250 ± 18	256 ± 16[a]	249 ± 18	225 ± 13[b]
78	264 ± 19	274 ± 19[a]	263 ± 21	246 ± 16[b]
108	288 ± 35	282 ± 31	289 ± 48	255 ± 27

[a]Statistically different from control group at $P \leq 0.05$.
[b]Statistically different from control group at $P \leq 0.01$.

TABLE 5-5. BODY WEIGHT TRENDS IN A CARCINOGENICITY STUDY OF UNLEADED GASOLINE VAPOR IN B6C3F1 MICE
(adapted from IRDC 1982)

	Mean body weight + S.D. (grams)			
Study week	control	67 ppm	292 ppm	2,056 ppm
Males				
0	22 + 2	21 + 2	22 + 2	22 + 2
13	30 ± 2	29 ± 2	31 ± 2[b]	31 ± 2
26	33 ± 2	32 ± 2[a]	32 ± 2[a]	34 ± 2[a]
52	38 ± 4	36 ± 3[b]	35 ± 3[b]	35 ± 3[b]
78	38 ± 4	37 ± 4	37 ± 3[a]	35 ± 3[b]
102	39 ± 4	37 ± 5	38 ± 3	35 ± 3
Females				
0	18 + 2	18 + 2	18 + 2	18 + 2
13	25 ± 1	25 ± 1	26 ± 1[a]	26 ± 1[b]
26	28 ± 1	28 ± 2	28 ± 1	29 ± 2
52	31 ± 3	32 ± 4	30 ± 2	30 ± 2[a]
78	35 ± 3	35 ± 5	34 ± 3[b]	32 ± 3[b]
112	34 ± 3	35 ± 4	34 ± 3	32 ± 3

[a]Statistically different from control group at $P \leq 0.05$.
[b]Statistically different from control group at $P \leq 0.01$.

TABLE 5-6. EFFECT OF CHRONIC EXPOSURE OF UNLEADED GASOLINE VAPOR ON KIDNEY WEIGHTS AND KIDNEY/BODY WEIGHT RATIOS IN MALE FISCHER 344 RATS (IRDC 1982)

Weight Measured	Month of study	Kidney weights and kidney/body weight ratios — Dose group: 0 ppm (control)	67 ppm	272 ppm	2,056 ppm
Kidney	3	2.39 ± 0.32[a](10)[b]	2.39 ± 0.32 (10)	2.41 ± 0.20 (10)	2.70 ± 0.24[c] (10)
Kidney/body	3	8.19 ± 0.80 (10)	7.82 ± 0.80 (10)	8.13 ± 0.54 (10)	9.35 ± 0.49[d] (10)
Kidney	6	2.62 ± 0.32 (10)	2.71 ± 0.26 (10)	2.64 ± 0.16 (10)	2.84 ± 0.17 (10)
Kidney/body	6	7.87 ± 0.55 (10)	7.76 ± 0.64 (10)	8.03 ± 0.36 (10)	8.86 ± 0.39[d] (10)
Kidney	12	3.07 ± 0.19 (10)	3.13 ± 0.19 (10)	3.30 ± 0.32 (10)	3.13 ± 0.38 (10)
Kidney/body	12	8.02 ± 0.55 (10)	7.94 ± 0.40 (10)	8.29 ± 0.31 (10)	8.78 ± 0.74[d] (10)
Kidney	18	2.70 ± 0.19 (10)	2.64 ± 0.19 (10)	2.73 ± 0.13 (10)	2.80 ± 0.11 (10)
Kidney/body	18	6.94 ± 0.49 (10)	7.26 ± 1.18 (10)	7.37 ± 0.51 (10)	7.83 ± 0.44[d] (10)
Kidney	24	2.80 ± 0.33 (26)	2.91 ± 0.34 (38)	2.87 ± 0.24 (34)	3.13 ± 0.34[d] (32)
Kidney/body	24	6.93 ± 0.95 (26)	7.38 ± 1.09 (38)	7.55 ± 0.71[c] (34)	8.75 ± 1.01[d] (32)

[a]Mean ± S.D.
[b]Number of animals evaluated.
[c]Statistically significant difference compared to control group ($P \leq 0.05$).
[d]Statistically significant difference compared to control group ($P \leq 0.01$).

material located within tubules or the collecting ducts of the renal pelvis, was observed in 20% of the high-dose males.

At the 12-month interim sacrifice, the occurrence of proteinaceous casts in the kidneys of male rats was nearly equal in all groups: 20, 30, 30, and 30% in control, low-, mid-, and high-dose male rats, respectively. Mineralization in the renal pelvis occurred in 20% of the mid-dose male rats and in 80% of the high-dose male rats. Progressive glomerulonephrosis was diagnosed in one high-dose male rat. Another new finding was karyomegaly (very large nuclei within renal tubular epithelial cells) in male rats.

The complexity of nonneoplastic morphologic alterations observed in the kidneys of all rats, especially males, increased after 18 months of exposure. Progressive glomerulonephrosis occurred with higher incidence than previously. The lesion was characterized by atrophied or sclerosed glomeruli, dilated renal tubules containing proteinaceous casts, tubular damage with regeneration or scarring, and the presence of foci of chronic inflammatory cells. The incidence of glomerulonephrosis in male rats was 20% in controls, 30% in the mid-dose group, and 20% in the high-dose group; the incidence in female rats was slightly lower. Proteinaceous casts in the kidneys of male rats were noted in 50, 50, 40, and 60% of control, low-, mid-, and high-dose male rats, respectively. Mineralization in the renal pelvis was seen in 20% of the mid-dose and 80% of the high-dose male rats. Renal congestion was commonly seen, and karyomegaly was again noted in male rats. A benign renal cortical adenoma was diagnosed in a high-dose male rat. Mononuclear cell leukemia was diagnosed in the kidney of a female rat that died during the 12- to 18-month interval.

At the final sacrifice, nearly all male rats exhibited progressive glomerulonephrosis. The incidence rates were 100, 95, 97, and 100% in control, low-, mid-, and high-dose male rats, respectively. A slightly lower rate

of occurrence was seen in female rats. Mineralization in the renal pelvis occurred in 0, 5, 63, and 91% of the control, low-, mid-, and high-dose males, respectively. Karyomegaly was observed occasionally in the male rats. One mid-dose male rat had renal tubular epithelial hyperplasia at termination. The lesion was characterized by the presence of a large dilated tubule containing a cystic lumen lined by epithelial cells. Renal cysts, epithelial cell pigmentation, hydronephrosis, chronic interstitial inflammation, congestion, cortical and pelvic mineralization in female rats, and necrosis were among the nonneoplastic lesions observed in the 18-month to terminal sacrifice period.

Pathologic examination of the rats revealed a small incidence of renal tumors in each treated group of male rats (Table 5-7). The first of these tumors was detected at the 18-month interim kill. Renal carcinomas were found in each treated group of male rats, with those in high-dose males being significantly ($P < 0.05$) increased compared to controls (Table 5-7). A statistical test for linear trend was significant at the 0.05 level. Renal carcinomas generally consisted of epithelial cells in a tubular or acinar pattern in the cortex, and renal adenomas mainly included small masses of epithelial cells forming tubular or papillary structures in the cortex. Renal sarcomas consisted primarily of spindle cells in a more pelvic location. The following percentages of final sacrificed male rats had mineralization of the renal pelvis: control, 0%; low-dose, 5%; mid-dose, 63%; high-dose, 91%. Mineralization of the renal pelvis was not found in each kidney with a tumor (Table 5-8); hence, mineralization of the renal pelvis does not appear to have been a requirement in the etiology of kidney tumor formation in rats exposed to unleaded gasoline vapor.

Spontaneous kidney tumor formation is rare in male Fischer 344 rats; for example, Goodman et al. (1979) reported a historical control incidence of one kidney adenoma (0.05%), two kidney adenocarcinomas (0.11%), and three benign

TABLE 5-7. KIDNEY TUMOR INCIDENCE IN MALE FISCHER 344 RATS FROM CHRONIC EXPOSURE TO UNLEADED GASOLINE VAPOR (IRDC 1982)

Tumor type	Exposure group (ppm gasoline vapor)				Time to first tumor (day)
	0 (control)	67	292	2,056	
Renal adenoma/ adenoma cortex	0/100[a]	0/100	2/100	1/100	546
Renal carcinoma/ renal carcinoma undifferentiated	0/100[b]	1/100[c]	2/100	6/100[c,d,f]	692
Renal sarcoma[e]	0/100	0/100	1/100	0/100	748
Total	0/100[b]	1/100	5/100[d,f]	7/100[c,d,f]	

[a]Number with tumor/number examined.
[b]Statistical analysis for linear trend was significant at the 0.05 level.
[c]In the IRDC (1982) interim report, renal carcinomas were diagnosed in 2 low-dose and 5 high-dose male rats. Further analysis of the kidney sections, as indicated in the subsequent final report on the study, resulted in the observation of renal carcinomas in 1 low-dose and 6 high-dose male rats.
[d]The 100 animals in each denominator in this table include 40 animals sacrificed at 3, 6, 12, and 18 months, and decedents and survivors in the remaining 60 animals which were allowed to survive for the duration of the study. If the 40 interim sacrificed animals are excluded from each denominator to allow replacement of the 100 total animals with the 60 animals allowed to survive for the duration of the study, the statistically significant differences shown in this table remain significant at $P < 0.05$.
[e]A renal sarcoma was also found in a female rat in the 292-ppm exposure group.
[f]Statistically significant ($P < 0.05$) increase compared to control group by Fisher's Exact Test.

TABLE 5-8. INDIVIDUAL DATA ON MINERALIZATION OF THE RENAL PELVIS AND KIDNEY TUMORS IN MALE AND FEMALE FISCHER 344 RATS EXPOSED TO UNLEADED GASOLINE VAPOR[a]

Dose group	Rat identification number[b]	Kidney tumor type (grade)[bc]	Renal pelvis mineralization (grade)[c]
Males			
67 ppm	1339	Carcinoma (3)	None
272 ppm	1442	Carcinoma (2)	None
	1381	Carcinoma, undifferentiated (0-4)	None
	1447	Sarcoma (4)	None
	1420	Adenoma (3)	Present (1)
	1380	Adenoma (2)	Present (3)
2,056 ppm	1467	Carcinoma (3)	Present (3)
	1550	Carcinoma (3)	None
	1481	Carcinoma (3)	Present (3)
	1490	Carcinoma	Present (3)
	1506	Carcinoma, undifferentiated (3)	Present (2)
	1544	Carcinoma, undifferentiated (3)	Present (3)[d]
	1501	Adenoma, cortex (2)	Present (2)
Females			
292 ppm	1842	Sarcoma (0-4)	None

[a]These data are taken from the IRDC (1982) report except where indicated.
[b]These data represent the final review of the kidney slides as presented by D. Kitchen (1983) at the Workshop on the Kidney Effects of Hydrocarbons.
[c]Grading system: 1 = very slight
2 = slight or small
3 = moderate
4 = severe
[d]Mineralization located in renal cortex instead of renal pelvis.

mixed kidney tumors (0.17%), for a total of six kidney tumors (0.33%) formed spontaneously in 1,794 untreated Fischer 344 rats evaluated in the National Cancer Institute's Carcinogenesis Testing Program from 1972 through 1978. The historical control incidence of 0.11% reported by Goodman et al. (1979) is 20-fold less than the 2% incidence of kidney adenocarcinomas in low-dose males shown in Table 5-7.

A significantly ($P < 0.01$) increased incidence of hepatocellular carcinomas alone and of hepatocellular adenomas and carcinomas combined was found in high-dose female mice as compared to control mice (Table 5-9). Except for a hepatocellular carcinoma in a high-dose female mouse that died at between 12 and 18 months, the observation of hepatocellular adenomas and carcinomas in female mice, as shown in Table 5-9, was confined to those animals necropsied from 18 months through final sacrifice. Hepatocellular carcinomas were described as having invasive, trabecular, and solid patterns with areas of necrosis and cytoplasmic vacuolization. Macroscopic findings in the liver of male and female mice included lobulated masses of raised red firm foci which correlated with histopathologic diagnoses of adenomas and carcinomas in liver. Mucoserous nasal discharge, possibly related to an irritant effect of gasoline vapor, was noted for male and female high-dose mice. A papillary cycstic adenoma of the renal cortex was found in a high-dose female mouse killed at final sacrifice, and bilateral renal tubular adenocarcinomas were found in a high-dose female mouse which died in the period between 18 months and final sacrifice.

In summary, exposure to unleaded gasoline vapor produced a small but statistically significant ($P < 0.05$) incidence of renal carcinomas in male Fischer 344 rats, and a statistically significant increase ($P < 0.01$) in hepatocellular carcinomas in female B6C3F1 mice under the conditions of this bioassay. Moderate body weight gain decreases with no reduction in survival in high-dose groups

suggest that a maximum tolerated dose was approached; however, mineralization in the kidney indicates that exposure to unleaded gasoline vapor produced toxicity in this organ in each treated group of male rats. Applying the International Agency for Research on Cancer (IARC) classification approach for carcinogens, the Carcinogen Assessment Group concludes that these studies furnish sufficient evidence for the carcinogenicity of unleaded gasoline vapor in animals under the conditions of the bioassay.

TABLE 5-9. HEPATOCELLULAR TUMOR INCIDENCE IN FEMALE B6C3F1 MICE FROM CHRONIC EXPOSURE TO UNLEADED GASOLINE VAPOR (IRDC 1982)

Hepatocellular tumor type	Exposure group (ppm gasoline vapor)			
	0 (control)	67	292	2,056
Adenoma	1/100[a]	4/100	3/100	7/100
Carcinoma	7/100	6/100	9/100	20/100[b,c]
Adenoma and carcinoma combined	8/100	10/100	12/100	27/100[b,c]

[a]Number with tumor/number examined.
[b]Statistically significant ($P < 0.01$) increase compared to control group.
[c]The 100 animals in each denominator in this table includes 40 animals sacrificed at 3, 6, 12, and 18 months and decedents and survivors in the remaining 60 animals which were allowed to survive for the duration of the study. If the 40 interim sacrificed animals are excluded from each denominator to allow replacement of the 100 total animals with the 60 animals allowed to survive for the duration of the study, the statistically significant differences shown in this table remain significant at $P < 0.01$.

5.2.2 90-Day Inhalation Exposure Study With Gasoline Vapor In Rats and Monkeys (MacFarland 1983)

A 90-day inhalation exposure study of the toxicity of unleaded gasoline vapor in Sprague-Dawley rats and squirrel monkeys was performed as a pre-chronic test in preparation for the carcinogenicity study with unleaded gasoline in rats and mice. In the 90-day study, rats and monkeys were exposed 6 hours/day, 5 days/week for 13 weeks to vapors of an unleaded EPA reference gasoline and a leaded commercial gasoline, as shown in Table 5-10. The hydrocarbon composition of the two gasolines was similar, but the unleaded gasoline contained 5 mg/gallon of lead and the leaded gasoline contained 1.94 g/gallon of lead. The animals were examined for mortality, body weight, food consumption, toxic signs, hematological changes, urinary changes, tissue lead levels, and pathology. Pulmonary function tests and cortical flash-evoked response tests were also done on the monkeys.

Some female monkeys in Groups III and V showed emesis. Body weights in male rats in Groups II and IV were significantly greater at termination. Female rats in Group III had increased reticulocyte counts, and some rats in Group V had increases in hematocrit and mean corpuscular volume, and decreases in white cell count and mean corpuscular hemoglobin concentration.

Male monkeys in Groups III and V had an increased minute volume. Female monkeys in Group III had a reduced respiratory rate, and female monkeys in Group V had a decreased tidal volume at termination.

Liver weights were increased in male rats in Groups II and IV and decreased in Group V female rats. Kidney weights were increased in Group IV female rats and Group V male monkeys. Thyroid weights were increased in male monkeys in Groups II and III. Heart/body weights were decreased in male rats

in Groups IV and V, and brain weights were decreased in male rats in Groups II and III. Group V female rats had decreases in liver/body and adrenal/body weights.

The initial pathological examinations showed no treatment-related effects. Histopathologic reexamination of tissue sections showed subtle but discernible changes in kidneys of Group III male rats. These changes were described as an increase in the incidence and severity of regenerative epithelium, and proteinaceous material in dilated tubules was found.

TABLE 5-10. DESIGN OF THE 90-DAY INHALATION EXPOSURE STUDY

Concentration group	Number[a] and Species of Animals		Dose (ppm)
	Rats	Monkeys	
I. Control	40	8	0
II. Unleaded gasoline	40	8	384
III. Unleaded gasoline	40	8	1,552
IV. Leaded gasoline	40	8	103
V. Leaded gasoline	40	8	374

[a]Equally divided as to sex.

5.2.3 Renal Toxicity of Gasoline and Related Petroleum Naphtha in Male Rats (Halder et al. 1983)

The renal effects of subchronic inhalation exposure of male and female Sprague-Dawley rats to vapors of unleaded gasoline and related petroleum naphthas described in Table 5-11 have been reported by C. A. Halder, T. M. Warne, and N. S. Hartoum at the Workshop on the Kidney Effects of Hydrocarbons, held in Boston, MA, on July 18-20, 1983. The results of this study are presented in Tables 5-12 through 5-20. This study is especially pertinent to the unleaded gasoline carcinogenicity study in that it gives an indication as to which fractions in unleaded gasoline can produce kidney toxicity.

Exposure of male and female Sprague-Dawley rats to unleaded gasoline for 21 days induced mild tubular degenerative and regenerative changes with increases in hyalin droplets in the renal cortex in males. Corticomedullary tubular dilatation and necrosis were found in one high-dose male rat.

A 90-day exposure to unleaded gasoline resulted in a treatment-related incidence of tubular dilatation and necrosis at the corticomedullary junction in male rats, along with a dose-related severity. The persistence of these lesions during a 4-week recovery period suggests an irreversible effect.

Similar 21-day exposures of rats to full-range alkylate naphtha, polymerization naphtha, light catalytic reformed naptha, and light straight-run naphtha induced renal lesions in males similar to those obtained with unleaded gasoline treatment. Milder renal lesions were found in males exposed to light catalytic cracked naphtha. No renal effects were noted with exposure to heavy catalytic reformed naphtha.

The results of these studies suggest that paraffin and alkene fractions are effective as renal toxicants and that aromatics are relatively non-toxic. The unleaded gasoline blend included some of the naphtha materials tested in

this study, and although the unleaded gasoline composition is proprietary, it was mentioned that it contained 22% full-range alkylate naphtha, a fraction which could be a significant factor in renal toxicity induced by unleaded gasoline exposure.

TABLE 5-11. SUMMARY OF THE COMPOSITION AND BOILING RANGES OF THE TEST MATERIALS

Material	Composition (%)			Boiling range (°F)	
	Paraffins[a]	Olefins	Aromatics	10% bp	90% bp
Light straight-run naphtha	96	0	4	71	222
Light catalytic-cracked naphtha	39	32	29	174	346
Light catalytic-reformed naphtha	67	2	31	137	230
Heavy catalytic-reformed naphtha	7	0	93	290	364
Full-range alkylate naphtha	98	2	0	124	315
Polymerization naphtha	8	92	<1	205	353
Unleaded gasoline blend	45[b]	12[b]	43[b]	112	326

[a]Includes cyclo-, normal, and branched.
[b]Estimated.

TABLE 5-12. NEPHROTOXIC EFFECTS IN RATS FOLLOWING A 21-DAY INHALATION EXPOSURE TO LIGHT STRAIGHT-RUN NAPHTHA

Group and concentrations[a]	Incidence[b] M	F
Environmental control	0/10	0/10
Sham control	0/10	0/10
1.50 mg/L (395 ppm)	0/10	0/10
5.13 mg/L (1349 ppm)	0/10	0/10
14.56 mg/L (3829 ppm)	3/10	0/10

[a]Analytical time-weighted average in mg/L (ppm).
[b]Incidence of tubular dilation and necrosis at corticomedullary junction.

TABLE 5-13. NEPHROTOXIC EFFECTS IN RATS FOLLOWING A 21-DAY INHALATION EXPOSURE TO LIGHT CATALYTIC CRACKED NAPHTHA

Group and concentrations[a]	Effects
Sham control	
0.20 mg/L (43 ppm)	
2.04 mg/L (434 ppm)	Evidence of early degenerative changes in kidneys of treated male rats.
13.06 mg/L (2,777 ppm)	

[a]Analytical time weighted average in mg/L (ppm).

TABLE 5-14. NEPHROTOXIC EFFECTS IN RATS FOLLOWING A 21-DAY INHALATION EXPOSURE TO LIGHT CATALYTIC REFORMED NAPHTHA

Group and concentrations[a]	Incidence[b]	
	M	F
Environmental control	0/10	0/10
Sham control	0/10	0/10
2.00 mg/L (544 ppm)	0/10	0/10
5.85 mg/L (1,591 ppm)	1/10	0/10
20.30 mg/L (5,522 ppm)	3/10	0/10

[a]Analytical time weighted average in mg/L (ppm).
[b]Incidence of tubular dilation and necrosis at corticomedullary junction.

TABLE 5-15. NEPHROTOXIC EFFECTS IN RATS FOLLOWING A 21-DAY INHALATION EXPOSURE TO HEAVY CATALYTIC REFORMED NAPHTHA

Group and concentrations	Incidence[b]	
	M	F
Environmental control	0/10	0/10
Sham control	NE[c]	NE
1.03 mg/L (215 ppm)	NE	NE
2.81 mg/L (587 ppm)	0/10	0/10
10.20 mg/L (2132 ppm)	0/10	0/10

[a]Analytical time weighted average in mg/L (ppm).
[b]Incidence of tubular dilation and necrosis at cortico-medullary junction.
[c]NE = Not examined. Pathology was not done due to lack of adverse effects at higher concentrations.

TABLE 5-16. NEPHROTOXIC EFFECTS IN RATS FOLLOWING A 21-DAY INHALATION EXPOSURE TO FULL-RANGE ALKYLATE NAPHTHA

Group and concentrations[a]	Incidence[b]	
	M	F
Environmental control	0/10	0/10
Sham control	0/10	0/10
1.54 mg/L (345 ppm)	10/10	0/10
4.92 mg/L (1,104 ppm)	10/10	0/10
15.31 mg/L (3,434 ppm)	10/10	0/10

[a]Analytical time weighted average in mg/L (ppm).
[b]Incidence of tubular dilation and necrosis at cortico-medullary junction

TABLE 5-17. NEPHROTOXIC EFFECTS IN MALE RATS FOLLOWING A REPEAT 21-DAY INHALATION EXPOSURE TO FULL-RANGE ALKYLATE NAPHTHA

Group and concentrations[a]	Incidence[b] in males
Sham control	0/40
0.015 mg/L (3 ppm)	0/20
0.152 mg/L (34 ppm)	4/10
1.538 mg/L (345 ppm)	11/20

[a]Analytical time-weighted average in mg/L (ppm).
[b]Incidence of tubular dilation and necrosis at corticomedullary junction.

TABLE 5-18. NEPHROTOXIC EFFECTS IN RATS FOLLOWING A 21-DAY INHALATION EXPOSURE TO POLYMERIZATION NAPHTHA

Group and concentrations[a]	Incidence[b]	
	M	F
Environmental control	0/10	0/10
Sham control	0/10	0/10
1.04 mg/L (215 ppm)	0/10	0/10
3.05 mg/L (632 ppm)	2/10	0/10
9.89 mg/L (2,049 ppm)	4/10	0/10

[a]Analytical time-weighted average in mg/L (ppm).
[b]Incidence of tubular dilation and necrosis at corticomedullary junction.

TABLE 5-19. NEPHROTOXIC EFFECTS IN RATS FOLLOWING A 21-DAY INHALATION EXPOSURE TO AN UNLEADED GASOLINE BLEND

Group and concentrations[a]	Incidence[b]	
	M	F
Environmental control	0/10	0/10
Sham control	0/10	0/10
0.11 mg/L (29 ppm)	0/10	0/10
1.58 mg/L (416 ppm)	0/10	0/10
12.61 mg/L (3,316 ppm)	1/10	0/10

[a]Analytical time-weighted average in mg/L (ppm).
[b]Incidence of tubular dilation and necrosis at corticomedullary junction.

TABLE 5-20. NEPHROTOXIC EFFECTS IN RATS FOLLOWING A 90-DAY INHALATION EXPOSURE TO AN UNLEADED GASOLINE BLEND

Group and concentration[a]	Incidence[b]			
	Terminal sacrifice		Four-week recovery	
	M	F	M	F
Environmental control	0/10	0/10	0/10	0/10
Sham control	0/10	0/10	0/10	0/10
0.15 mg/L (40 ppm)	1/10	0/10	1/10	0/10
1.44 mg/L (379 ppm)	7/10	0/10	5/10	0/10
14.70 mg/L (3,866 ppm)	5/10	0/10	4/10	0/10

[a]Analytical time-weighted average in mg/L (ppm).
[b]Incidence of tubular dilation and necrosis at cortico-medullary junction.

5.2.4 Renal Effects of Decalin in Several Laboratory Mammalian Species (Alden et al. 1983)

A comparison of the renal effects in various laboratory mammalian species exposed to decalin, a prototype volatile hydrocarbon, was discussed in "The Pathogenesis of the Nephrotoxicity of Volatile Hydrocarbons in the Male Rat," by Carl L. Alden, R. L. Kanerva, G. Ridder, and L. C. Stone, at the Workshop on the Kidney Effects of Hydrocarbons, held in Boston, MA, on July 18-20, 1983. One of the observations presented was that a 91-day inhalation exposure to 5 ppm and 50 ppm decalin induced renal toxicity in male Fischer 344 rats and not in females, male or female mice, male or female dogs, and male or female guinea pigs (Table 5-21). The observed renal effects in male rats included hyalin droplets in the cytoplasm of proximal convoluted tubular epithelial cells, granular casts at the junction of the inner and outer band of the outer zone of the medulla, and augmented chronic glomerulonephropathy. These droplets consist of an $alpha_{2u}$ globulin, a protein synthesized in the male rat liver under the control of testosterone and endogenous corticosterone. They occur spontaneously in sexually mature male rats but not in castrated males, in female rats, or in humans.

TABLE 5-21. BIOLOGICAL TESTING OF DECALIN, A PROTOTYPE VOLATILE HYDROCARBON

Species tested	Renal injury	Reference
Rat (male/female)	+/-	AFAMRL-TR-79-121 (Wright-Patterson AFB)
Mice (female)	-	
Dog (male/female)	-/-	
Guinea pig (male/female)	-/-	AFMRL-TR-78-55 (Wright-Patterson AFB)
Mice (male)	-	Dr. Logan Stone (personal communication)

5.2.5 Toxicity of Synthetic Fuels and Mixed Distillates In Laboratory Animals (MacNaughton and Uddin 1983)

Toxicity studies on mixed distillates and synthetic fuels in experimental animals have been or are being done by the United States Air Force, and preliminary results of these studies were reported by M.G. MacNaughton and D.E. Uddin at the Workshop on the Kidney Effects of Hydrocarbons, held in Boston, MA, on July 18-20, 1983. The studies are summarized below. The design of the experiments, in which the agents were given by inhalation, is shown in Table 5-22. Beagle dogs, Fischer 344 rats, Golden Syrian hamsters, and C57BL/6 mice were used.

5.2.5.1 Studies with RJ-5 Synthetic Fuel

RJ-5 fuel consists of hydrogenated dimers of norbornadiene with a vapor pressure of 1.3 kPa at 103°C.

Results of the studies with a one-year exposure to 30 mg/m^3 and 150 mg/m^3 were as follows:

1. Decreased body weight gain in rats and dogs, with possible appetite suppression.
2. Acute lung inflammation and some bronchopneumonia in rats and dogs sacrificed immediately after 6 months of treatment.
3. After a 1-year holding period, there was a 25% incidence of alveolargenic carcinomas in CF-1 mice (the strain shown in the workshop proceedings), a strain predisposed to this tumor type.

The results of the studies in which dogs, mice, hamsters, and rats were exposed to 30 or 150 mg/m^3 for 1 year followed by a 1-year holding period were as follows:

1. Decreased body weight gain in exposed male rats and male hamsters throughout the study. Increased body weight gain in exposed rats during treatment was reversed during the post-treatment period.

TABLE 5-22. DESCRIPTION OF FUEL INHALATION EXPOSURES

Fuel	Exposure (months)	Concentration (mg/m^3)	Species[a]	End date
Synthetic				
JP-10	12, int[b]	560	D,R,M/F,H	Completed
RJ-5	6, cont[c]	155	D,R,M/F,M	Completed
RJ-5	12, int	30, 150	D,R,M/F,H	Completed
Mixed Distillate				
JP-4	8, int	2,500, 5,000	D,R,M,M/F	Completed
JP-4	3, cont	500, 1,000	D,R,M/F	Dec. 1983
JP-4	12, int	500, 1,000	R,M/F	Jul. 1984
JP-5	3, cont	150 750	D,R,M/F	Completed
JP-5 (S)[d]	3, cont	250, 750	D,R,M/F	Completed
JP-7	12, int	150, 750	R,M/F	Dec. 1985
JP-8	3, cont	500, 1,000	R,M/F	Jul. 1985
JP-TS	12, int	200, 1,000	R,M/F	Dec. 1985
DFM	3, cont	50, 300	R,M/F	Completed
DFM(S)	3, cont	50, 300	R,M/F	Completed

[a]D (dogs); R (rats); M (monkeys); M/F (mice, female); H (hamsters).
[b]Intermittent (6 hours/day, excluding weekends and holidays).
[c]Continuous.
[d]Shale.

2. Decreased ($P < 0.05$) kidney/body weights in exposed female rats.
3. Four (7%) renal cell adenomas and five (8%) renal cell carcinomas in high-dose male rats; one (2%) renal cell carcinoma in a low-dose male rat. No renal cell carcinomas were seen in controls.
4. Other kidney lesions in exposed male rats were:

Lesion	Incidence		
	150 mg/m^3	30 mg/m^3	Control
Renal medullary mineralization	57/62 (92%)	2/59 (3%)	0%
Moderate pelvic urothelial hyperplasia	58%	7%	2%
Hyalin droplets	18%	19%	2%
Cortical cysts	24%	2%	0%

5.2.5.2 Studies with JP-10 Synthetic Fuel

JP-10 fuel is a bicyclic, bridged compound; exotetrahydrodi(cyclopentadiene), with a vapor pressure of 1.87 kPa.

Results of the studies with a 1-year exposure at 562 mg/m^3 followed by a 1-year recovery period were:

1. Slight weight loss in exposed rats and hamsters.
2. Hepatocellular vacuolization in 50% of the control and 75% of the exposed female mice.
3. Nine renal cell carcinomas in treated male rats compared to one in contols; poorly differentiated malignant neoplasms in one control and one treated male rat.
4. Other renal effects in male rats were:

Lesion	Incidence	
	Treated	Control
Augmented renal tubule degeneration compatible with old-rat nephropathy	43/49 (87%)	32/49 (65%)
Medullary mineral deposits (mineralized cell debris)	100%	0%
Papillary hyperplasia of renal pelvic epithelium	26/49 (53%)	2/49 (4%)

5. No toxic lesions in female mice.
6. Adrenal cortical adenomas and carcinomas were found in 27% of the control and 28% of the treated male hamsters; however, adrenal zona glomerulosa adenomas and adrenal zona glomerulosa hyperplasia were found in 14% and 72% of treated male hamsters, respectively, and 5% and 45% of control male hamsters, respectively.

5.2.5.3 Studies with JP-4 Mixed Distillate

JP-4 mixed distillate has characteristics similar to gasoline and has a vapor pressure of 13 kPa. JP-4 represents 85% of the turbine fuel used by the Department of Defense.

Results of studies with an 8-month intermittent exposure to 2,500 and 5,000 mg/m^3 (containing 80 mg/m^3 benzene) were:

1. Increased organ and organ/body weights for kidney, liver, spleen, and lung in exposed male rats.
2. A 27% incidence of bronchitis in exposed rats.
3. A transient increase in red blood cell fragility in female dogs.

Results of studies with a 90-day continuous exposure to 500 and 1,000 mg/m^3 with a 19-month holding period were:

1. Increases in serum globulin and total protein and BUN in low-dose and high-dose dogs.
2. Decreased body weight gain in exposed male and female rats during treatment.
3. Centrilobular hepatocellular fatty change in 88% of the low-dose and 89% of the high-dose female mice.
4. Kidneys of all exposed male rats contained hyalin droplets in the proximal tubular epithelium, and focal dilatation of renal tubules near the corticomedullary junction with plugging by cellular debris was found in 96% of the low-dose and 100% of the high-dose male rats.

Results of studies with a 1-year exposure to 500 and 1000 mg/m^3 were:

1. Decreases in body weight and in kidney and liver weights in high- and low-dose male rats.
2. Decreases in spleen and kidney weights in low dose female rats.
3. Histopathological examination of tissues is ongoing.

5.2.5.4 Studies with JP-5

JP-5 mixed distillate is the other major turbine engine fuel besides JP-4. Results of studies with a 90-day continuous exposure of dogs, rats, and mice to 150 and 750 mg/m^3 and a 19 month post-exposure period were:

1. Decreased body weight in exposed male rats.
2. Increased BUN and serum creatinine in male and female high-dose rats.
3. Mild, diffuse fatty change with small vacuoles in hepatocytes of 3% of the control, 73% of the low-dose, and 24% of the high-dose mice. "Foamy" hepatocellular cytoplasmic vacuoles were found in 18% of the control, 15% of the low-dose, and 44% of the high-dose female mice.

4. Male rats sacrificed at the end of the 90-day exposure period had dilated renal tubules filled with granular necrotic debris at the cortico-medullary junction.
5. By 19 months post-treatment, old-rat nephropathy was evident in 96%, 96%, and 84% of the high dose, low dose, and control males, respectively. Old-rat nephropathy was more severe in treated males. Renal medullary tubular mineralization was found in 82% of the high-dose, 59% of the low-dose, and none of the control male rats. A dose-related focal hyperplasia of the renal pelvis was reported.

In summary, chronic exposure to RJ-5 and JP-10 synthetic fuels induces a common pattern of nephrotoxicity leading to renal carcinomas. Similar studies with JP-4 and JP-5 show the same preneoplastic lesions, but histopathological analysis is currently incomplete and no information is available about neoplastic response.

5.2.6 Influence of Benzene on the Renal Carcinogenic Effects of Unleaded Gasoline Vapor in Male Rats

According to an October 1983 draft report of a bioassay of benzene by the National Toxicology Program (NTP 1983) in which Fischer 344 rats and B6C3F1 mice were tested, several organ sites in mice and rats had benzene induced carcinomas. These included Zymbal gland and oral cavity carcinomas in male and female rats; skin carcinomas in male rats; Zymbal gland, prepugial gland and lung carcinomas in male mice and mammary, ling, and hepatocellular carcinomas in female mice.

Data from the NTP report are discussed in section 5.4.2.6 and Appendix B, and from the Maltoni et al. experiment are discussed here. In these experiments, male and female Sprague-Dawley rats were either dosed with 50 or 250 mg/kg of benzene by gavage 5 days/week for 52 weeks, exposed by inhalation 4 to 7

hours/day to 200 ppm benzene for 15 weeks or to 200-300 ppm benzene for 104 weeks, or dosed with 500 mg/kg of benzene by gavage 4-5 days/week for 104 weeks. Animals in these studies were allowed to survive until spontaneous death before pathological examination. Treated animals were compared with controls.

In the study in which rats were dosed with 50 and 250 mg/kg of benzene, there was a dose-related increase in mortality. Dose-related increases in Zymbal gland carcinomas, "haemolymphoreticular" neoplasms, and mammary carcinomas were reported. Two carcinomas of the oral cavity, one subcutaneous angiosarcoma, and one hepatoma were also found in treated animals.

No carcinogenic effect from 15 weeks exposure of 200 ppm benzene was observed. Exposure to 200-300 ppm benzene for 104 weeks increased mortality, and Zymbal gland carcinomas and hepatomas were attributed to treatment in this study.

Gavage treatment with 500 mg/kg of benzene decreased body weight and induced hematological effects. Zymbal gland carcinomas and carcinomas of the oral cavity were concluded to be treatment-related.

None of the tumor types attributed to benzene treatment in the studies by Maltoni et al. (1982) and NTP (1983) were found as treatment related effects in the carcinogenicity study with unleaded gasoline vapor (104-week exposure) in male Fischer 344 rats. Conversely, the kidney tumors in male Fischer 344 rats in the study with unleaded gasoline vapor were not a treatment-related effect in the studies of Maltoni et al. (1982) in Sprague-Dawley rats. Nonneoplastic renal lesions of similar morphology have been found in male Sprague-Dawley rats and male Fischer 344 rats exposed to unleaded gasoline blend as well as other hydrocarbons in toxicity studies

with inhalation exposure. Renal toxicity was not indicated in the Maltoni et al. (1982) report. Taking all of this evidence together, it would appear that the kidney has not yet been shown to be a target organ for benzene carcinogenicity in male rats. Furthermore, the benzene level compared to other ingredients in unleaded gasoline, which was completely volatilized in the carcinogenicity study, was relatively low at 2%, and comparison with similar toxic and carcinogenic effects induced in the kidneys of male rats by other hydrocarbons, including mixed distillates, synthetic fuels, and other hydrocarbons without benzene, indicates that the hydrocarbon nature of unleaded gasoline was pivotal in the induction of renal carcinomas in male Fischer 344 rats. Snyder et al. (1980) found an increased number of hematopoietic neoplasms in male C57BL mice exposed to benzene, but there was no indication of renal neoplasia from exposure to benzene in this study.

5.2.7 Conclusions of the UAREP Report (1983) on Toxicological Interpretation of Hydrocarbon-Induced Kidney Lesions

An analysis of the toxicology and carcinogenicity of unleaded gasoline and other hydrocarbons, issued by the Universities Associated for Research and Education in Pathology, Inc. (UAREP) was published in December, 1983. This section summarizes the review and interpretation of the data presented in that document.

5.2.7.1 Asessment of the API Chronic Inhalation Study with Unleaded Gasoline Vapor in Rats and Mice

1. There were significant increases in renal adenoma and carcinoma incidence in male Fischer 344 rats and in hepatocellular adenoma and carcinoma incidence in female B6C3F1 mice exposed to unleaded gasoline vapor. The bioassay was well designed and conducted, and there were several independent examinations of the kidney slides.

2. Male Rat Kidney Lesions in Exposed Groups

   a. Three-month findings: Focal degeneration in the proximal tubules, hyalin droplets in the proximal tubules, granular casts at the junction between the inner and outer stripes of the outer medulla, some evidence of regeneration. The lesions seemed dose-related, with the greatest prominence in the high-dose group.

   b. Findings by 12 months: Karyomegaly, probably in the P_3 segment of the proximal tubules. Old-rat nephropathy, in treated as well as control rats, shown as atrophy of the P_1 segment of the proximal tubule with basement membrane thickening, mesangial thickening in the glomeruli, interstitial fibrosis with chronic inflammation, and periodic acid-Schiff (PAS)-positive tubular colloid casts in the distal nephron. Calcium hydroxyapatite deposition in the papilla was evident in the exposed groups.

   c. Findings after 12 months: Areas of hyperplasia. Progression of the severity of old rat nephropathy, which was greater in the treated groups than in controls, as well as preneoplastic and neoplastic lesions.

   d. Findings at 18 months: Renal adenoma in one rat.

   e. Findings at 24 months (final sacrifice): Renal adenomas and carcinomas in treated rats.

3. Liver Pathology in Female Mice

   No treatment-related lesions were found between 3 and 18 months. It could not be determined whether preneoplastic lesions preceded the liver tumors. Acute effects, such as fatty metamorphosis from exposure of mice to other hydrocarbons, were neither reported nor looked for in the carcinogenicity study with unleaded gasoline.

5.2.7.2 Interpretation of the Toxicological Carcinogenic Findings in the Carcinogenicity Study with Unleaded Gasoline by UAREP

1. Male Rat Kidney - Nonneoplastic Lesions

Signs of acute and chronic renal toxicity were evident. There was some necrosis, but more often there was cell degeneration and/or blebbing with release of cell debris forming casts between the pars recta (P_3 segment) and the thin limb. The kidney lesions in exposed male rats were unique in that they were unlike those induced by known nephrotoxins such as mercuric chloride, halogenated hydrocarbons, or nitrilotriacetic acid.

The mechanism of gasoline-induced nephrotoxicity is obscure. There was no uniform necrosis in the P_3 segment, and many lesions were found in the P_1 and P_2 segments. It is currently not possible to accurately characterize the nature and location of the toxic lesions in male rat kidneys in this study.

The toxic kidney lesions were clearly distinguishable from old-rat nephropathy, which in the latter involved the whole kidney and showed atrophy of the P_1 segment of the proximal tubule and glomerular sclerosis. However, exposure to unleaded gasoline vapor augmented the severity of old-rat nephropathy.

The most striking chronic nonneoplastic lesion in exposed rats was severe mineralization of the tubules in the papilla. The deposits were characterized as calcium hydroxyapatite. The etiology behind the mineralization is uncertain, but it was hypothesized that chronic damage in the proximal tubule and higher segments in the nephron leads to phospholipid vesicle-induced calcification. The observed calcification

pattern is unique to hydrocarbon exposures, and chronic exposure of male rats to JP-10 synthetic missile fuel has also induced mineralization in the kidney.

2. Male Rat Kidney - Preneoplastic and Neoplastic Lesions

The neoplastic process resulting from treatment of male rats with unleaded gasoline resembles that induced by several other renal carcinogens: karyomegaly, probably in the P_3 segment, followed by hyperplasia, followed by adenomas that were often cystic, and carcinomas. These preneoplastic and neoplastic lesions were found in association with the increased severity of old-rat nephropathy, but it was not possible to characterize their precise location, nature, or progression. It was not possible to establish the influence of old-rat nephropathy on these lesions, and the mechanism for the induction of these preneoplastic and neoplastic lesions is unknown.

3. Liver Lesions in Female Mice

Acute toxicity or preneoplastic lesions were not found in the livers of female mice, according to the carcinogenicity study report; however, hematoxylin and eosin staining is not sensitive for the detection of preneoplastic lesions. Acute toxicity studies with other hydrocarbons in mice have revealed fatty metamorphosis in the liver. The neoplasms in livers of female mice exposed to unleaded gasoline vapor resembled mouse liver neoplasms found in other studies, but such neoplasms are often phenotypically similar regardless of etiology.

5.2.7.3 Review of Human Kidney Lesions

1. Acute - There are no thorough studies on human kidney lesions from acute exposure to hydrocarbons. Case reports indicating structural

and functional changes in the kidney show the main lesions as variants of an immume complex type of glomerular nephritis, a type of lesion that has not been found in rodents. The mechanism of acute toxicity in the case reports is difficult to pinpoint because of confounding factors.

2. Chronic - There is no persuasive evidence that human exposure to gasoline is associated with renal cancer. There is no evidence for the calcification of papilla or calculi in the bladder or kidney from human exposure to gasoline. Human renal adenocarcinomas are morphologically similar to those found in male rats chronically exposed to unleaded gasoline vapor, as well as to well-characterized models of rodent renal neoplasia induced by chemical carcinogens. However, renal adenocarcinomas develop in human kidneys that are normal except for a putative increase in hyperplasia and adenomas, whereas adenocarcinomas in the kidneys of rats chronically exposed to unleaded gasoline vapor occurred with a background of chronic, and often severe, renal disease. An apparent increase in the incidence of adenomas and carcinomas in the kidneys of dialysis patients is the only possible equivalent to adenocarcinoma induction in rats. This possible similarity between humans and rats needs to be further investigated, but it is consistent with the view that any type of chronic renal injury, e.g., old-rat nephropathy, can possibly act as a promoter and/or cocarcinogen in the induction of renal neoplasia.

Human renal cancer can occur along with chronic interstitial nephritis, e.g., chronic analgesic nephropathy. This type of cancer arises in the renal pelvic epithelium to yield transitional cell carcinomas totally different in location and structure from the lesions seen in the chronic rat study with unleaded gasoline.

5.2.7.4 Species and Sex Comparison of the Kidney

Renal morphology has been most thoroughly studied in the rat, rabbit, and dog. There has been no detailed ultrastructural study in the mouse kidney. A detailed ultrastructural study has been done with renal biopsies from 10 human males who were screened for renal dysfunction.

The human kidney is multilobular, without the distinct zonation caused by the alignment of nephrons in the unilobar rodent kidney. The rodent kidney has a long loop of Henle and long papillae to allow extensive concentration of urine. The human kidney has nothing like the outer stripe of the outer medulla in the rodent kidney, which contains the pars recta (P_3 segment) of the proximal tubule and the ascending limb of the loop of Henle. The human kidney has an ultrastructurally simple proximal tubule in contrast to the kidney of the rat, mouse, and rabbit. No morphological differences between the kidneys of male and female humans have been described.

The size and number of lysosomes in the P_1, P_2, and P_3 segments in the male rat kidney are larger than in the female rat kidney. This may be correlated with the unique production of alpha-2-microglobulin and resorption of this protein in the proximal tubule in the male rat kidney. Endoplasmic reticulum and microbodies are more prominent in female than in male rat kidneys, which may indicate a difference between them in metabolic capacity. Castration and hypophysectomy of male rats decreases the differences in the proximal tubule, particularly lysosomes, between male and female rat kidneys.

Hyalin droplets in the P_1 and P_2 segments have been found in kidneys of male rats exposed to hydrocarbons other than unleaded gasoline vapor, e.g., decalin. These droplets consist mainly of protein, including alpha

2-microglobulin, within the phagolysosomal system of the male rat kidney. In the male rat kidney it is presumed that, with no evidence of acute glomerular lesions, these droplets represent accumulations of endogenous proteins that are produced in the male rat liver and resorbed by the kidney, to be phagocytized by lysosomes. Protein accumulation by the kidney could be due to increased synthesis and uptake and/or decreased degradation. The mechanism of hydrocarbon nephrotoxicity is presently unclear. Exposure to decalin produces hyalin droplets in male but not in female rats, and these droplets have been observed to disappear quickly after cessation of treatment with decalin. However, it is not known whether chronic lysosomal overload can produce cell injury in rat kidney proximal tubules.

Less is known about renal mixed function oxidase (MFO) than about liver MFO. There are marked species, strain, and sex differences in the metabolic capability of the rodent kidney. There are potentially significant quantitative and, to a lesser degree, qualitative differences in renal MFO components and activity among species. In all species studied thus far, MFO activity has been localized in the proximal tubule and usually in the P_3 and/or P_2 segments. Little data exist on MFO in the human kidney. Metabolites from other organs can possibly go to the kidney to produce toxicity in vivo. Specific studies on renal MFO and unleaded gasoline toxicity are lacking, but there is some evidence that renal MFO may play a role in the renal toxicity of other hydrocarbons.

5.2.7.5 Rodent Kidneys and Other Hydrocarbons

The only hydrocarbon fuel other than unleaded gasoline that has been tested in a chronic rodent bioassay is the synthetic missile fuel JP-10. Exposure to JP-10 was found to induce renal carcinomas in male Fischer 344 rats.

Most solvents and hydrocarbons have induced similar acutely toxic lesions in the rodent kidney; however, no specific mechanism of action has been determined, nor has an ultrastructural analysis been done.

Paraffin and isoparaffin fractions have been found to be more acutely toxic in rodent kidneys than are other fractions of petroleum products.

The mutagenicity of unleaded gasoline has been reported as negative. However, in the in vitro assays, only S-9 fractions from rat liver were used, which may not relate to other organs: also, there may have been a problem with the volatility and solubility of the gasoline in these assays.

None of the tested hydrocarbons has produced a uniform necrosis in the pars recta epithelium, as is commonly seen in the rodent kidney with other renal toxins, such as mercuric chloride.

Acute lesions in the rodent kidney from hydrocarbon exposure have been characterized as hyalin droplet accumulations, focal areas of degeneration and necrosis, epithelial regeneration, and granular casts in the corticomedullary junction.

5.2.7.6 Old-Rat Nephropathy

Old-rat nephropathy is characterized by interstitial fibrosis, thickening of tubular basement membranes, interstitial chronic inflammation, vascular thickening in interlobular and afferent arterioles, glomerular hyalinization, and tubular atrophy, especially in the P_1 segment of the proximal tubule.

In the unleaded gasoline carcinogenicity bioassay, increased numbers of mitoses, hyperplasia, karyomegaly, and other preneoplastic lesions were not seen in control rats.

Old-rat nephropathy may start at an early age. Old females show a lesser degree of nephropathy than males. The severity of old-rat nephropathy varies among strains.

Old-rat nephropathy was morphologically different from preneoplastic lesions in the API carcinogenicity study of unleaded gasoline vapor. However, a possible etiologic relationship between old-rat nephropathy and toxic lesions from exposure to unleaded gasoline vapor cannot be ruled out.

In humans, chronic renal disease has been associated with renal adenocarcinoma only in kidney dialysis patients. Lesions in the kidneys of these patients are morphologically similar to those seen in male rats in the chronic unleaded gasoline vapor study, as well as in other rodent studies with hydrocarbons. No control human group has been studied along with the dialysis patients.

Old-rat nephropathy resembles focal and segmental glomerulosclerosis in human disease and, to a lesser extent, arteriolar and arterial nephrosclerosis in aging humans. Several patients with renal carcinoma following dialysis had renal failure secondary to nephrosclerosis, and one of these patients had multiple calculi in the kidney.

Studies with the liver indicate a greater ability of younger than of older rats to metabolize carcinogens; however, a similar comparison with the kidney still needs to be explored.

5.2.7.7 Comparative Nephrotoxicity and Nephrocarcinogenicity

Species, strain, and sex differences in response to nephrotoxins are clearly evident. Many chemical classes of nephrotoxins induce similar morphological effects, and most nephrotoxic chlorinated hydrocarbons affect primarily the P_3 segment of the proximal tubule, which is apparantly lacking in humans. However, few agents have well-characterized mechanisms of acute and chronic renal toxicity. Some chlorinated hydrocarbons, e.g., chloroform, have chronic, carcinogenic effects that do not always correspond to acute effects in terms of target organ response.

Chloroform induces a selective and uniform degeneration and necrosis of proximal tubule epithelial cells, with the effect being greatest in areas with the greatest MFO activity, as supported by studies in rats and dogs. Similar patterns of proximal tubule degeneration from exposure to chloroform have been observed in these two species.

There is evidence that similarity of morphological endpoints does not necessarily indicate similarity between mechanisms. For example, nitriloacetic acid may act as a promoter in the induction of renal adenocarcinomas in rats, but the renal adenocarcinomas induced by this agent are morphologically similar to those observed in rats in the chronic unleaded gasoline vapor study. Acute, but not chronic, renal lesions from mercuric chloride treatment are unlike those induced by exposure to hydrocarbons. Mercuric chloride, as well as chlorinated hydrocarbons, initially induces selected necrosis in the renal P_3 segment, and with higher doses also induces progressive necrosis in the P_1 and P_2 segments.

5.2.7.8 Significance to Humans of the Chronic Inhalation Study with Unleaded Gasoline Vapor in Rats and Mice

1. The relationship between old-rat nephropathy and renal neoplasia in the chronic unleaded gasoline study is presently uncertain. Although renal neoplasia in male rats exposed to unleaded gasoline does not appear to stem from basophilic cells in the old-age renal lesions, a role of the old-age lesions in the etiology of renal neoplasia presently cannot be ruled out. In humans, chronic renal disease has been associated with an increased incidence of renal cancer.
2. No statistically significant association between renal epithelial neoplasia and environmental agents, except for cigarette smoke, has been found in humans. Tumors of the renal pelvis in humans have been associated with exposure to environmental agents.

3. Anatomical and physiological differences between rat and human kidneys may contribute to differences in renal responses to environmental agents, including unleaded gasoline. This issue needs further study.

5.2.8 Summary of Animal Studies

A lifetime inhalation bioassay of unleaded gasoline in Fischer 344 rats and B6C3F1 mice induced a statistically significant incidence (6/100) of renal carcinomas in the kidney cortex of male rats and a larger, also statistically significant incidence (20/100) of hepatocellular carcinomas in female mice. Female rats and male mice had no significant treatment-related induction of tumors at any organ site. The incidence of renal tumors was statistically significant at the highest dose tested (2,056 ppm) but not at the two lower doses (292 ppm and 67 ppm). In mice the incidence of liver carcinomas alone and adenoma and carcinoma combined was also statistically significant in the highest but not the two lower dose groups. Moderate decrements in body-weight gain in the high-dose groups indicate that the maximum tolerated dose was reached. Glomerulonephrosis occurred in nearly all male rats, and mineralization of the pelvis was correlated with dose. However, there was no correlation between animals with tumors and those with mineralization.

The acute and subchronic renal toxicity of decalin, a volatile hydrocarbon of the same general type as those contained in gasoline, is confined to male rats and does not occur in female rats or in mice, dogs, or guinea pigs. In a series of 21-day inhalation exposures of male rats to a variety of chemical fractions of gasoline, renal toxicity was correlated with the paraffin components and not with the aromatic compounds in the mixture. The same pattern of renal toxicity as well as a positive renal tumor response occurs in response to chronic inhalation of two synthetic fuels (RJ-5 and JP-10). Chronic inhaltion studies with the jet fuels used by the Air Force and Navy (JP-4 and

JP-5) have shown the same nephrotoxic lesions, but no statements can be made about the carcinoma response until histopathological analysis has been completed. The renal toxicity pattern observed with exposure to hydrocarbon mixtures, involving protein accumulation in renal tubules, is clearly different than the kidney lesions occurring spontaneously in old rats, and occurs in males of both Fischer 344 and Spraque-Dawley strains, but not in females of these strains or in mice or monkeys.

Mutagenesis tests of unleaded gasoline have been carried out in Salmonella, yeast, mouse lymphoma in vivo cytogenetics, and mouse dominant lethal systems. Various gasoline feedstocks have been tested in mouse lymphoma and in vivo cytogenetics assays. The results of most of these assays have not met the criteria for positive responses. A detailed examination of their adequacy is in process.

5.3 Epidemiologic Studies of Petroleum Workers

Animal studies involving mice and rats have indicated that unleaded gasoline exposure may increase the risk of cancer, especially kidney and liver cancers, in humans. The purpose of this section is to review the epidemiologic literature in order to determine whether there is any epidemiologic evidence suggested by the animal findings. Three epidemiologic studies have been reviewed: two published [Thomas et al. (1980, 1982)] and one unpublished [Rushton and Alderson (1982)].

5.3.1 Thomas et al. (1980)

Death records of individuals, who at the time of death were active members of the Oil, Chemical, and Atomic Workers International Union (OCAW), were reviewed for specific causes of death by the Environmental Epidemiology Branch of the National Cancer Institute (Thomas et al. 1980). The study group

consisted of 3,105 males whose deaths were reported to the OCAW International Headquarters by Union locals in Texas between 1947 and 1977 and for whom death certificates were obtained. Death certificates were not available for 10% of the reported deaths. Approximately 40% of the decedents were less than 50 years of age at death. Also, 40% of the decedents were union members for less than 10 years.

The individual plants in which members had worked were classified into one of five major categories according to major processes. The most interesting and the one for which approximately 70% of the deaths were classified was the petroleum refinery and petrochemical plant category. In the discussion presented here, the results will be restricted to this category.

Proportionate mortality ratios (PMRs), adjusted for age and calendar time using the United States general population, were computed and tested for statistical significance.

The PMR for all cancer deaths (1.26) was significantly elevated for whites ($P < 0.01$) but not for blacks ($P > 0.05$). Also, the relative frequency for arteriosclerotic heart disease deaths was elevated significantly for both racial groups ($P < 0.01$). However, the relative frequencies for respiratory and digestive disease deaths were quite a bit lower than expected for whites and blacks. Both races had significantly elevated ($P < 0.01$) PMRs for non-motor vehicle accidents, whereas the PMR for motor vehicle accidents was significantly greater for whites only.

With regard to the relative frequencies of cause-specific cancer deaths, greater than expected frequencies ($P < 0.05$) were observed for cancers of the digestive organs and peritoneum, respiratory system, and skin for whites.

The PMRs for cancer of the stomach (2.69) and kidney (2.14) were significantly elevated ($P < 0.05$) only for white males who joined the union 20 or

more years prior to death. Black males whose lengths of union membership were either less than 10 years or 10 or more years experienced significantly greater than expected frequencies (PMRs of 2.42 and 2.80, respectively) of stomach cancer deaths.

These results provide very weak evidence for the carcinogenicity of gasoline vapors because of the following study limitations. The underlying cause of death was unable to be determined for 10% or approximately 350 of the total number of deaths. This underreporting of the causes of death could heavily influence the cause-specific mortality frequencies, especially if the underreporting were occurring for a small number of causes of death.

A serious problem is inherent in the usefulness of PMRs. If the study group has a lower mortality rate than the comparison group for all causes of death, PRMs represent inflated estimates of cause-specific risks. Furthermore, excesses for one or more causes will automatically force others to be in deficit.

This study has the obvious limitation of resricting its investigation to active members of the union, thereby excluding union members who retired or left the union for other reasons as well as excluding non-union members. The results of this study, therefore, may overrepresent diseases with very low survival rates and underrepresent diseases which tend to occur in retirees.

No exposure information regarding mesured levels of gasoline vapor is given. Furthermore, the question has to be raised as to whether this study actually investigates the risk of leaded gasoline exposure or health in contrast to unleaded gasoline exposure. This study examined the mortality experience of active OCAW members between 1947 and 1977. The advent of unleaded gasoline use did not take place until the late 1970s.

Along with the problem of determining the exact agent to which the study population was exposed, there is a question with regard to the adequacy of the latency period. A surrogate measure of latency is length of union membership. Approximately 40% and 25% of the decedents were OCAW members for less than 10 years and more than 20 years, respectively. Thus, for a significantly large proportion of the study population, the period between first exposure and death was less than the generally accepted average latency period of 10 to 30 years for environmentally induced cancers.

5.3.2 Thomas et al. (1982)

For three of the refineries included in their earlier study, Thomas et al. (1982) examined the cause-specific mortality experience of an expanded group of union members. The number of male deaths from the original study of these three refineries was 1,161. This number was expanded to include 1,194 retiree deaths and 154 additional active union member deaths. In the earlier study, reported deaths were for the period between 1947 and 1977. The period of observation for the present study was extended through 1979. Thus, 2,509 active and retired members of the OCAW were available for analysis in the present study. As with the earlier study, death certificates were unable to be located for 8% of the reported deaths. However, no information was given regarding length of union membership.

The PMR for stomach cancer was significantly elevated ($P < 0.05$) for whites (1.41) and nonwhites (1.96). The relative frequencies of deaths attributable to cancer of the pancreas (1.42), prostate (1.46), brain (2.28), and hematopoietic and lymphatic system (1.72), [including leukemia (1.89) for whites only] were significantly greater than expected ($P < 0.05$). Although the PMR (1.51) for kidney cancer was elevated for white males, it was not statistically significant ($P > 0.05$).

There were differences in mortality patterns for white males among active and retired union members. Significant relative excesses ($P < 0.05$) of stomach, pancreatic, and brain cancer deaths were seen among active members. However, among retired members, the PMRs were significantly elevated for prostrate cancer, Hodgkin's disease, multiple myeloma, and leukemia deaths.

The limitations of this study are similar to those already noted in relation to the earlier study. There are serious concerns regarding the loss of individuals due to the unavailability of death certificates, lack of exposure information, and the inherent validity of PMRs.

5.3.3 Rushton and Alderson (1982)

Rushton and Alderson (1982), in an unpublished report, presented the results of a retrospective cohort mortality study of workers at distribution centers from three oil companies in Great Britain. This study was funded by 23 oil companies in Great Britain, and was coordinated by the Institute of Petroleum. The study population consisted of men employed for at least one year between January 1, 1950 and December 31, 1975. The comparison population used was the entire male population of England and Wales.

A total of 762 distribution centers contributed 23,358 men to the study population. Ninety-nine percent of the population was followed sucessfully to determine their vital status as of December 31, 1975. The study population accounted for 397,568.60 person-years, with an average follow-up period of 17.1 years.

The number of deaths of the study population was decidedly lower than that of the comparison population both from all causes (3,925 observed, 4,632 expected) and from all neoplasms (1,002 observed, 1,157 expected).

These deficits may in part reflect the "healthy worker" effect. However, consideration must also be given for the criteria of inclusion of participants

into the study. Although a feasibility study suggested that a minimum of 10 years of employment should be required for admittance into the study population, it was decided to reduce this requirement to one year in order to increase the number of people in the study. Although this action did, in fact, increase the study population by one and one-half, it undoubtedly contributed to an over-estimation of the expected numbers of deaths, causing the observed number of deaths to be in deficit.

With regard to cancer of the kidneys and suprarenals, there were slightly more deaths than expected (23 observed, 19.05 expected). However, among drivers the 12 deaths observed were significantly larger ($P < 0.05$) than the 7.03 deaths to be expected. It also should be noted that all but two of the drivers had started work before 1940 and had over 20 years of service.

It is indeed unfortunate, given the size and scope of this epidemiologic survey, that this report prepared by Rushton and Alderson is best characterized as superficial, anecdotal, and generally incomplete. For example, years of employment are parenthetically discussed in the authors' explanation for the selection criteria of workers into the study. No accompanying tables are presented. It can only be inferred that 36% of the study population had under 10 years of employment. Virtually no other information regarding years of employment and, hence, latency period can be gleaned from this document, with the exception of an occasional reference in the discussion of the mortality from a few diseases.

Pertinent information regarding measured levels of gasoline vapor at the 762 distribution centers is missing. Also missing is exposure data for the various occupational categories at these centers. Specifically, to what extent and amount were the drivers subject to gasoline vapors? Did the drivers of gasoline tank trucks assist in the unloading of gasoline into tanks?

Furthermore, what constitutes a distribution center? The background material is at best incomplete in this report.

5.3.4 Summary of Epidemiologic Studies

Three epidemiologic studies of workers exposed to petroleum products including gasoline and cancer have been reviewed: two published [Thomas et al. (1980, 1982)] and one unpublished [Rushton and Alderson (1982)].

Thomas et al. (1980, 1982) reported on two studies in which the death records of men who worked in oil refineries and petrochemical plants were reviewed for specific causes of death. The 1980 paper examined the records of workers who at the time of death were active members of the Oil Chemical and Atomic Workers International Union (OCAW). The 1982 paper was expanded to include retired members of the OCAW at the time of death. Proportionate Mortality Ratios (PMRs) adjusted for age and calendar time using the United States general population were computed.

The 1980 study showed that the PMRs for kidney and stomach cancers were significantly increased ($P < 0.05$) for white males who joined the union 20 or more years prior to death. However, black males whose lengths of union membership were less than 10 years, as well as those whose union membership were 10 or more years, experienced significantly greater than expected frequencies of stomach cancer deaths. As indicated in the 1982 paper, there were differences in mortality patterns for white males among both active and retired union members. Significant relative excesses ($P < 0.05$) of stomach, pancreatic, and brain cancer deaths were seen among active members. However, among retired members, the PMRs were significantly elevated for prostate cancer, Hodgkin's disease, multiple myeloma, and leukemia deaths.

Rushton and Alderson (1982) presented the results of a retrospective cohort mortality study of male workers at distribution centers from three

oil companies in Great Britain. The notable result was that among drivers there were 12 kidney cancer deaths which were significantly in excess ($P < 0.05$) compared to the 7.03 expected number of deaths.

All three studies suffer from insufficient documentation of exposure and employment histories and questionable applicability for determining the carcinogenicity of unleaded gasoline. The studies by Thomas et al. (1980, 1982) present inadequate definitions of the study populations and methodologies. Moreover, the limitations inherent in proportionate mortality ratios (PMRs) are in themselves sufficient to cast doubt on the results of these studies. PMRs reflect inflated estimates of mortality if the study group has a lower mortality rate than the comparison group for all causes of death. Also, excesses for one or more causes may automatically lead to a deficit in others. Because of its incomplete nature, the study by Rushton and Alderson (1982) is judged to be inadequate.

5.4 Quantitative Risk Estimation

This quantitative section deals with the estimation of cancer risk due to exposure to unleaded gasoline vapor. The unit risk is defined here as the lifetime incremental cancer risk from exposure to 1 ppm of gasoline vapor in air. Uncertainties about the risk estimate and the possible role of benzene content in gasoline vapor are also addressed in this section.

The risk estimate for gasoline vapor represents an extrapolation below the dose range of experimental data. There is currently no solid scientific basis for any mathematical extrapolation model that relates exposure to cancer risk at the extremely low concentrations, including the unit concentration given above, that must be dealt with in evaluating environmental hazards. For practical reasons the correspondingly low levels of risk cannot be measured

directly either by animal experiments or by epidemiologic studies. Low-dose extrapolation must, therefore, be based on current understanding of the mechanisms of carcinogenesis. At the present time the dominant view of the carcinogenic process involves the concept that most cancer-causing agents also cause irreversible damage to DNA. This position is based in part on the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal response that is characteristic of mutagenesis is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at high doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). Some supporting evidence also exists from animal experiments (e.g., the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the non-threshold model which is linear at low doses, has been adopted by CAG as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with such a model should be regarded as conservative, representing the most plausible upper limit for the risk (i.e., the true risk is not likely to be higher than the estimate, but it could be lower).

For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification as regards carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent as compared with other carcinogens. Such estimates are, of course, more reliable when the comparisons are based on studies in which the test species, strain, sex, and routes of exposure are similar.

The quantitative aspect of carcinogen risk assessment is addressed here because of its possible value in the regulatory decision-making process, e.g., in setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, the imprecision of presently available technology for estimating cancer risks to humans at low levels of exposure should be recognized. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk from exposure to a unit concentration of gasoline vapor (i. e., with this model it is not likely that the true risk would be much more than the estimated risk, but it could be considerably lower).

The risk estimates in this paper relate only to exposure to gasoline vapor. Risks related to the entire range of compounds that may be present in air are not estimated here.

5.4.1 Procedures for the Determination of Unit Risk

5.4.1.1 Low-Dose Extrapolation Model

The mathematical formulation chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form:

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_1 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[-(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate in the animal control group at dose d.

The point estimate of the coefficients q_i, $i = 0, 1, 2, \ldots, k$, and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The maximum likelihood estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program, GLOBAL79, developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1^* \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and R/q_1^* is a 95% lower confidence limit on the dose, producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper-limit q_1^* is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value q_1^* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation:

$$2 (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear nonthreshold concept discussed earlier. The slope, q_1^*, is taken as an upper-bound of the potency of the chemical in inducing cancer at low doses.

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose is deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

5.4.1.2 Selection of Data

For some chemicals, several studies in different animal species, strains, and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of the data sets from several studies to use in the model. It may also be appropriate to correct for metabolism differences between species and for absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are as follows:

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher

than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the set of data having the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex, and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment. The geometric mean of numbers A_1, A_2, ..., A_m is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}.$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

5.4.1.3 Calculation of Human Equivalent Dosages

Following the suggestion of Mantel and Schneiderman (1975), it is assumed that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the two-thirds power of the weight, as would be the case for a perfect sphere, the exposure in mg/day per two-thirds power of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner:

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e), and

W = average weight of the experimental animal

Then, the lifetime exposure is:

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

5.4.1.3.1 Oral.

Often exposures are not given in units of mg/day, and it becomes necessary to convert the given exposures into mg/day. Similarly, in drinking water studies, exposure is expressed as ppm in the water. For example, in most feeding studies exposure is given in terms of ppm in the diet. In these cases, the exposure in mg/day is:

$$m = ppm \times F \times r$$

where ppm is parts per million of the carcinogenic agent in the diet or water, F is the weight of the food or water consumed per day in kg, and r is the absorption fraction. In the absence of any data to the contrary, r is assumed to be equal to one. For a uniform diet, the weight of the food consumed is proportional to the calories required, which in turn is proportional to the surface area, or two-thirds power of the weight. Water demands are also assumed to be proportional to the surface area, so that

$$m \propto ppm \times W^{2/3} \times r$$

or

$$\frac{m}{rW^{2/3}} \propto \text{ppm}.$$

As a result, ppm in the diet or water is often assumed to be an equivalent exposure between species. However, this is not justified for the present study, since the ratio of calories to food weight is very different in the diet of man as compared to laboratory animals, primarily due to differences in the moisture content of the foods eaten. For the same reason, the amount of drinking water required by each species also differs. It is therefore necessary to use an empirically-derived factor, f = F/W, which is the fraction of an organism's body weight that is consumed per day as food, expressed as follows:

Species	W	Fraction of body weight consumed as f_{food}	f_{water}
Man	70	0.028	0.029
Rats	0.35	0.05	0.078
Mice	0.03	0.13	0.17

Thus, when the exposure is given as a certain dietary or water concentration in ppm, the exposure in $mg/W^{2/3}$ is

$$\frac{m}{rW^{2/3}} = \frac{\text{ppm} \times F}{W^{2/3}} = \frac{\text{ppm} \times f \times W}{W^{2/3}} = \text{ppm} \times f \times W^{1/3}$$

When exposure is given in terms of mg/kg/day = m/Wr = s, the conversion is simply

$$\frac{m}{rW^{2/3}} = s \times W^{1/3}.$$

5.4.1.3.2 Inhalation.

When exposure is via inhalation, the calculation of dose can be considered for two cases where 1) the carcinogenic agent is either a completely water-soluble gas or an aerosol and is absorbed proportionally to the amount of air breathed in, and 2) where the carcinogen is a poorly water-soluble gas which reaches an equilibrium between the air breathed and the body compartments. After equilibrium is reached, the rate of absorption of these agents is expected to be proportional to the metabolic rate, which in turn is proportional to the rate of oxygen consumption, which in turn is a function of surface area.

5.4.1.3.2.1 Case 1

Agents that are in the form of particulate matter or virtually completely absorbed gases, such as sulfur dioxide, can reasonably be expected to be absorbed proportionally to the breathing rate. In this case the exposure in mg/day may be expressed as:

$$m = I \times v \times r$$

where I = inhalation rate per day in m^3, v = mg/m^3 of the agent in air, and r = the absorption fraction.

The inhalation rates, I, for various species can be calculated from the observations of the Federation of American Societies for Experimental Biology (FASEB 1974) that 25 g mice breathe 34.5 liters/day and 113 g rats breathe 105 liters/day. For mice and rats of other weights, W (in kilograms), the surface area proportionality can be used to find breathing rates in m^3/day as follows:

$$\text{For mice, } I = 0.0345\ (W/0.025)^{2/3}\ m^3/\text{day}$$

$$\text{For rats, } I = 0.105\ (W/0.113)^{2/3}\ m^3/\text{day}$$

For humans, the value of 30 m^3/day* is adopted as a standard breathing rate (International Commission on Radiological Protection 1977). The equivalent

exposure in $mg/W^{2/3}$ for these agents can be derived from the air intake data in a way analogous to the food intake data. The empirical factors for the air intake per kg per day, $i = I/W$, based upon the previously stated relationships, are tabulated as follows:

Species	W	i = I/W
Man	70	0.29
Rats	0.35	0.64
Mice	0.03	1.3

Therefore, for particulates or completely absorbed gases, the equivalent exposure in $mg/W^{2/3}$ is

$$d = \frac{m}{W^{2/3}} = \frac{Ivr}{W^{2/3}} = \frac{iWvr}{W^{2/3}} = iW^{1/3}vr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the fraction absorbed, r, is assumed to be the same for all species.

5.4.1.3.2.2 Case 2

The dose in mg/day of partially soluble vapors is proportional to the O_2 consumption, which in turn is proportional to $W^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the O_2 consumption as $O_2 = k\ W^{2/3}$, where k is a constant independent of species, it follows that:

$$m = k\ W^{2/3} \times v \times r$$

or

$$d = \frac{m}{W^{2/3}} = kvr$$

*From "Recommendation of the International Commission on Radiological Protection," page 9. The average breathing rate is 10^7 cm^3 per 8-hour workday and 2 x 107 cm3 in 24 hours.

As with Case 1, in the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain concentration in ppm or ug/m^3 in experimental animals is equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps et al. 1977). When the animals are exposed via the oral route and human exposure is via inhalation or vice versa, the assumption is made, unless there is pharmacokinetic evidence to the contrary, that absorption is equal by either exposure route.

5.4.1.4 Calculation of the Unit Risk from Animal Studies

The risk associated with d $mg/kg^{2/3}/day$ is obtained from GLOBAL79 and, for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp(-q^*d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. This value is estimated simply by finding the number of $mg/kg^{2/3}/day$ that corresponds to one unit of X, and substituting this value into the above relationship. Thus, for example, if X is in units of ug/m^3 in the air, then for case 1, $d = 0.29 \times 70^{1/3} \times 10^{-3}$ $mg/kg^{2/3}/day$, and for case 2, d = 1, when ug/m^3 is the unit used to compute parameters in animal experiments.

If exposures are given in terms of ppm in air, the following calculation may be used:

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas)}}{\text{molecular weight (air)}} \text{ mg/m}^3$$

Note that an equivalent method of calculating unit risk would be to use mg/kg for the animal exposures, and then to increase the j^{th} polynomial coefficient by an amount:

$$(W_h/W_a)^{j/3} \quad j = 1, 2, \ldots, k,$$

and to use mg/kg equivalents for the unit risk values.

5.4.1.4.1 Adjustments for Less Than Lifespan Duration of Experiment

If the duration of experiment L_e is less than the natural lifespan of the test animal L, the slope q_1^*, or more generally the exponent g(d), is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose d is continued, the age specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the third power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, it is expected that the cumulative tumor rate would increase by at least the third power of age. Using this fact, it is assumed that the slope q_1^*, or more generally the exponent g(d), would also increase by at least the third power of age. As a result, if the slope q_1^* [or g(d)] is calculated at age L_e, it is expected that if the experiment had been continued for the full lifespan L at the given average exposure, the slope q_1^* [or g(d)] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Daffer et al. (1980), where the probabiity of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp[-f(t) \times g(d)].$$

5.4.2 Lifetime Risk Estimates

5.4.2.1 Data Available for Risk Estimation

The chronic inhalation study of unleaded gasoline vapor conducted by the International Research and Development Corporation (IRDC 1983) and sponsored by the American Petroleum Institute (API) is the only study that can be used to derive the carcinogenic potency of unleaded gasoline vapor. Tables 5-23 and

5-24 present dose-response data used in these calculations. The data in Table 5-23 were taken from Tables 21 and 22 of Volume 5 of the API report. The data in Table 5-24 were taken from Tables 23 and 24 of Volume 6 of the API report. All of the tumors reported in Tables 5-23 and 5-24 were observed after 18 months of study. One kidney tumor that was observed in the 40 animals sacrificed before 18 months in the highest dose group is not included in Table 5-23.

5.4.2.2 Choices of Low-Dose Extrapolation Models

In addition to the multistage model currently used by the CAG for low-dose extrapolation, estimates of risk from exposure to gasoline vapor were also determined using two other models (the probit and the Weibull models). These models cover almost the entire spectrum of risk estimates that could be generated from existing mathematical extrapolation models. These models are generally statistical in character, and are not derived from biological arguments, except for the multistage model, which has been used to support the somatic mutation hypothesis of carcinogenesis (Armitage and Doll 1954, Whittemore 1978, Whittemore and Keller 1978.) The main difference among these models is the rate at which the response function, P(d), approaches zero or P(0) as dose, d, decreases. For instance, the probit model would usually predict a smaller risk at low doses than the multistage model because of the difference of the decreasing rate in the low-dose region. However, it should be noted that one could always artificially give the multistage model the same (or even greater) rate of decrease as the probit model by making some dose transformation and/or by assuming that some of the parameters in the multistage model are zero. This, of course, is not reasonable without knowing, a priori, what the carcinogenic process for the agent is. Although the multistage model appears to be the most reasonable or at least the most general model to use, the maximum likelihood estimate generated from this model does not help to determine the shape of the dose-response curve

TABLE 5-23. INCIDENCE RATES OF TOTAL KIDNEY TUMORS IN MALE FISCHER 344 RATS EXPOSED TO UNLEADED GASOLINE VAPOR
(International Research and Development Corporation 1983)

Experimental dose (ppm)	Standardized lifetime dose (ppm)[a]	Incidence rate
0	0	0/49
67	11.96	2/59 (3.4%)
292	52.14	5/56 (8.9%)
2056[b]	367.14	5/45 (11.1%)

[a]The dose in ppm is assumed to be equivalent between humans and animals. Since the doses were given only 6 hours/day and 5 days/week, the lifetime dose is calculated by multiplying the factor (5 x 7) x (6/24) to each of the experimental doses.
[b]The data from this group is not used in calculation.

TABLE 5-24. INCIDENCE RATES OF HEPATOCELLULAR TUMORS IN FEMALE MICE (B6C3FL) EXPOSED TO UNLEADED GASOLINE VAPOR
(International Research and Development Corporation 1983)

Experimental dose (ppm)	Standardized lifetime dose (ppm)	Carcinoma/adenoma incidence rate	Carcinoma incidence rate
0		8/57 (14.0%)	7/57 (12.3%)
67	11.96	10/52 (19.2%)	6/52 (11.5%)
292	52.14	13/57 (22.8%)	9/57 (15.8%)
2056	367.14	28/56 (50.0%)	20/56 (35.7%)

beyond experimental exposure levels. Furthermore, maximum likelihood estimates at low doses extrapolated beyond experimental doses could be unstable depending on the amount of the lowest experimental dose; the upper-bound estimates from the multistage model at low doses are relatively more stable than maximum likelihood estimates. The upper-bound estimate can be taken as a plausible estimate at low doses if the true dose-response curve is actually linear. The upper-bound estimate means that the risks are not likely to be higher, but could be lower if the compound has a concave upward dose-response curve or a threshold at low doses. Because the estimated risk is a probability conditional to the assumption that an animal carcinogen is also a human carcinogen, the actual risk could range from a value near zero to an upper-bound estimate.

5.4.2.3 Calculation of Unit Risk (Risk at 1 ppm)

In the calculation of unit risk, ppm in air is assumed to be equivalent between animals and humans. The data from the highest dose group in Table 5-23 is excluded in the calculation because the model dose not fit well if these data are included (See 5.4.1.1 above). Furthermore, the data seem to indicate the toxic effect in the highest dose group because only two-thirds of the animals survived beyond 18 months. Using the tumor incidence data and the corresponding lifetime dose presented in Tables 5-23 and 5-24, the cancer risks at 1 ppm are calculated using the multistage model. The results are presented in Table 5-25. Both the 95% upperbound estimate and the maximum likelihood estimate are given. Because the maximum likelihood estimate of the linear component in the multistage model is not zero, the upper-bound estimate is only about two times the corresponding point estimate. The cancer risk estimates in Table 5-25 can be used to represent the carcinogenic potency of unleaded gasoline vapor. The kidney data in rats and the combined hepatocellular adenoma/carcinoma

data in mice are closely similar, spanning a range from 2.1×10^{-3} to 3.5×10^{-3}. This range represents one measure of uncertainty in the upper limit of potency, the lower limit being zero potency.

TABLE 5-25. ESTIMATES OF CARCINOGENIC POTENCY DUE TO EXPOSURE TO 1 PPM OF UNLEADED GASOLINE VAPOR

Data base	q_1^* 95% upper-bound estimate	Maximum likelihood estimate
(1) Kidney tumor in male rats	3.5×10^{-3}	2.0×10^{-3}
(2) Hepatocelluar carcinoma/ adenoma in female mice	2.1×10^{-3}	1.4×10^{-3}
Hepatocellular carcinoma in female mice	1.4×10^{-3}	8.5×10^{-4}
Geometric mean of (1) and (2)	2.7×10^{-3}	1.7×10^{-3}

5.4.2.4 Comparison of Risk Estimates by Different Low-dose Extrapolation Models

For comparison, the probit and the Weibull models are also used to calculate cancer risks at various dose levels. The calculated results are presented in Table 5-26. The maximum likelihood estimates of the parameters in each model are presented in Appendix B. The results shown in Table 5-26 indicate that all three of the models predict comparable risks (within an order of magnitude) at

TABLE 5-26. 95% UPPER-BOUND (AND MAXIMUM LIKELIHOOD) ESTIMATION OF LIFETIME RISK AT VARIOUS DOSE LEVELS, USING THREE DIFFERENT LOW-DOSE EXTRAPOLATION MODELS

		Dose in ppm						
Data base	Model	0.001	0.005	0.01	0.05	0.10	0.5	1.0
Kidney tumor, male rats	Multistage	3.53×10^{-6} (2.01×10^{-6})	1.77×10^{-5} (1.01×10^{-5})	3.53×10^{-5} (2.01×10^{-5})	1.77×10^{-4} (1.01×10^{-4})	3.53×10^{-4} (2.01×10^{-4})	1.77×10^{-3} (1.01×10^{-3})	3.53×10^{-3} (2.01×10^{-3})
	Probit	1.18×10^{-5} (4.92×10^{-7})	1.16×10^{-4} (6.24×10^{-6})	2.82×10^{-4} (1.72×10^{-5})	1.78×10^{-3} (1.49×10^{-4})	3.57×10^{-3} (3.50×10^{-4})	1.42×10^{-2} (2.09×10^{-3})	2.30×10^{-2} (4.18×10^{-3})
	Weibull	6.42×10^{-4} (5.95×10^{-5})	1.64×10^{-3} (1.77×10^{-4})	2.45×10^{-3} (2.83×10^{-4})	6.01×10^{-3} (8.43×10^{-4})	8.74×10^{-3} (1.35×10^{-3})	2.00×10^{-2} (4.00×10^{-3})	2.78×10^{-2} (6.40×10^{-3})
Hepatocellular carcinoma/ adenoma, female mice	Multistage	2.14×10^{-6} (1.44×10^{-6})	1.07×10^{-5} (7.20×10^{-6})	2.14×10^{-5} (1.44×10^{-5})	1.07×10^{-4} (7.20×10^{-5})	2.14×10^{-4} (1.44×10^{-4})	1.07×10^{-3} (7.20×10^{-4})	2.14×10^{-3}) (1.44×10^{-3})
	Probit	9.77×10^{-11} (2.85×10^{-12})	1.84×10^{-8} (6.91×10^{-10})	1.42×10^{-7} (5.97×10^{-9})	9.67×10^{-6} (5.50×10^{-7})	4.79×10^{-5} (3.14×10^{-6})	1.17×10^{-3} (1.12×10^{-4})	3.72×10^{-3} (4.24×10^{-4})
	Weibull	1.79×10^{-4} (1.94×10^{-5})	5.63×10^{-4} (6.87×10^{-5})	9.17×10^{-4} (1.18×10^{-4})	$2.81/10^{-3}$ (4.19×10^{-4})	4.53×10^{-3} (7.21×10^{-4})	1.34×10^{-2} (2.55×10^{-3})	2.11×10^{-2} (4.38×10^{-3})
Hepatocellular carcinoma only, female mice	Multistage	1.39×10^{-6} (8.53×10^{-7})	6.95×10^{-6} (4.27×10^{-6})	1.39×10^{-5} (8.53×10^{-6})	6.95×10^{-5} (4.27×10^{-5})	1.39×10^{-4} 8.53×10^{-5})	6.95×10^{-4} (4.27×10^{-4})	1.39×10^{-3} (8.53×10^{-4})
	Probit	6.79×10^{-14} (1.07×10^{-15})	5.67×10^{-11} (1.09×10^{-12})	7.88×10^{-10} (1.71×10^{-12})	1.91×10^{-7} (5.61×10^{-9})	1.56×10^{-6} (5.27×10^{-8}	1.08×10^{-4} (5.35×10^{-6})	5.13×10^{-4} (3.04×10^{-5})
	Weibull	9.26×10^{-6} (4.75×10^{-7})	4.33×10^{-5} (2.52×10^{-6})	8.38×10^{-5} (5.17×10^{-6})	3.81×10^{-4} (2.75×10^{-5})	7.17×10^{-4} (5.61×10^{-5})	3.17×10^{-3} (2.99×10^{-4})	5.88×10^{-3} (6.14×10^{-4})

1 ppm, but the difference becomes greater as the dose becomes smaller. For instance, on the basis of hepatoceulular tumors, the miltistage model predicts a much higher risk than that predicted by the probit model at a dose level of 0.001 ppm. This observation is not surprising, since the tangent (slope) of the probit curve approaches zero as dose approaches zero, while the slope of the multistage curve is linear at low doses. The risks predicted by the Weibull model on the basis of kidney tumors and hepatocellular carcinoma/adenoma are higher on the entire exposure range (0.001 ppm to 1.0 ppm) than the multistage model because the Weibul model shows a sub-linear dose-response relationship which is not considered biologically plausible (see section 5.4). For this reason, low-dose linearity has intuitive appeal. For example, the incidence of hepatocellular tumors at the lowest experimental dose (11.96 ppm) is 10/52, and the incidence in controls (0 ppm) is 8/57. In the absence of knowledge as to the shape of the dose-response relationship below the lowest experimental dose level, the only reasonable method of estimating cancer potency without having the possibility of seriously underestimating the true risk is to use linear extrapolation. That is, the slope (potency) is calculated by:

$$(10/52 - 8/57)/11.96 = 4.3 \times 10^{-3}/\text{ppm}$$

This crude estimate is about threefold greater than the maximum likelihood estimate (1.44×10^{-3}/ppm) calculated from the multistage model which utilizes all the data points, including the lowest data point used in the above calculation.

If one assumes that the dose-response curve is concave upward at low doses, the risk calculated by the low-dose linear model can be considered an upper-bound estimate of the true risk; it is the only plausible estimate that does not have the potential for underestimating the true risk on the basis of the

data given. Any more precise estimate would require either further assumptions about the shape of the dose-response curve or biological knowledge of the mechanism of carcinogenic action.

In comparing the models in Table 5-26, note that the multistage model has higher maximum likelihood estimates than the probit model for all doses lower than 0.01 ppm. This result is attributed to the low dose linearity characteristic of the multistage model's prediction of the dose-response relationship. In fact, the multistage model produces a linear relationship over the entire range of exposure estimates (0.001 ppm to 1.0 ppm) that would be produced by emissions of controlled and uncontrolled gasoline vapors. Based on its low dose linearity characteristic, the multistage model is selected as the model EPA should rely upon to estimate risk of exposure to gasoline vapor because it provides conservative estimates at low doses (i. e., below 0.01 ppm) and an adequately conservative approximation of risk at higher doses. At higher dose levels in the range of ambient exposure levels that would result from continued release of uncontrolled gasoline vapor emissions (i.e., between 0.01 ppm and 1.0 ppm), the multistage and probit models produce approximately the same maximum likelihood risk estimates (within the error band of these models estimates). Although the multistage model maximum likelihood risk estimates are about one-half of the probit model estimates at dose levels of 0.05 ppm to 1.0 ppm, this result is not considered to be a significant factor given the inherent uncertainties in developing risk estimates. In fact, the maximum likelihood risk estimates of each model being within a factor of 2 of one another is considered good agreement.

5.4.2.5 Uncertainties of Quantitative Risk Assessment

5.4.2.5.1 Uncertainties Associated with Potency Estimates

It is well known that different models, all of which might fit well with a given set of data over the experimental dose range, might nevertheless predict drastically different responses at low doses. Gasoline vapor data are no exception. As shown in Table 5-26, the multistage model predicts much higher risk than the probit model at 0.001 ppm, on the basis of liver tumor incidence. The risk estimate at low doses for unleaded gasoline vapor is calculated by using the linearized multistage model, which is linear at low doses. The potency estimate derived from such a model has been considered an upper-bound estimate on the assumption that the shape of the dose-response curve is upwardly concave at low dose levels. The carcinogenic potency, q_1^*, as derived from the multistage model, represents the 95% upper-bound confidence estimate, reflecting only the statistical variability of the response data.

The low-dose risk estimate derived from animal data must further be extrapolated to humans. There are many factors that must be considered in extrapolating risk from animals to humans. Included among these factors are differences between humans and animals with respect to life span, body size, genetic variability, and pharmacokinetic effects such as metabolism and excretion patterns. In assessing the risks of gasoline vapor, it was assumed that ppm in air will induce the same tumor response in humans as in animals. It is questionable, however, whether this simple assumption is capable of accounting for all the differences between humans and the animals that were used in the gasoline experiment of the IRDC (1983).

An important but often neglected factor in risk assessment is the weight of the evidence that gasoline vapor is carcinogenic to humans. The risk

estimate derived from animal data gives only a conditional probability of cancer on the assumption that the agent is carcinogenic to humans.

5.4.2.5.2 Uncertainties Associated with the Use of Potency Estimates to Predict Individual Risks in Real-life Exposure Patterns

The carcinogenic potency estimate for unleaded gasoline can be used to predict the human cancer risk from continuous gasoline exposure, subject to the uncertainties previously discussed. The actual human exposure to gasoline vapor, however, is likely to be only a few minutes per week. The questions then arise as to whether this exposure can be averaged over the entire week in order to arrive at a continuous exposure estimate, and whether overestimation or underestimation of the risk of intermittent doses would result from such averaging. The available data for analogous situations indicate that either of the two possibilities may be true. In studying factors modulating the carcinogenicity of benzidine, Vesselinovitch et al. (1975) demonstrated that twice-weekly administration of benzidine by stomach intubation was less effective in inducing liver and harderian gland tumors but more effective in inducing lung adenomas than the continuous (daily) feeding of equivalent doses.

Another example which may or may not be relevant to the case of gasoline vapor exposure are the studies of low-linear energy transfer (LET) radiation. After reviewing all the data on radiation-induced genetic and tumorigenic effects in plants, "simple" biological systems, animals, and humans, the National Council on Radiation Protection and Measurements (NCRP 1980) concluded that, for a given total dose, the high-dose rate exposure is more effective than the low-dose rate exposure in producing the response and that the difference in response between the two exposure patterns diminishes as the total dose decreases.

The applicability of this observation to the human exposure to gasoline vapor is not known. If one assumes that the gasoline vapor has the same dose-rate effect as the low-LET radiation exposure, then the use of averaging dose would not overestimate the risk and would give a close approximation to the true risk when the exposure level is small. In this discussion, it is assumed that the dose-response relationship obtained previously predicts accurately the true risk when the dose is continuous.

In general, three possible situations can occur in estimating cancer risk due to gasoline vapor exposure in the real-life situation when the averaging dose is used:

1. The real-life (intermittent) exposure pattern and the continuous (averaging dose) exposure patterns are equally effective. In this case the risk estimate is unbiased.

2. The real-life exposure pattern is more effective than the continuous exposure pattern. In this case, the risk is underestimated when the dose is averaged.

3. The real-life exposure pattern is less effective than the continuous exposure pattern. In this case, the risk is overestimated when the dose is averaged.

Not enough is known about the mechanism of action to state which possibility is the most likely or to know the magnitude of either the overestimation or the underestimation.

5.4.2.6 Cancer Risk Attributable to Benzene Content in Gasoline Vapor

To estimate the cancer risk which could be quantitatively attributable to the benzene content in gasoline vapor, the following assumptions are made:

1. Tumor response due to benzene content in gasoline vapor is additive. That is, benzene does not act synergistically or antagonistically with other chemical compounds in the gasoline vapor complex mixture.

2. Tumor responses due to benzene exposure need not be site-specific among different species or strains. This assumption is made for the purpose of quantitative analysis, but may not be valid from a biological point of view.

3. Part per million (ppm) in air is assumed to be equally effective in inducing tumors among different species.

4. The absorption rate for rats is similar irrespective of the route of exposure (gavage or inhalation).

Table 5-27 summarizes the cancer risk of benzene at 1 ppm. Both 95% upper-bound and maximum likelihood (point) estimates are presented. Details on the data and calculations are presented in Appendix C. It should be noted that the potencies presented in Table 5-27 are to be used solely for determining the fraction of tumor response in the gasoline vapor study that is attributable to benzene content, and should not be construed as the CAG's estimates of the carcinogenic potency of benzene in humans. The fraction of the unleaded gasoline tumor response attributable to benzene content can be expressed as:

$$AR = \text{potency (benzene)} \times 0.02/\text{potency (gasoline)}$$

where 0.02 is the reported benzene content. Since both gasoline and benzene potency estimates calculated on the basis of different data sets are comparable, it is appropriate to use the geometric means presented respectively in Table 5-25 and Table 5-27 to calculate AR.

TABLE 5-27. ESTIMATES OF THE CARCINOGENIC POTENCY OF BENZENE (RISK AT 1 PPM)

Data base	Upper-bound estimate	Maximum likelihood estimate
Female rats[a]	1.3×10^{-2}	8.0×10^{-3}
Male rats[b]	7.9×10^{-3}	5.2×10^{-3}
Female rats[b]	1.3×10^{-2}	8.7×10^{-3}
Male mice[c]	1.4×10^{-2}	6.9×10^{-3}
Geometric mean	1.2×10^{-2}	7.1×10^{-3}

[a]Zymbal gland carcinoma (gavage); Maltoni et al. (1982).
[b]Zymbal gland carcinoma (gavage); NTP (1983).
[c]Hematopoietic neoplasms (inhalation); Snyder et al. (1980).

When upper bound estimates are used:

$$AR = 1.2 \times 10^{-2} \times 0.02/2.7 \times 10^{-3} = 0.09$$

When maximum likelihood estimates are used:

$$AR = 7.1 \times 10^{-3} \times 0.02/1.7 \times 10^{-3} = 0.08$$

These calculations indicate that from the quantitative viewpoint alone, the benzene content accounts for less than 10% of the tumor responses observed in the IRDC (1983) unleaded gasoline study.

Another way to determine the quantitative benzene contribution to the tumor response is to calculate the expected increase of tumor-bearing animals and compare it with the corresponding observed response (after adjusting for the background rate) at each of the two lowest experimental doses in which toxic effects were not observed. These calculations (not shown here) also indicate that about 10% of responses to gasoline could be due to benzene content. One of the main uncertainties associated with the conclusion made above is the assumption that the risk due to benzene is additive to that of gasoline vapor. There is no evidence to support or deny this assumption. However, it can be shown that, under the multistage theory of carcinogenicity, if two carcinogens act on different stages of carcinogenesis, a multiplicative effect will result. There is abundant evidence that a carcinogen or a non-carcinogen could modify (enhance or inhibit) the carcinogenic action of another compound. Since gasoline vapor contains more than one chemical compound, such interactive effects are likely. Further research is needed to identify which compound (e.g., benzene) or fraction of compounds is responsible for the carcinogenic effect.

5.4.3 Summary of Quantitative Risk Estimation

Data from the API study on kidney tumors in male rats and liver adenomas and carcinomas in female mice were used to derive an estimate of the incremental upper-limit unit risk due to continuous human exposure to 1 ppm of unleaded gasoline. Since the animals breathed the complete mixture under laboratory conditions, whereas humans are expected to breathe only the more volatile components of the mixture, the estimates are uncertain. The estimates from the mouse and rat data are similar: 2.1×10^{-3} $(ppm)^{-1}$ from mouse data and 3.5×10^{-3} $(ppm)^{-1}$ from rat data.

The presence of 2% benzene in the unleaded gasoline mixture could theoretically contribute to the response, although the mouse liver and rat kidney have not been target organs in animal experiments with benzene. Based on those experiments, it is estimated that the contribution of benzene to the response observed in the API unleaded gasoline studies could be on the order of 10%. However, there is no qualitative evidence that benzene actually is contributing to the response.

5.5 Summary and Conclusions

5.5.1 Summary

5.5.1.1 Qualitative

5.5.1.1.1 Animal Studies

A lifetime inhalation bioassay of unleaded gasoline in Fischer 344 rats and B6C3F1 mice has induced a statistically significant incidence (6/100) of renal carcinomas in the kidney cortex of male rats and a larger, also statistically significant, incidence (20/100) of hepatocellular carcinomas in female mice. Female rats and male mice had no significant treatment-related induction of tumors at any organ site. The incidence of renal tumors was statistically significant at the highest dose tested (2,056 ppm) but not at the two lower

doses (292 ppm and 67 ppm). In mice, the incidence of liver carcinomas alone and adenoma and carcinoma combined, was also statistically significant in the highest but not the two lower dose groups. Moderate decrements in the body weight gain in the high-dose groups indicate that the maximum tolerated dose was reached. Glomerulonephrosis occurred in nearly all of the male rats, and mineralization of the pelvis was correlated with dose. However, there was no correlation between animals with tumors and those with mineralization.

The acute and subchronic renal toxicity of decalin, a volatile hydrocarbon of the same general type as those in gasoline, is confined to male rats and does not occur in female rats or in mice, dogs or guinea pigs. In a series of 21-day inhalation exposures of male rats to a variety of chemical fractions of gasoline, renal toxicity was correlated with the paraffin components and not with the aromatic compounds in the mixture. The same pattern of renal toxicity, as well as a positive renal tumor response, occurred in response to chronic inhalation of two synthetic fuels (RJ-5 and JP-10). Chronic inhalation studies with the jet fuels used by the Air Force and Navy (JP-4 and JP-5) have shown the same nephrotoxic lesions, but no statements can be made about the carcinoma response until histopathological analyses are completed. The renal toxicity pattern observed with exposure to hydrocarbon mixtures involving protein accumulation in renal tubules, is clearly different than the kidney lesions occurring spontaneously in old rats, and occurs in males of both Fischer 344 and Sprague-Dawley strains but not in females of these strains or in mice or monkeys. Mutagenesis tests of unleaded gasoline have been carried out in Salmonella, yeast, mouse lymphoma *in vivo* cytogenetics and mouse dominant lethal systems. Various gasoline feedstocks have been tested in mouse lymphoma and *in vivo* cytogenetics assays. The results of most of these assays have not met the criteria for positive responses. A detailed examination of their adequacy is in process.

5.5.1.1.2 Epidemiologic Studies

Three epidemiologic studies of workers exposed to petroleum products including gasoline have been reviewed: two published [Thomas et al., (1980, 1982)] and one unpublished [Rushton and Alderson (1982)].

Thomas et al. (1980, 1982) reported on two studies in which the death records of male individuals who worked in oil refineries and petrochemical plants were reviewed for specific causes of death. The 1980 paper examined the records of workers who at the time of death were active members of the Oil Chemical and Atomic Workers International Union (OCAW). The 1982 paper was expanded to include retired members of the OCAW at the time of death. Proportionate Mortality Ratios (PMR) adjusted for age and calendar time using the United States general population were computed. The 1980 study showed that the PMRs for kidney and stomach cancers were significantly increased ($P < 0.05$) for white males who joined the union 20 or more years prior to death. However, black males whose lengths of union membership were less than 10 years, as well as those whose union membership were 10 or more years, experienced significantly greater than expected frequencies of stomach cancer deaths. As indicated in the 1982 paper, there were differences in mortality patterns for white males among both active and retired union members. Significant relative excesses ($P < 0.05$) of stomach, pancreatic, and brain cancer deaths were seen among active members. However, among retired members, the PMRs were significantly elevated for prostate cancer, Hodgkin's disease, multiple myeloma, and leukemia deaths.

Rushton and Alderson (1982) presented the results of a retrospective cohort mortality study of male workers at distribution centers from three oil companies in Great Britain. The notable result was that there were 12 kidney cancer

deaths among drivers, which represented a significant excess ($P < 0.05$) in comparison with the 7.03 expected number of deaths.

The studies by Thomas et al. (1980, 1982) present problems in their definitions of the study population and in their methodology. The limitations inherent in proportionate mortality ratios (PMRs) are sufficient to cast doubt on the results of these studies. PMRs reflect inflated estimates of mortality if the study group has a lower mortality rate than the comparison group for all causes of death. Also, excesses for one or more causes may automatically lead to a deficit in others.

Because of the incomplete nature of the study by Rushton and Alderson (1982), it is judged to be inadequate. All three studies suffer from insufficient documentation of exposure and employment histories and questionable applicability for assessing the carcinogenicity of unleaded gasoline.

5.5.1.2 Quantitative

Data from the API study on kidney tumors in male rats and liver adenomas and carcinomas in female mice were used to derive an estimate of the incremental upper-limit unit risk due to continuous human exposure to 1 ppm of unleaded gasoline. Since the animals breathed the complete mixture under laboratory conditions, whereas humans are expected to breathe only the more volatile components of the mixture, the estimates are uncertain. The estimate from the mouse and rat data are similar: 2.1×10^{-3} $(ppm)^{-1}$ from mouse data and 3.5×10^{-3} $(ppm)^{-1}$ in rat data.

The presence of 2% benzene in the unleaded gasoline mixture could theoretically contribute to the response, although the mouse liver and rat kidney have not been the target organs in animal experiments with benzene. Based on those experiments, it is estimated that the contribution of benzene to

the response observed in the API unleaded gasoline studies could be on the order of 10%. However, there is no qualitative evidence that benzene actually is contributing to the response.

5.5.2 Conclusions

The occurrence of a small but definite kidney tumor response in male rats and a significant hepatocellular response in female mice furnish sufficient evidence, using the criteria of the International Agency for Research on Cancer (IARC), for the carcinogenicity of unleaded gasoline in animals. The similar pattern of response in rats to the synthetic fuels RP-5 and JP-10, and the renal toxicity observed in chronic bioassays with JP-4 and JP-5, support the findings with unleaded gasoline, indicating that some agent or combination of agents common to these mixtures is responsible for the observed effects.

The scattered reports of kidney cancer in workers exposed to gasoline-related compounds hint that some effect may be occurring in humans, but the evidence is judged to be too poor to justify anything but a classification of inadequate under the IARC criteria for epidemiologic evidence. Therefore, unleaded gasoline should be placed in IARC category 2B, meaning that unleaded gasoline is a probable human carcinogen.

6.0 ISSUES TO BE ADDRESSED BY THE SCIENCE ADVISORY BOARD

Before EPA can evaluate the risk gasoline vapor emissions may pose to human health, EPA needs the Science Advisory Board's advice on the soundness of the scientific studies performed by the American Petroleum Institute and other relevant studies discussed in section 5.0. Advice is also needed on methdology for deriving unit risk factors from these studies. It would be especially helpful if the Science Advisory Board would also address the following questions:

6.1 Quality of Evidence

1. Do you see any defect in the design or conduct of the animal studies that would cause you to seriously question the results?

2. Do you agree with our conclusion that the test gasoline is an animal carcinogen?

3. Does the available evidence permit any conclusion on the likelihood that gasoline vapor is carcinogenic in humans?

   a. does the available human evidence support the API animal results?

   b. is the difference in the composition of the test gasoline compared to ordinary gasoline a serious drawback to the relevancy of the animal studies for estimating human risks?

   c. does the fact that the studies used completely volatilized gasoline rather than the mixture of higher volatiles characteristic of partial evaporation represent a serious drawback to the interpretation of the results for human exposure?

4. Does the SAB agree with the explanation given for the absence of benzene-like carcinogenic response in the API studies? Were any factors overlooked? Is the assumption that the risks from benzene and gasoline vapor are additive valid for the purposes of analysis?

6.2 Quantitative Risk Assessment

1. Given the need for quantitative estimates of gasoline vapor health risks, does EPA's methodology for the derivation of the unit risk factors constitute a reasonable approach?

   a. is there reason to believe that the use of a linear model for dose/response extrapolation is inappropriate in this case?

   b. is it reasonable to combine malignant and benign tumors in extrapolating cancer risks from the animal data?

2. Are the rat and mouse strains used in the API studies equally applicable as the basis for risk extrapolation to humans?

3. Does the lifetime exposure regimen characteristic of the animal bioassays seriously compromise the use of these studies in estimating risks for human populations intermittently exposed to gasoline vapor (e.g. self-service refueling)?

4. Are the uncertainties in the gasoline vapor unit risk factors adequately described?

APPENDIX A

SUMMARY OF

API INHALATION STUDY

A CHRONIC INHALATION STUDY WITH UNLEADED GASOLINE VAPOR

H.N. MacFarland,[1] C.E. Ulrich,[2] C.E. Holdsworth,[3]
D.N. Kitchen,[4] W.H. Halliwell,[5] and S.C. Blum[6]

[1] Gulf Life Sciences Center, 260 Kappa Drive, Pittsburgh, PA 15238, to whom reprint requests should be addressed.

[2] International Research and Development Corporation, Mattawan, MI

[3] American Petroleum Institute, Washington, D.C.

[4] Biolabs, Inc., Las Cruces, NM

[5] Westpath, Inc., Ft. Collins, CO

[6] Exxon Research and Engineering Co., Linden, NJ

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ABSTRACT

A chronic inhalation study of unleaded gasoline vapor was conducted in mice and rats. The gasoline employed was typical of gasoline used in the U.S. and contained 2 percent benzene. Groups of both sexes of B6C3F$_1$ mice and Fischer 344 rats were exposed to three concentrations of vapor, 67, 292, and 2056 ppm. Exposures were for 6 hours per day, 5 days per week, for periods ranging from 103 to 113 weeks. Interim sacrifices were conducted at 3, 6, 12, and 18 months. Laboratory studies, including hematological and biochemical determinations, were performed on rats at the interim sacrifices and at termination. Histopathological studies were conducted on both species at every interval.

No consistent compound-related changes were seen in pharmacotoxic signs, mortality, hematological or biochemical indices in either species. Significant depression of body weight gain was seen in both sexes of rats and male mice exposed to the highest level of gasoline vapor. On gross necropsy, a compound-related increase in liver nodules and masses was seen in female mice exposed to the high level.

The most interesting observations were made on histopathological examination of the rats' tissues and, of these, pathological changes in the kidneys were the most striking. Renal carcinomas or sarcomas, in the cortex or near the renal poles, were seen in the male rats at all dose levels, with some evidence of a dose-response relationship. One female rat in the intermediate dose group exhibited a renal sarcoma. Two mice had renal tumors, considered to be spontaneous neoplasms. Mention is made of new studies that have been prompted by the present findings.

INTRODUCTION

Although gasoline, a fuel for the internal combustion engine, has been manufactured and used for several decades, no chronic investigation of its toxicological properties has been undertaken. To rectify this gap in our knowledge, the American Petroleum Institute began in the early 1970s to sponsor a program of longer term studies. A 90-day inhalation investigation with leaded and unleaded gasoline in rats and monkeys was completed in 1976 and later a paper was written for publication (Kuna and Ulrich, 1983). During the long hiatus between the original 90-day study report and the later paper of Kuna and Ulrich, a careful re-evaluation of the study's kidney tissues was undertaken for toxic signs consistent with those being observed for other hydrocarbon solvents. Upon reexamination by pathologists familiar with nephrotoxic lesions, subtle regenerative changes were discovered in the renal tubules. These minimal changes were seen only in male rats.

Shortly after the completion of the 90-day study in 1976, but before re-evaluation of the kidney slides from that study, the present chronic study was begun in rats and mice . The study protocol was adapted from that recommended by the National Cancer Institute (NCI)(1976). Unleaded gasoline was utilized in an inhalation investigation in which exposures were continued for 24 to 26 months.

Nephrotoxic lesions were seen in the chronic study. An unexpected finding was primary renal neoplasms in male rats near or at termination of the study. Both nephrotoxic and nephrocarcinogenic findings in male rats have stimulated further exploratory programs now in progress.

MATERIALS AND METHODS

Gasoline Sample

The unleaded automotive motor fuel (gasoline) used in the study was prepared to conform with the specifictions of unleaded gasoline in use in the United States in 1976, as determined by a road octane survey (DuPont Road Octane Survey, Summer 1976). At the time the gasoline was blended for the study, benzene concentrations in U.S. gasolines averaged about 1 percent with a maximum approaching 2 percent; therefore, benzene content of the gasoline was adjusted to the upper limit of U.S. gasolines. The specifications are shown in Table 1, but more detailed information on chemical composition is provided in Appendix 1.

Table 1

Animals

Fischer 344 albino rats and B6C3F_1 mice, each species equally divided as to sex, were utilized. After a 2-week quarantine period just prior to initiation of exposures, weight ranges were as follows:

Rats, male	95-129 g.
Rats, female	79-105 g.
Mice, male	14--26 g.
Mice, female	12--20 g.

At this time, both mice and rats were approximately 6 weeks of age. They were provided with Purina® Laboratory Chow® #5001 up to

week 38; thereafter Purina® Laboratory Chow® #5002 was used. Tap water and chow were available ad libitum except during the actual exposures.

Design

From larger groups of a given species and sex, only animals which appeared healthy were selected. They were further restricted as to weight range, using only those rats, both sexes, and female mice whose weights were within ±1.5 standard deviations of the group mean; ±1.6 standard deviations was permitted for the male mice. The animals were assigned at random, with 100 animals of each species and sex, i.e., a total of 400 per chamber in each group, in the design shown in Table 2.

Table 2

Interim sacrifices of 10 randomly selected animals of each species and sex were performed at 3, 6, 12, and 18 months.

Chamber Operations

Exposures were conducted in 16 m^3 stainless steel and glass exposure chambers (Fig. 1), designed by Leong (Leong, 1976; Drew, 1978). the supply air was filtered and controlled for temperature and humidity, and flow rates between 900 and 1900 liters per minute, depending on the desired chamber concentrations, were established by the main exhaust pump. Temperature and humidity were measured each day at the start of exposure and at 1, 3, and 5 hours. Gasoline was delivered from a liquid metering pump to a heated countercurrent

vaporization column and completely volatilized. Dry nitrogen at 5-6 liters per minute was used to carry the vapor into the main inlet pipe of the chamber. The exposure pattern was 6 hours per day, 5 days per week, for periods which ranged from 103 to 113 weeks. The target concentrations of gasoline were 50, 275, and 1500 ppm.

Chemical Analysis

Nominal concentrations were determined daily and calculations of concentration in ppm were made by using weight loss data and assuming an average molecular weight of 108 for the gasoline.

Analytical concentrations were determined by drawing samples from the chambers into a gas chromatograph equipped with a flame ionization detector. The operating conditions for the chromatograph are shown in Table 3.

Table 3

These conditions resulted in the appearance of a single peak for the complex hydrocarbon mixture, thereby facilitating expression of results as total hydrocarbon concentration.

Standard curves for calibration were prepared by injecting a known volume of liquid gasoline into a 25-liter Saran bag filled with nitrogen. It was found, after the experiment had been in progress for 24 weeks, that the gas chromatograph responded differently to gasoline standards prepared in nitrogen as compared to chamber samples of gasoline vapor in air. The magnitude of the correction

factor to be applied for each of the three concentrations under investigation was determined; it varied nonlinearly depending on the absolute concentration. This showed that the target concentrations of 50, 275, and 1500 ppm had been, in fact, 67, 292, and 2056 ppm, with standard deviations of ±3.1; 11.0, and 110.4, respectively, and the study was continued at these concentrations.

Biological Estimations

Animals were observed twice daily for signs of toxicity, behavioral changes, general appearance, and deaths. Each animal was individually examined for clinical signs and palpable tissue masses once a month. Individual body weights were recorded monthly for the first 17 months and biweekly thereafter.

Serum biochemical determinations were performed on seven male and seven female rats randomly selected from each group at the interim sacrifices (3, 6, 12, and 18 months) and at termination. The rats were fasted overnight, blood withdrawn from the orbital sinus, and the following enzyme activities determined as recommended by NCI (1976): alkaline phosphatase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, ornithine carbamyl transferase, and isocitrate dehydrogenase.

Hematologic evaluations were conducted at the 18-month interim and terminal sacrifice on the same rats used for biochemical

determinations at those time points. The following variables were measured: hemoglobin, hematocrit, erythrocyte count, total and differential leucocyte count, platelet count, reticulocyte count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

Gross and microscopic examinations of tissues were performed on animals dying during study, those obtained at the interim sacrifice periods, and those sacrificed at termination. A 40 percent survivability criterion was used to terminate each group; this resulted in the termination times shown in Table 4.

Table 4

At the 3, 6, and 12 month interim sacrifies, ten rats and ten mice of each sex were asphyxiated with carbon dioxide, and a complete necropsy was performed. At the 18-month interim sacrifice and at termination, animals were sacrificed by sodium pentobarbital anesthesia and exsanguinated. The trachea and lungs were removed at maximum inspiration and examined while inflated and deflated. The contents of the abdominal, thoracic, and cranial cavities were examined in situ and after dissection.

After trimming of fat and connective tissue, the tissues listed in Table 5 were weighed.

Table 5

The tissues listed in Table 6 were fixed in phosphate-buffered neutral formalin; hematoxylin and eosin stained paraffin sections were prepared for microscopic examination.

Table 6

Statistical Procedures

Body weight, hematologic, and serum biochemical data were tested for homogeneity of variance (Steel and Torrie, 1960), followed by a parametric analysis of variance. When a significant F-ratio was obtained, individual group comparisons were performed, utilizing student's t-test when variances were heterogeneous and Dunnett's test (1964) when homogeneous.

In some cases where the number of animals was small and the variances heterogeneous, the nonparametric multiple-group test of Kruskal-Wallis was applied and where appropriate, individual group comparisons were made with the Mann-Whitney U test (Siegel, 1956).

Data from male rats were analyzed for mortality, all renal tumors, malignant tumors, and renal adenomas, carcinomas and undifferentiated tumors combined, using procedures outlined in Thomas et al. (1977). Life table curves were computed and tested for homogeneity by both approximate and exact methods. A pair-wise comparison of groups was made. In addition, each datum set was examined for linear trend in the proportions, using both unadjusted and time-adjusted tests. The exact test for trend and approximate test for homogeneity and departure from trend were performed. Differences in pairs of proportions were examined.

RESULTS

Chamber Conditions

As indicated above, the actual concentrations of gasoline vapor in the chambers (67, 292, 2056 ppm) were higher than the originally planned target concentrations (50, 275, 1500 ppm), but when the calibration discrepancy was recognized, it was decided to continue the animal exposures at these higher concentrations throughout the study.

The temperatures and humidities in the four chambers ±S.D. ranged from 24 ±1.4 to 26 ±1.3C, and 52 ±9.5 to 56 ±7.2, respectively.

General Animal Observations

Some minor signs were noted intermittently in the study, including ocular discharge and apparent irritation in all four groups of rats. In mice, a significant number of animals developed alopecia, ranging in size from a small restricted area to a generalized hair loss over as much as two-thirds of the animals' bodies. The alopecia was seen in all groups, including controls, with approximately equal incidence.

No significant differences in spontaneous death rate were seen in female rats and mice. Male control rats, Group I, exhibited a significantly higher death rate after week 80 than any of the exposed groups. The male rats in Groups II, 67 ppm, had a partic-

ularly low spontaneous death rate. The following significant differences were noted in male mice: Groups II and III, 67 and 292 ppm, had a higher death rate than controls, but the Group IV male mice, 2056 ppm, exhibited a lower death rate when compared to controls.

Some statistically significant depressions in body weight were encountered. Male rats in Group IV had significantly lower body weights than controls from Week 13 to termination. Female rats in Group IV showed a similar depression which was significant from Week 26 to the end of the study. Male mice in Group IV exhibited a lower body weight than controls; the differences were significant from Week 66 to termination. In addition changes were noted in relative (in relation to body weight) and absolute organ weights in rats. The kidney weights of male rats of Group IV were elevated, both absolutely and relatively, from the 3-month interim sacrifice through to termination. At termination, the relative kidney weights of Group III male rats and Group IV female rats were also elevated. There was a dose-related relative increase in the testes and ovaries of Groups III and IV rats, and a slight depression in absolute heart weights was noted in Group IV males and females.

In mice, statistically significant alterations in organ weights were noted sporadically throughout the study, but none of these changes showed consistent trends, and thus they were not considered to be exposure related. Neither kidney nor liver weights were remarkable.

Clinical Observations

The usual slight variability in the various hematological indices was noted during the course of the study, but not considered to be related to the gasoline exposure.

The evaluated biochemical variables were unremarkable throughout the study. The serum ornithine carbamyl transferase values were judged unreliable because of methodological problems and were discounted.

Pathological Findings - Mice

The microscopic examination of tissues from the mice showed a large variety of neoplastic and nonneoplastic changes throughout the study which were not dose-related and were seen in both control and treated groups. In the 18-month to final sacrifice period and at final sacrifice, the female mice of Group IV exhibited an increased incidence of hepatocellular tumors. The incidence for all groups during the 18-month to final sacrifice time period was 45, 36, 45, and 44 percent in male mice, and 14, 19, 21, and 48 percent in the female mice, Groups I-IV, respectively.

There was some indication of a trend in the female mice in Groups I, II, and III; however, the high incidence, 48 percent, in the Group IV females was considered to be related to the exposure to gasoline.

The tumors were of two types. Hepatocellular adenomas were usually small and less than 1 cm in diameter. They were generally spherical, did not contain distinct sinusoids or portal areas, and were composed of hepatocytes that were usually larger than those of

the surrounding parenchyma. The juncture of the tumor with the surrounding parenchyma was distinct, and there was usually evidence of compression of the surrounding hepatocytes. The hepatocellular carcinomas were characterized by great variability of cell size, some containing large nuclei. The border of the tumor with the surrounding hepatocytes was indistinct with evidence of invasion of the surrounding parenchyma. The pattern of growth varied and included trabecular and solid patterns with areas of necrosis or hemorrhage.

Several of the hepatocellular carcinomas in mice metastasized to the lungs. In the final sacrifice, tumors in 7 percent of the male mice in Group III and 2 percent in Group IV metastasized to the lungs. No hepatocellular carcinomas in the final sacrifice female mice metastasized to the lungs. In the moribund male mice and those that died on test, tumors in 20 percent in Group I metastasized to the lungs. In the moribund female mice and those that died on test, tumors in 6 percent in Group I, 10 percent in Group III, and 7 percent in Group IV metastasized to the lungs.

Two female mice in Group IV exhibited renal tumors. One mouse, killed at final sacrifice, had a papillary cystic adenoma of the cortex. This adenoma consisted of a cystic space into which projected small papillae composed of cells morphologicaly similar to renal tubular epithelium. There was no evidence of peripheral invasion; it had distinct and discrete morphologic limits. The other mouse, which died during the 18-month to final sacrifice period,

exhibited bilateral renal tubular adenocarcinomas. These tumors replaced large portions of each kidney and contained large coalescing areas of necrosis and hemorrhage.

Pathological Findings - Rats

At the 3-month interim sacrifice, dose-related histopathological changes were observed in the male rats. These consisted of cortical multifocal renal tubular basophilia, protein casts, and chronic interstitial inflammation. The basophilia was characterized by the presence of renal tubules containing basophilic epithelial cells. The proteinaceous tubular casts occurred within dilated renal tubules and were commonly located at the corticomedullary junction. The incidence was 70 and 100 percent in Groups III and IV, respectively. Chronic interstitial inflammatory foci with a predominantly lymphoid cell type were observed at 20 and 70 percent incidence in Groups III and IV, respectively. In addition, renal congestion and very small foci of renal cortical mineralization were noted in several rats.

In animals dying in the 3- to 6-month interval or sacrificed at 6 months, the renal changes in male rats described above were again evident. The incidence of tubular basophilia was 0, 40, 100, and 100 percent in Groups I to IV, respectively. Proteinaceous casts were observed in 27 percent of the rats of Group I, 80 percent in Group III, and 100 percent in Group IV. The incidence of chronic interstitial inflammation was 18, 20, 100, and 100 percent in Groups I to IV, respectively. Mineralization in a radial pattern within the renal

pelvis, with material located within tubules or the collecting ducts of the renal pelves, was observed in 20 percent of the males in Group IV.

At the 12-month interim sacrifice, the occurrence of proteinaceous casts in the kidneys of male rats was nearly equal in all groups, 20, 30, 30, and 30 percent in Groups I to IV, respectively. Mineralization in the renal pelvis occurred in 20 percent of the male rats of Group III and in 80 percent in Group IV. Progressive glomerulonephrosis was diagnosed in one male rat from Group IV. Another new finding was karyomegaly, very large nuclei within renal tubular epithelial cells in male rats.

The complexity of morphologic alterations observed in the kidneys of all rats, especially males, increased after 18 months of exposure. Progressive glomerulonephrosis occurred in higher incidence than previously. The lesion was characterized by atrophied or sclerosed glomeruli, dilated renal tubules containing proteinaceous casts, tubular damage with regeneration or scarring, and the presence of foci of chronic inflammatory cells. The incidence of glomerulonephrosis in male rats was 20 percent in Group I, 30 percent in Group III, and 20 percent in Group IV; the incidence in female rats was lightly lower. Proteinaceous casts in kidneys of male rats were noted in 50, 50, 40, and 60 percent in Groups I to IV, respectively. Mineralization in the renal pelvis was seen in 20 percent of Group III and 80 percent of Group IV male rats. Renal congestion was commonly seen and karyomegaly was again noted in male rats. A benign renal

cortical adenoma was diagnosed in a Group IV male rat. Mononuclear cell leukemia was diagnosed in the kidney of a female rat that died during the 12- to 18-month interval.

At the final sacrifice, nearly all male rats exhibited progressive glomerulonephrosis. The incidence rates were 100, 95, 97, and 100 percent in Groups I to IV, respectively. A slightly lower rate of occurrence was seen in female rats. Mineralization in the renal pelvis occurred in 5, 63, and 91 percent of the males in Groups II, III, and IV, respectively. Karyomegaly was observed occasionally in the male rats. One male rat in Group III had renal tubular epithelial hyperplasia at termination. The lesion was characterized by the presence of a large dilated tubule containing a cystic lumen lined by epithelial cells. Renal cysts, epithelial cell pigmentation, hydronephrosis, chronic interstitial inflammation, congestion, cortical and pelvic mineralization in female rats, and necrosis were among the nonneoplastic lesions observed in the 18-month to terminal sacrifice period.

Of great interest were primary renal neoplasms diagnosed at termination or in those rats which died after 18 months. The total number of these primary renal tumors was 14, with zero, one, six, and seven in Groups I to IV, respectively, as shown in Table 7.

Table 7

All but one of these primary renal neoplasms occurred in male rats making the occurrence in males three adenomas, nine carcinomas,

and one sarcoma. The neoplasm in the female was a renal sarcoma or a mixed malignant tumor.

The renal adenomas were characterized by the presence of cuboidal to columnar epithelial cells, generally located in the cortex, which formed tubular or papillary structures. The masses were small, circumscribed, and the mitotic index was low.

The renal carcinomas varied in cellular morphology but generally contained epithelial cells arranged in a tubular or acinar pattern. Cellular pleomorphism, cellular anaplasia, central hemorrhage and/or necrosis was common. The mitotic index varied but was generally moderate to high. The histologic appearance varied greatly within some individual neoplasms and contained well-formed to ill-defined tubules. Other areas contained cells arranged in solid sheets with little structural arrangement and a scanty connective tissue stroma. Figure 2 is a photomicrograph of a typical renal carcinoma obtained from a Group IV male rat at termination.

Histologically, the renal sarcomas displayed a variety of cell types. The predominant type was a spindle cell, commonly seen invading the edge of the lesion and infiltrating between normal renal tubules. Other areas contained more solid sheets of spindle cells arranged in a whorl-like pattern. Some areas within the neoplasms were very anaplastic and pleomorphic in nature.

The renal adenomas and carcinomas were generally located in the cortex, but several were located near the renal poles. The

sarcomas had a central or pelvic anatomic location.

After 12 months, both sexes of rats exhibited a mild, multifocal, pulmonary inflammatory response characterized by an accumulation of alveolar macrophages in the alveolar spaces of the lungs. At termination, the incidence of these aggregates of macrophages was 19, 5, 43, and 38 percent in males, and 40, 46, 34, and 67 percent in females, in Groups I to IV, respectively.

DISCUSSION

Rats exhibited ocular discharge and appeared to be susceptible to the irritant effects of the airborne gasoline vapor. Death rates in male rats exhibited some differences among groups throughout the study, but none of these were considered to be related to the exposure. The depression in body weights seen in both sexes of rats exposed to the high concentration, Group IV, is regarded as a toxic stress effect of the gasoline exposure. Increases in kidney weights, both absolute and relative, were noted particularly in the male rats in the intermediate and high dose groups. There was also a slight increase in the relative weights of gonads in these groups. These changes in gonad weight may be, in part, a reflection of decreased body weights. The hematological and biochemical findings in rats were unremarkable.

The nephrotoxic changes seen at the 3-month and 6-month interim sacrifices are in accord with the observations of several investigators. Carpenter et al. (1975 a, b; 1977) reported renal tubular regenerative changes and dilated tubules containing

eosinophilic debris at the corticomedullary junction in male Harlan-Wistar rats exposed to the vapors of Stoddard solvent, 60 solvent, and High Naphthenic solvent, all derived from petroleum. These studies were performed under contract for the American Petroleum Institute, as were the 90-day inhalation studies in Sprague-Dawley rats and squirrel monkeys with leaded and unleaded gasolines reported to API in 1976 and subsequently written for publication by Kuna and Ulrich (1983). An initial reading of the slides of the kidney sections from this latter investigation revealed no remarkable observations but, after a careful reexamination some years later, subtle changes were detected in the male rats exposed to a high concentration (approx. 1500 ppm) of unleaded gasoline vapor. These consisted of an increase in the incidence and severity of regenerative epithelial changes, and dilated tubules containing proteinaceous material were observed. Other investigators have also noted similar alterations following administration of certain petroleum solvents. Other characteristics of the early nephropathy in the present study included interstitial inflammatory focal reactions and a progressive cortical mineralization. At the 12-month point, there was a decrease and equalization in the incidence of proteinaceous casts, increase in mineralization, and occurrence of karyomegaly in the renal tubular epithelial cells of male rats.

The further progression of the early nephropathy becomes increasingly obscured by the advent of "old rat nephropathy," a progressive glomerulonephrosis. This condition was first diagnosed in

one male rat in the high concentration group at the 12-month interim sacrifice. By 18 months, 20 to 30 percent of the male rats were affected and the incidence in the females was only slightly lower. However, the mineralization in the renal pelves and karyomegaly in male rats, seen prior to the onset of old rat nephropathy, were still readily distinguishable at 18 months. At termination, essentially all male rats and nearly all female rats exhibited old rat nephropathy. The incidence of pelvic mineralization was increased and karyomegaly was observed occasionally in the male rats.

It should be noted that, in the second year, two disease processes seemed to be occurring in parallel, the old rat nephropathy and a number of preneoplastic changes that appeared not to be concomitants of old rat nephropathy. These changes included karyomegaly, hyperplasia, and an early benign neoplasm.

The surprising finding at termination was the primary renal neoplasms, 13 of which were diagnosed in the male rats with evidence of a dose relationship, and one sarcoma seen in a female rat in the intermediate dose group. The spontaneous incidence of primary renal tumors in the Fischer 344 rat is extremely low in both sexes (Coleman et al. 1977; Goodman et al. 1979). It must, therefore, be concluded that the dose-related incidence of such tumors in male rats in the present study is to be ascribed to the exposure to wholly vaporized gasoline.

The nonneoplastic pulmonary inflammatory response, seen after 12 months and at a slightly higher incidence in female rats, may be

related to the slight irritant effect of the gasoline vapor. It is interesting to note that no evidence of the progressive focal interstitial fibrosis reported by Lykke and Stewart (1978) was found in the present study. These authors exposed rats to 100 ppm of the vapors of a leaded gasoline for periods ranging from 6 to 12 weeks.

In mice, alopecia was a frequent occurrence during the exposure phase, but was seen in all groups, including controls; thus, it does not appear to be related to the gasoline exposure. No remarkable changes in death rate or organ weights were seen in the mice.

The pathological finding of interest in the mice was an increased incidence of hepatocellular tumors, noted in the females in the period from the 18-month sacrifice to termination. These tumors are commonly seen in mice and have a significant spontaneous incidence which is higher in males (Tarone et al., 1981). Whether the exposure promoted the appearance of additional tumors or even initiated them cannot be determined from the present study. In some cases, metastasis to the lungs and kidneys were noted.

The most important findings in this chronic study are the early and progressive renal tubular disease seen in male rats in the first year, the advent and enhanced development of old rat nephropathy in the second year with a parallel appearance of certain preneoplastic changes, and the final appearance of primary renal neoplasms in the male rats. The hypothesis has been advanced that there may be causal connections between the early nephropathies and the late appearance of renal neoplasms, with the preneoplastic changes

in the second year as a possible link. New studies are planned to explore this question.

In analyzing the results of this study, attention has been directed to the gasoline, which is a complex mixture of several hundred hydrocarbons (See Appendix 1). There are five main classes: n-alkanes, isoalkanes, cycloalkanes, alkenes, and aromatics. Some evidence is beginning to accrue which suggests that the renotoxic effect of whole gasoline may be largely due to the presence of one or two of the main types of hydrocarbons. In particular, the isoalkanes are suspect (Cockrell et al. 1983; Pitts et al. 1983). Studies are in progress to examine the relative activity of the five hydrocarbon classes and individual molecular species.

Finally, the relevance of the results of this study to man is under active investigation. Collectively, epidemiological studies of populations that are exposed to gasoline in occupational situations has not revealed any statistically significant increase in renal carcinoma although slight increases have been detected in some studies (Hanis et al. 1979; Hanis et al. 1982). It should be noted that, in real-life situations where gasoline vapors are released, the vapors tend to be richer in the low-boiling constituents. Analyses of such atmospheres reveal total hydrocarbon concentrations generally less than 60 ppm for approximately two minutes (0.28 ppm based on an 8-hour Time Weighted Average).

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Table 1

SPECIFICATIONS OF UNLEADED MOTOR GASOLINE

	Sample Used in Study	Unleaded Commercial Average*
Research Octane No.	92.0	92.1
Motor Octane No.	84.1	83.6
(R+M)/2	88.1	87.9
Reid Vapor Pressure, lbs.	9.5	9.9
Distillation, ASTM D-86		
IBP	93	92
5	105	
10	116	124
20	138	
30	164	
40	190	
50	216	220
60	238	
70	256	
80	294	
90	340	332
95	388	
EP	428	412
Recovery	97%	
10% Evap., °F	112	
50% Evap., °F	211	
90% Evap., °F	331	
API Gravity	60.6	59.3
Gum, ASTM D381, mg/gal	1	1
Sulfur, ppm	97	
Phosphorus, g/gal	<0.005	
Lead, g/gal	<0.05	
Stability, hrs	24+	
HC Analysis, ASTM D1319		
Aromatics	26.1 Vol. %	27%
Olefins	8.4 Vol. %	7%
Saturate	65.5 Vol. %	66%
Benzene Content	2.0%	1.0%**

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* DuPont Road Octane Survey, Summer 1976

** Average benzene content typical of U.S. gasolines.

Table 2

DESIGN OF STUDY

Group	Designation	Target Concentration
I	Chamber Control	0 ppm
II	Low Concentration	50 ppm
III	Intermediate Concentration	275 ppm
IV	High Concentration	1500 ppm

Table 3

CHROMATOGRAPH OPERATING CONDITIONS

Gas Chromatograph:	Varian 2400
Detector:	Flame Ionization
Column:	5' x 1/8 inch O.D. Stainless Steel 1.5% OV-101 on 100/120 Mesh Chromosorb GHP
Sample Loop Size:	5 cc
Column Temp.:	200° C
Detector Temp.:	270° C
Injector Temp.:	250° C
Air Flowrate:	300 ml/min
N_2 Flowrate:	60 ml/min
H_2 Flowrate:	30 ml/min
Range:	10^{-11}
Attenuation:	1024 for 1500 ppm 8 for 275 ppm 64 for 50 ppm
Chart Speed:	2.5 cm/min for 1500 ppm 0.25 cm/min for 275 ppm 2.5 cm/min for 50 ppm

Table 4

TERMINATION TIMES FOR ANIMAL GROUPS

Group No.	Species/ Sex	No. of Animals at Initiation	Duration of Exposure (Weeks)
I	Rat-M	100	107
	Rat-F	100	109
	Mouse-M	100	107
	Mouse-F	100	113
II	Rat-M	100	107
	Rat-F	100	109
	Mouse-M	100	103
	Mouse-F	100	113
III	Rat-M	100	107
	Rat-F	100	109
	Mouse-M	100	103
	Mouse-F	100	113
IV	Rat-M	100	107
	Rat-F	100	109
	Mouse-M	100	107
	Mouse-F	100	113

---

103 weeks = 23.9 months

107 weeks = 24.7 months

109 weeks = 25.2 months

113 weeks = 26.1 months

Table 5

TISSUES SELECTED FOR WEIGHING

brain	thyroid/parathyroid complex*
heart	kidneys**
liver	pituitary*
testis	lung with trachea**
ovaries*	adrenals*

---

* Tissues weighed after fixation

** Tissues weighed in toto, prior to dissection

Table 6

TISSUES PREPARED FOR MICROSCOPIC EXAMINATION

Both Species*

- gross lesions and tissue masses (and regional lymph nodes, if possible)
- blood smear (as required by the pathologist)
- mandibular lymph node
- salivary gland
- sternebrae, femur, or vertebrae including marrow
- thyroids
- parathyroids
- jejunum
- colon
- liver
- gallbladder (mice)
- prostate
- testes
- ovaries
- lungs and mainstream bronchi
- larynx
- nasal cavity
- heart
- esophagus
- stomach
- uterus
- brain (three sections, including frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons)
- thymus
- trachea
- pancreas
- spleen
- kidneys
- adrenals
- urinary bladder
- pituitary
- spinal cord
- eyes

Rats Only

- optic nerve
- Harderian gland
- Zymbal gland
- oral mucous membrane
- duodenum
- ileum
- cecum
- mammary gland
- mesenteric lymph node
- skeletal muscle
- sciatic nerve
- skin
- epididymides
- seminal vesicles
- cervix
- Fallopian tubes
- head

*As recommended by NCI. 1976.

Table 7

PRIMARY RENAL NEOPLASMS IN RATS

Test Group	Neoplasm	Number of Neoplasms	
		Males	Females
I	None	0	0
II	Carcinoma	1	0
III	Adenoma	2	0
	Carcinoma	2	0
	Sarcoma	1	1
IV	Carcinoma	6	0
	Adenoma*	1	0

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* Occurred in male rat at 18 months.

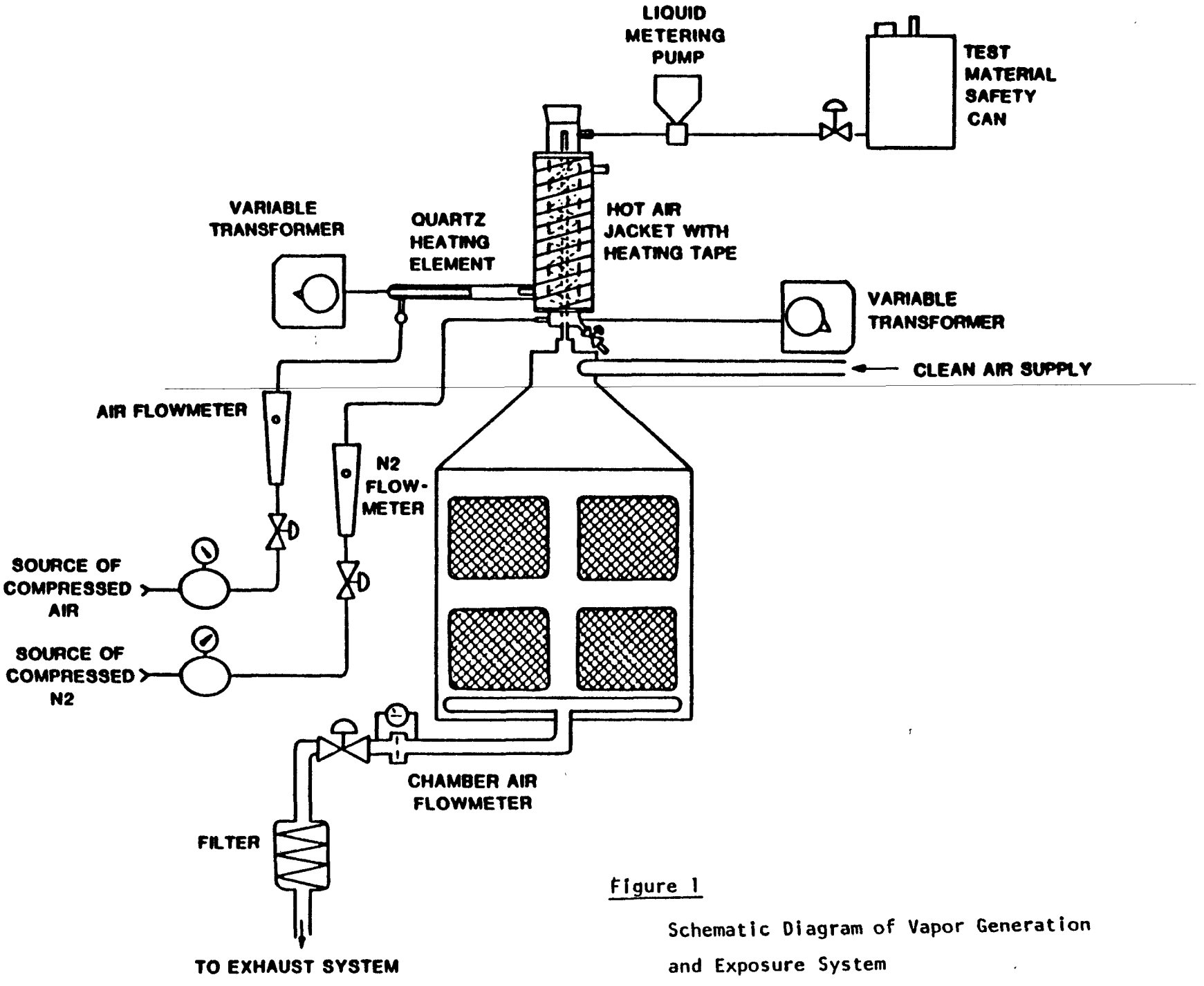

Figure 1

Schematic Diagram of Vapor Generation and Exposure System

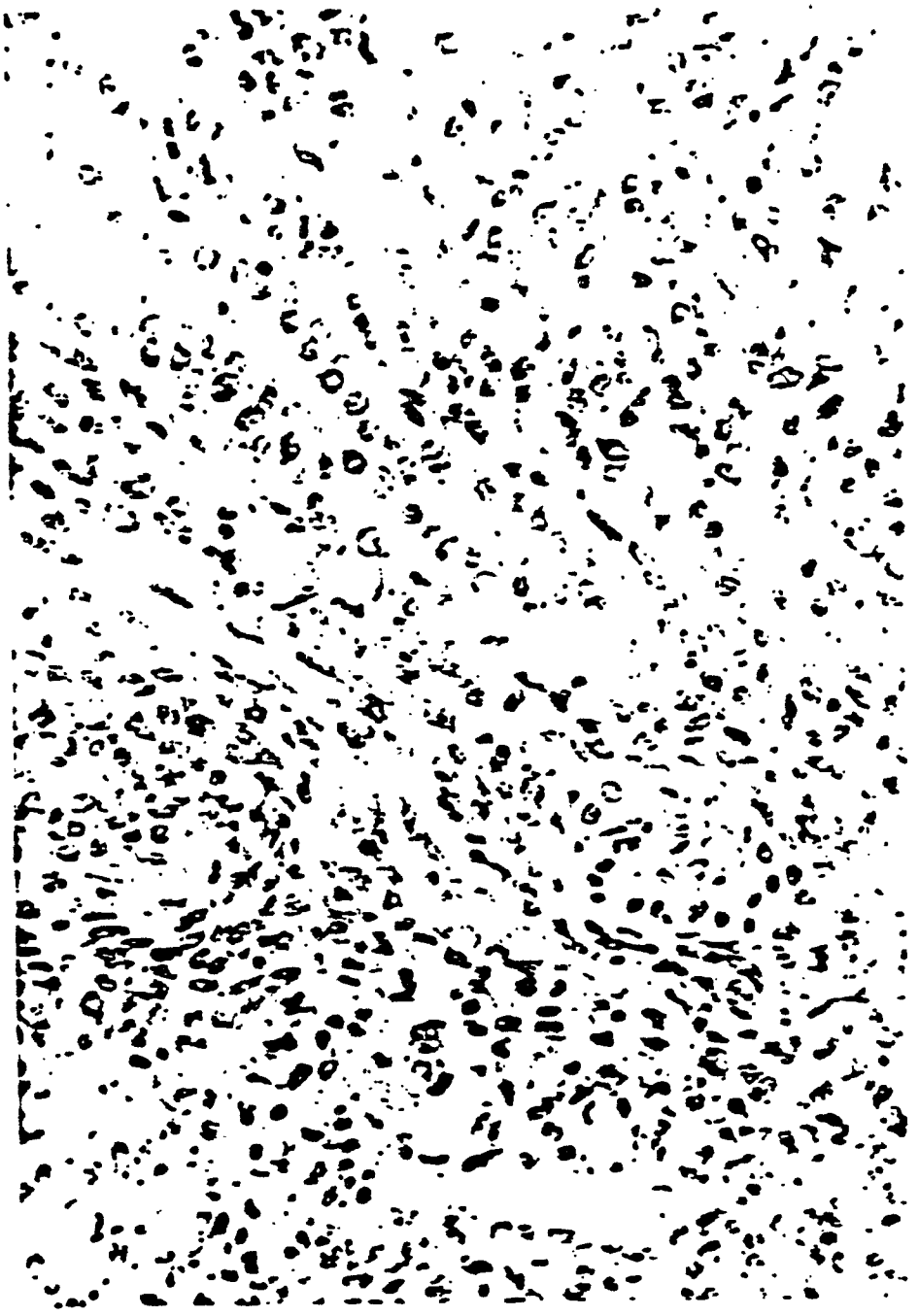

Figure 2

Histologic appearance of a renal carcinoma composed of epithelial cells arranged in a tubulo-acinar pattern. Note cellular pleomorphism and anaplasia.

APPENDIX 1 - COMPOSITION OF GASOLINE

The specifications used to define petroleum products such as gasoline are directed towards performance characteristics, usually stated in terms of physical properties; little attempt is made to determine detailed chemical composition, as can be seen in the data of Table 1 in the text. The gasoline used in the present study was formulated by blending four refinery streams, as shown in Table 1A.

Table 1A

The antioxidant consisted of 76 percent 2,6-di-tertiary butylphenol, with the remainder about equal parts of 2-tertiary butylphenol and 2,4,6-tri-tertiary butylphenol. The metal deactivator was a 50 percent solution of N, N'-disalicylidene-1,2-diaminopropane in commercial xylene. The concentration of 5 lbs/1000 bbl corresponds to approximately 20 ppm w/w or 14 ppm w/v.

Like gasoline, the four refinery streams in Table 1A are specified largely by physical parameters, with only minimal chemical compositional information, as shown in Tables 2A, 3A, 4A, and 5A.

Tables 2A, 3A, 4A, 5A

The most detailed compositional information available on the unleaded gasoline employed in this study, based on gas chromatographic and mass spectrometric analyses, covers 151 compounds out of over 542 that are possible. These data are provided in Table 6A.

Table 6A

The specific individual compounds identified as major contributors in Table 6A are listed in Table 7A.

Table 7A

It should be noted that about 75 percent of the gasoline is comprised of 42 of the compounds determined. In Table 1A, benzene adjustment to approach 2 percent is indicated, based on an infrared analytical method. However, when the more precise gas chromatographic-mass spectrometric analytical procedure was used to obtain the results shown in Table 6A, the benzene content was estimated to be 1.69 percent. More recent re-analyses of the gasoline by an improved method indicates that the actual benzene content was 1.80 to 1.96 percent, a satisfactory approximation to 2 percent.

We thank Richard W. King of Sun Tech, Inc., for providing the detailed information on the chemical composition of the gasoline.

Table 1A

Formulation of Unleaded Gasoline

Generic Stream [1]	CAS Number	Volume %
Light Catalytic Cracked Naphtha	64741-55-5	7.6
Heavy Catalytic Cracked Naphtha	64741-54-4	44.5
Light Catalytic Reformed Naphtha	64741-63-5	21.3
Light Alkylate Naphtha	64751-66-8	22.0
Benzene added to bring to 2%		0.8
Butane added to increase Reid Vapor Pressure		3.8
plus:		
Antioxidant		5 lbs/1000 bbl
Metal Deactivator		5 lbs/1000 bbl

[1] Toxic Substances Control Act (TSCA) PL 94-469: Candidate List of Chemical Substances, Addendum I, Generic Terms Covering Petroleum Refinery Processed Streams, January 1978.

Table 2A

Specifications of Light Catalytic Cracked Naphtha

A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominately in the range of C4 through C11 and boiling in the range of approximately -20 degrees C to 190 degrees C (-4 to 374 degrees F). It contains a relatively large proportion of unsaturated hydrocarbons.

Tests	Range of Company Data*
Gravity, degrees API	50-75
Sulfur, weight %	0.02-0.3
Nitrogen, ppm	10-50
Reid Vapor Pressure, psia	2-12
Distillation (ASTM D-86), °F	
IBP	80-125
10%	103-160
50%	152-265
90%	235-408
95%	240-430
EP	295-460
Paraffins, %	21-44
Olefins, %	15-68.5
Napthenes, %	10-16
Aromatics, %	6-28
Saturates, %	-

---

* Based on data submitted by 11 companies.

Table 3A

Specifications of Heavy Catalytic Cracked Naphtha

A complex combination of hydrocarbons produced by a distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C_6 through C_{12} and boiling in the range of approximately 65°C to 230° C (148°F to 446°F). It contains a relatively large proportion of unsaturated hydrocarbons.

Tests	Range of Company Data*
Gravity, °API	36 - 47.1
Sulfur, wt. %	0.08- 0.3
Nitrogen, ppm	21 -110
Reid Vapor Pressure, psia	0.3 - 4.1
Distillation, °F (ASTM D-86 Equiv.)	
IBP	118 -275
10%	245 -333
50%	324 -372
90%	388 -412
95%	412 -422
EP	420 -450
PONA, % by MS	
Paraffins	22.8 - 32.7
Olefins	9.8 - 20.8
Naphthenes	10.6
Aromatics	45.0 - 56.6
Saturates	40.0
Aniline Pt., °F	64.0
MON (Clear)	77.6 - 81.3
RON (Clear)	85.0 - 90.8

---

* Based on data submitted by 6 companies.

Table 4A

Specifications of Light Catalytic Reformed Naphtha

A complex combination of hydrocarbons produced from the distillation of products from a catalytic reforming process. It consists of hydrocarbons having carbon number predominantly in the range of C_5 through C_{11} and boiling in the range of approximately 35 degrees C to 190 degrees C (95 to 374 degrees F). It contains a relatively large proportion of aromatic and branched chain hydrocarbons. This stream may contain 10 vol. % or more benzene.

Tests	Range of Company Data*
Gravity, degrees API	40 - 59
Sulfur, weight %	-
Nitrogen, ppm	-
Reid Vapor Pressure, psia	3.7- 11
Distillation (ASTM D-86), °F	
IBP	74 -149
10%	136 -225
50%	186 -299
90%	229 -360
95%	292 -381
EP	356 -448
Paraffins, %	28 - 55
Olefins, %	0 - 2.4
Napthenes, %	0.5- 4.4
Aromatics, %	30.9- 69.9
Saturates, %	-
Benzene, vol. %	0.6- 3.97

---

* Based on data submitted by 9 companies.

Table 5A

Specifications of Light Alkylate Naphtha

A complex combination of hydrocarbons produced by distillation of the reaction products of isobutane with monoolefinic hydrocarbons usually ranging in carbon numbers from C_3 through C_5. It consists of predominantly branched chain saturated hydrocarbons having carbon numbers predominantly in the range of C_7 through C_{10} and boiling in the range of approximately 90°C to 160°C (194°F to 320°F).

Tests	Range of Company Data*
Gravity, °API	70.4 - 74.1
Sulfur, Wt. %	0.002- 0.01
Nitrogen, ppm	1.1
Flash Pt., °F	122
Aniline Pt., °F	166
RVP, lbs.	4.2 - 6.9
Distillation, °F (ASTM D-86)	
IBP	104 -120
10%	154 -175
50%	208 -230
90%	235 -300
95%	-
EP	258 -335
P, %	99+
O, %	0.01 - 0.5
N, %	1.0
A, %	0.0 - 1.0
Saturates, %	98.5
RON (clear)	93.8 - 95.2
MON (clear)	90.5 - 92.5

---

* Based on data submitted by 3 companies.

Table 6A

Detailed Composition of Gasoline

Compound Class	Carbon No. Range	No. of Isomers Possible	No. of Isomers Analyzed For	Volume % in Fuel	No. of Major Contributors	Accounting For	
Alkanes	C_3 thru C_{10}	8	8	11.40	3	10.19	90%
Isoalkanes	C_4	1	1	1.14	1	1.14	
	C_5	2	2	10.26	1	10.26	
	C_6	4	4	8.99	3	8.81	
	C_7	8	8	4.77	4	4.54	
	C_8	17	14	16.73	4	11.75	
	C_9	34	22	2.01	4	1.51	
	C_{10} thru C_{13}	>75	-	2.65	no information		
Total Isoalkanes	C_4 thru C_{13}	>141	51	46.55	17	38.01	82%
Cycloalkanes	C_5	1	1	0.15	1	0.15	
	C_6	2	2	1.05	1	0.97	
	C_7	7	7	1.09	3	0.77	
	C_8	23	16	0.74	-	-	
	C_9	76	23	1.03	-	-	
	C_{10} thru C_{13}	>76	-	0.62	no information		
Total Cycloalkanes	C_5 thru C_{13}	>185	49	4.68	5	1.89	40%
Alkanes	C_2	1	1	0.00			
	C_3	1	1	0.03	1	0.03	
	C_4	4	4	0.90	2	0.75	
	C_5	6	6	1.29	3	1.22	
	C_6	17	17	1.40	2	1.26	
	C_7 thru C_{12}	>128	-	5.34	no information		
Total alkenes	C_2 thru C_{12}	>157	29	8.96	8	3.26	36%
Benzene	C_6	1	1	1.69	1	1.69	
Alkybenzenes	C_7	1	1	3.99	1	3.99	
	C_8	4	4	9.83	4	9.83	
	C_9	8	8	7.73	3	5.33	
	C_{10}	22	-	2.11	no information		
	C_{11}	>22	-	0.52	no information		
	C_{12}	>>22	-	0.21	no information		
Total Alkylbenzenes	C_6 thru C_{12}	>>36	14	26.08	9	20.84	80%
Indans/Tetralins	C_9 thru C_{13}	large	-	1.54	no information		
Naphthalenes	C_{10} thru C_{12}	15	-	0.74	no information		
Total Aromatics	C_6 thru C_{13}	>51	14	28.36	9	20.84	73%

Summary

Alkanes	C_3 thru C_{10}	8	8	11.4	3	10.2	90%
Isoalkanes	C_4 thru C_{13}	>141	51	46.5	17	38.0	82%
Cycloalkanes	C_5 thru C_{13}	>185	49	4.7	5	1.9	40%
Alkenes	C_2 thru C_{12}	>157	29	9.0	8	3.3	36%
Aromatics	C_6 thru C_{13}	>51	14	28.4	9	20.8	73%
TOTAL		>542	151	100.0	42	74.2	

Table 7A

Identification of Major Contributors

Alkanes (3)

- n-butane
- n-pentane
- n-hexane

Isoalkanes (17)

- isobutane
- isopentane
- 2-methylpentane
- 3-methylpentane
- 2,3-dimethylbutane
- 2-methylhexane
- 3-methylhexane
- 2,3-dimethylpentane
- 2,4-dimethylpentane
- 2,2,4-trimethylpentane
- 2,3,4-trimethylpentane
- 2,3,3-trimethylpentane
- 2,2,3-trimethylpentane
- 2-methyloctane
- 3-methyloctane
- 4-methyloctane
- 2,2,5-trimethylpentane

Cycloalkanes (5)

- methylcyclohexane
- 1,cis, 3-dimethylcyclopentane
- 1,trans, 3-dimethylcyclopentane
- cyclopentane
- methylcyclopentane

Alkenes (8)

- propylene
- trans butene-2
- cis butene-2
- pentene-1
- trans pentene-2
- cis pentene-2
- 2-methylpentene-1
- 2-methylpentene-2

Aromatics (9)

- benzene
- toluene
- ethylbenzene
- o-xylene
- m-xylene
- p-xylene
- 1-methyl, 3-ethylbenzene
- 1-methyl, 4-ethylbenzene
- 1,2,4-trimethylbenzene

APPENDIX B

COMPARISON AMONG DIFFERENT EXTRAPOLATION MODELS

Three models used for low-dose extrapolation, assuming the independent background, are:

Multistage: $$P(d) = 1 - \exp\left[-(q_1 d + \ldots + q_k d^k)\right]$$

where q_i are non-negative parameters;

Probit: $$P(d) = \int_{-\infty}^{A + B \ln(d)} f(x)\, dx$$

where f(.) is the standard normal probability density function; and

Weibull: $$P(d) = 1 - \exp\left[-bd^k\right]$$

where b and k are non-negative parameters.

The maximum likelihood estimates (MLE) of the parameters in the multistage model is calculated by means of the program GLOBAL82, which was developed by Howe and Crump (1982). The MLE estimates of the parameters in the probit and Weibull models are calculated by means of the program RISK81, which was developed by Kovar and Krewski (1981). Table B-1 presents the MLE of parameters in each of the four models.

TABLE B-1. MAXIMUM LIKELIHOOD ESTIMATES OF THE PARAMETERS FOR THE THREE EXTRAPOLATION MODELS BASED ON THREE DATA SETS IN API UNLEADED GASOLINE STUDY
(International Research and Development Corporation 1983)

Data base	Multistage model	Probit model	Weibull model
Kidney tumor in male rats	$q_1 = 2.01 \times 10^{-3}$ $q_2 = 0$	A = -2.64 B = 0.33	$b = 6.42 \times 10^{-3}$ k = 0.68
Hepatocellular carcinoma/adenoma in female mice	$q_1 = 1.44 \times 10^{-3}$ $q_2 = q_3 = 0$	A = -3.29 B = 0.52	$b = 5.15 \times 10^{-3}$ k = 0.78
Hepatocellular carcinoma in female mice	$q_1 = 8.53 \times 10^{-4}$ $q_2 = 3.83 \times 10^{-8}$	A = -3.98 B = 0.57	$b = 6.95 \times 10^{-4}$ k = 1.04

APPENDIX C

CARCINOGENIC POTENCY OF BENZENE

The carcinogenic potencies calculated in this appendix are to be used only for determining the contribution of benzene content to the tumor response observed in the unleaded gasoline vapor bioassay. In this endeavor, it is not necessary to consider the species conversion factor where the data from the gavage study are used to estimate cancer risk via the inhalation route.

Benzene has been shown to produce Zymbal gland carcinoma in rats (Maltoni et al. 1982 and NTP 1983) and in male mice (NTP 1984) and hematopoietic neoplasms in male mice (Snyder et al. 1980). These data are used to calculate the carcinogenic potency for benzene. Preliminary calculation of the benzene potency on the basis of the male mice data (not presented here) shows comparable results. A complete document on benzene risk assessment, including human and animal data, is currently being prepared by the CAG. In the present appendix, only information relevant to the objectives stated above is presented. The presentation has been kept as simple as possible because the time allocated to this document is very limited. Details will be provided in the CAG risk assessment document on benzene which is now in preparation.

In estimating the relative potency of benzene, the CAG has utilized the following equivalency ratio:

$$1 \text{ ppm of benzene} = 3{,}250 \text{ ug/m}^3$$

The volumetric breathing rate for a rat weighing 300 grams is 0.2 m^3/day (see section 5.4.1.3.2.1). For purposes of convenience, the body weight for

all of the rats used in the Maltoni et al. (1982) and the NTP (1984) studies is assumed to be 300 grams.

Thus, 1 ppm of benzene in air is calculated as being equivalent to

$$3{,}250\ ug/m^3 \times 0.2\ m^3/day \times 10^{-3}\ mg/ug/0.30\ kg = 2.17\ mg/kg/day$$

Tables C-1 to C-4 present the tumor incidence data that have been used by the CAG to calculate the carcinogenic potency of benzene. The maximum likelihood estimate of the parameters in the multistage model, and the 95% upper-bound estimate, q_1^*, of the linear component, are also presented at the bottom of each table.

TABLE C-1. INCIDENCE OF ZYMBAL GLAND CARCINOMAS IN FEMALE SPRAGUE-DAWLEY RATS ADMINISTERED BENZENE BY GAVAGE (Maltoni et al. 1982)

Experimental dose (mg/kg/day)	Response
0	0/30
50	2/30
250	8/32

Remarks:

1. The number of animals surviving at the 26th week are used for the denominators.

2. Animals were treated by gavage 4 to 5 times a week for 52 weeks. The lifetime dose is calculated by d x (4.5/7) x (52/104) = 0.32 d, where d is the experimental dose.

3. The 95% upper-bound estimate of the linear component in the multistage model is $q_1^* = 5.97 \times 10^{-3}$/mg/kg/day or, equivalently, $q_1^* = 1.29 \times 10^{-2}$/ppm using the fact that 1 ppm of benzene in air is equivalent to a dose of 2.13 mg/kg/day. The maximum likelihood estimates of the parameters in the multistage model are q_1 = 8.03 x 10-3/ppm, $q_2 = 0$.

TABLE C-2. INCIDENCE OF HEMATOPOIETIC NEOPLASMA IN C57BL MALE MICE EXPOSED BY INHALATION (Snyder et al. 1980)

Dose (ppm)	Response
0	2/40
300	8/40

Remarks:

1. Mice were exposed by inhalation to 300 ppm of benzene 6 hours/day, 5 days/week for 488 days, at which time all of the benzene-treated animals died.

2. Lifetime dose is calculated as d = 300 x (6/24) x (5/7) = 53.57 ppm.

3. Because the lifespans of the treated animals were shorter than those of the controls, the risk calculated from the data is further adjusted by multiplying by a factor of $(630/488)^3$, where 630 days are assumed to be the lifespan of the control mice. The carcinogenic potency of benzene is calculated to be $q_1^* = 1.4 \times 10^{-2}$/ppm. The maximum likelihood estimate is $q_1 = 6.9 \times 10^{-3}$/ppm.

TABLE C-3. INCIDENCE OF ZYMBAL GLAND CARCINOMAS IN MALE RATS (F344) ADMINISTERED BENZENE BY GAVAGE (NTP 1984)

Dose (mg/kg/day)	Response
0	2/48
50	6/50
100	10/50
200	17/50

Remarks:

1. Benzene was administered by gavage 5 days/week for 103 weeks.

2. The lifetime dose is calculated by d x (5/7), where d is the experimental dose.

3. The carcinogenic potency of benzene is estimated to be:

$$q^* = 3.64 \times 10^{-3}/mg/kg/day$$

or, equivalently,

$$q_1^* = 7.90 \times 10^{-3}/ppm$$

The maximum likelihood estimates of parameters using the multistage model are:

$$q_1 = 5.16 \times 10^{-3}/ppm,\ q_2 = 5.56 \times 10^{-6}/(ppm)^2,\ q_3 = 0$$

TABLE C-4. INCIDENCE OF ZYMBAL GLAND CARCINOMAS IN FEMALE RATS (F344) ADMINISTERED BENZENE BY GAVAGE (NTP 1984)

Dose (mg/kg/day)	Response
0	0/50
25	5/50
50	5/50
100	14/49

Remarks:

1. The study design is the same as that described in Table B-3.

2. The carcinogenic potency of benzene is calculated as:

$$q^* = 5.96 \times 10^{-3}/mg/kg/day$$

or, equivalently,

$$q_1^* = 1.29 \times 10^{-2}/ppm$$

The maximum likelihood estimates of the parameters in the multistage model are:

$q_1 = 8.68 \times 10\text{-}3/ppm$, $q_2 = 0$ and $q_3 = 5.56 \times 10\text{-}7/(ppm)3$.

APPENDIX D

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