HEI HEALTH EFFECTS INSTITUTE

# Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research

- Report of the Institute's Health Review Committee, September 1985

- Supplement January 1988

SPECIAL REPORT

The Health Effects Institute (HEI) is a non-profit corporation founded in 1980 to assure that objective, credible, high-quality scientific studies are conducted on the potential human health effects of motor vehicle emissions. Funded equally by the U.S. Environmental Protection Agency (EPA) and 27 automotive manufacturers or marketers in the United States, HEI is independently governed. Its research projects are selected, conducted, and evaluated according to a careful public process, including a rigorous peer review process, to assure both credibility and high scientific standards. HEI makes no recommendations on regulatory and social policy. Its goal, as stated by former EPA Administrator William D. Ruckelshaus, is "simply to gain acceptance by all parties of the data that may be necessary for future regulations."




Designed and printed by Prime Communications, Inc.

SPECIAL REPORT

Health Effects Institute

CONTENTS

# Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research

## THE SEPTEMBER 1985 REPORT

In July 1984, the Motor Vehicle Manufacturers Association (MVMA) requested that HEI undertake a review of existing data on the health effects of unburnt gasoline vapors. The MVMA asked that HEI evaluate the need, if any, for a research program designed to address major unresolved issues, especially in regard to the carcinogenicity of gasoline vapors. Because significant differences in interpretation of the available scientific data appeared to exist between the U.S. Environmental Protection Agency (EPA) and the automotive industry, the HEI Board of Directors thought that this was the kind of problem for which HEI was established. The Board therefore accepted the request that HEI act as a neutral, independent third party, but asked that the EPA join industry in calling for HEI involvement. In February 1985, the agency formally requested that HEI consider unburnt gasoline vapors among its research priorities.

The Institute initiated its review of the existing toxicological literature on gasoline vapors by obtaining reviews from two private consulting firms. These reviews were discussed at a workshop held on May 16, 1985, and were subsequently revised on the basis of the reviewers' comments. The Health Review Committee had primary responsibility for preparation and review of the 1985 gasoline vapor report. Dr. Robert Kavet prepared the report with the assistance of Dr. Arthur Upton, and Dr. Ken Sexton. On September 3, 1985, HEI's Board of Directors approved publication of the report "Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research."

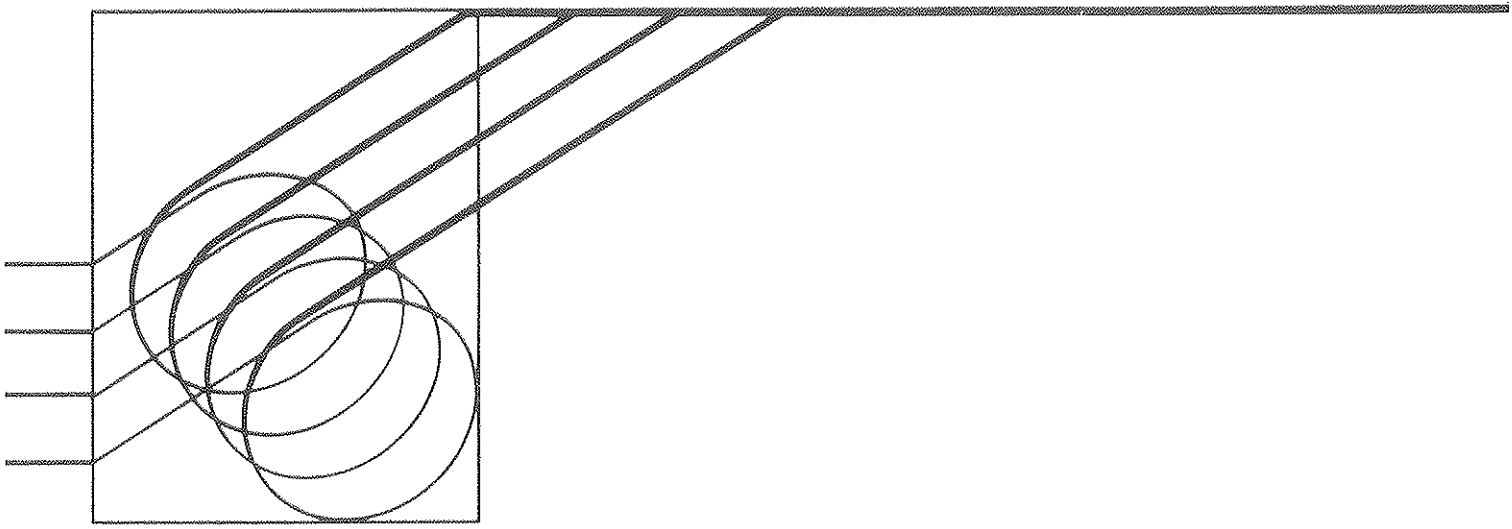
## THE JANUARY 1988 SUPPLEMENT

On August 19, 1987, the EPA published a proposed regulatory strategy to reduce hydrocarbon emissions from mobile sources by requiring (a) manufacturers of gasoline-fueled vehicles to make changes in the design of existing refueling evaporative emission control systems (i.e., the on-board canisters to catch gasoline vapors) (52 *Federal Register* 31162), and (b) gasoline refiners to reduce the volatility of commercial fuels (52 *Federal Register* 31274). In response to the proposed rulemaking, and at the suggestion of its sponsors, HEI decided to prepare a supplement to the 1985 report that would summarize the significant research on the health effects of gasoline vapors that has been published since HEI issued its report in September 1985.

The HEI Research Committee and the Review Committee reviewed the initial draft of the 1988 supplement and were in favor of placing HEI's supplement, as well as the HEI 1985 report, into the public record. It was decided that HEI should use the same mechanism that it used in producing the first document on gasoline vapors, i.e., the document would be prepared under the direction of the Institute's scientific committees and be peer-reviewed. Since HEI's Review Committee had primary responsibility for conducting the 1985 evaluation and review, it assumed the same responsibility for the 1988 update.

Dr. Kathleen M. Nauss of HEI's scientific staff was responsible for preparing the supplement with the assistance of Dr. Arthur Upton and Dr. Rashid Shaikh. Dr. Robert Kavet (Environmental Research Information, Inc.) did some of the library research for this document and prepared an earlier draft. This draft was reviewed by three outside scientists with expertise in the fields of liver and kidney carcinogenesis. Ms. Shirley Roses edited the document. A revised draft was circulated to members of the Research and Review Committees who decided that the 1988 update should be considered as a supplement to HEI's original 1985 gasoline vapor report, and the two documents should be bound in a single volume.

H E I



# Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research

Report of the Institute's Health Review Committee
September 1985

HEALTH EFFECTS INSTITUTE

## Table of Contents

## List of Figures and Tables

### Figures:



### Tables:

# Foreword

## FROM THE HEI BOARD OF DIRECTORS

In July 1984 the HEI industrial sponsors who are members of the Motor Vehicle Manufacturers Association asked the Health Effects Institute to "immediately undertake a review of the issue concerning potential adverse health effects from refueling vapor." EPA followed with a similar request in February 1985 when Dr. Bernard Goldstein, Assistant Administrator for Research and Development, asked HEI to "complete an examination of available and ongoing research on adverse health effects from exposure to gasoline vapors" and to "identify important gaps that should be addressed."

We accepted these requests for several reasons:

- They are consistent with our continuing responsibility to assess the state of the science prior to our own research involvement;
- They fit squarely within the charter of HEI, i.e. "to conduct or support the conduct of, and to evaluate, research and testing relating to the health effects of emissions from motor vehicles, and to provide the results of such research and testing and evaluations to the public and interested government agencies;"
- They spoke directly to former Administrator Ruckelshaus' hopes for HEI, that the "goal is simply to gain acceptance by all parties of the data that may be necessary for future regulations".

Enclosed is the report of the Institute's Health Review Committee, to which we gave primary responsibility for conducting the evaluation. This Committee is chaired by Dr. Robert I. Levy, Professor of Medicine at Columbia University and former Director of the National Heart, Lung and Blood Institute of the National Institute of Health. Development of the report was led by a member of that Committee, Dr. Arthur Upton, Chairman of the Department of Environmental Medicine at New York University, and former Director of the National Cancer Institute. The report is unanimously endorsed by all members of the Health Review Committee, as well as by individual members of the HEI Health Research Committee, who were also asked to review the document.

The report was shaped over the last nine months through a process that ensured objectivity and impartiality. We commissioned independent technical reviews of the existing toxicology to ensure that our Committees would have the most current information and could understand the differing views of outside consultants. A workshop was held in May 1985 in Cambridge, at which all Committee members had the opportunity to review the toxicological and epidemiological data relevant to this issue. Independent external reviews were also obtained from outstanding scientists in both the United States and Canada.

After careful review, we endorse the findings of the report. The report fundamentally finds that current scientific information is sufficient to classify wholly vaporized gasoline vapors as animal carcinogens, according to accepted scientific standards. In the absence of evidence to the contrary, it might also be prudent to assume that vapors from wholly vaporized gasoline are human carcinogens.

However, these vapors may be quite different from those to which consumers or workers are usually exposed. Accordingly, the unanimous scientific view is that the biological and exposure elements of the gasoline vapor problem are extremely complicated and, as yet, unresolved. Consequently, the usefulness of available animal and human data in helping to determine health risks is quite limited. Unburnt gasoline vapors may, upon further investigation, prove to present significant carcinogenic risks for humans. The evidence is not available to make that statement today. Significant additional research would have to be undertaken to understand important mechanisms of action, physiological differences between test animals and people and the extent and nature of exposures.

Archibald Cox
Chairman of the Board

Donald Kennedy
Member of the Board

William O. Baker
Member of the Board

September 3, 1985

## THE HEALTH EFFECTS INSTITUTE AND ITS RESEARCH PROCESS

The Health Effects Institute is a non-profit corporation which, according to its charter, is "organized and operated . . . specifically to conduct or support the conduct of, and to evaluate, research and testing relating to the health effects of emissions from motor vehicles."

It is organized to pursue this purpose in the following ways:

### Independence in Governance

HEI is structured to assure credible scientific investigation on the issues it selects. It is governed by a three-member board of directors whose members are William O. Baker, Chairman Emeritus of Bell Laboratories and Chairman of the Board of Rockefeller University, Archibald Cox, Carl M. Loeb University Professor (Emeritus) at Harvard University, and Donald Kennedy, President of Stanford University. Professor Cox chairs the Board. These individuals, who select their own successors, were initially chosen by then Environmental Protection Agency Administrator Douglas M. Costle. The current Administration has reiterated its support of the Institution.

### Two-Sector Financial Support

The Institute is financed through a unique mechanism. It receives half of its funds from the United States government through the Environmental Protection Agency and half from the automotive industry. Twenty-four leading manufacturers of vehicles or engines that are certified for use on U.S. highways contribute to the Institute's budget in shares which are proportionate to the number of vehicles or engines they sell.

### Research Planning and Project Evaluation

HEI is structured to define, select, support and review research to promote the application of the best in scientific research and to better define the possible health effects of mobile source emissions. Its research program is devised and selected by the Health Research Committee, a multi-disciplinary group of scientists who are knowledgeable about the complex problems involved in determining the health effects of mobile source emissions. The Committee seeks advice from its sponsors, and from other sources prior to independently determining the research priorities of the Institute.

After the Health Research Committee has defined an area of inquiry, the Institute announces to the scientific community that requests for application are available. Applications are reviewed first for scientific quality by an appropriate expert panel. They are then reviewed by the Health Research Committee both for quality and for relevance to the mission-oriented research program. Studies recommended by the Committee undergo final evaluation by the Board of Directors, which also reviews the procedures and assures the independence and quality of the selection process.

When a study is completed, a draft final report is reviewed by a separate Health Review Committee, which has no role in the selection process. This group assesses the scientific quality of each study and evaluates the contribution of the research to unsolved scientific questions. The study is assigned about a year in advance of completion to a member of the Review Committee, who acts as "primary reviewer." When the report is received, the primary reviewer directs a peer review which involves: (1) the referral of the report to appropriate technical experts and, when appropriate, (2) the involvement of the Review Committee biostatistician, who reviews the report to determine whether the data support the conclusions. The primary reviewer then drafts a review which is examined by the full Review Committee, revised as necessary, and made available to the sponsors and to the public along with the final report after evaluation by the Board. All HEI investigators are urged to publish the results of their work in the peer reviewed literature. The timing and nature of HEI Report release is and will be tailored to ensure that the Review Committee's report does not interfere with the journal publication process. The report of the Review Committee will be as thorough as necessary to explain any individual report. Its nature and content may change from study to study.

**Special Reports**

From time to time, the Institute also undertakes reviews of science not directly funded by HEI. These reviews are generally requested by the sponsors, but may also be done at the initiative of the Board, the Research Committee, or the Review Committee, in order to meet their responsibilities under the bylaws.

Primary assistance to the Health Review Committee in the development of this report was provided by Dr. Robert I. Kavet and Dr. Ken Sexton.

ABSTRACT

Gasoline vapors are released into the air at many points along the chain of fuel transfer operations that begins at the refinery and ends at the local service station. Recently, a two-year animal bioassay found increased kidney (or renal) tumors in male rats and increased liver tumors in female mice, following chronic exposure to wholly vaporized unleaded gasoline. While these findings raise concerns about potential human carcinogenicity, several key issues remain to be resolved before health risks, if any, from gasoline vapor exposure can be assessed accurately: 1) how does the experimental vapor composition compare to that typically experienced by humans; 2) to what degree are the effects observed in the two species applicable to humans; 3) what is the nature and magnitude of human exposure to gasoline vapors in occupational and nonoccupational settings; and 4) how should relevant epidemiologic studies be interpreted. This report examines the scientific uncertainties surrounding the question of gasoline vapor carcinogenicity and points out specific areas where further research is needed to reduce those uncertainties. Discussions about whether mandatory controls should be implemented on the basis of the available estimates of public health risk are matters of policy, and as such, are outside the scope of this report.

## Summary

### HEALTH EFFECTS INSTITUTE (HEI) INVOLVEMENT

In July 1984, the EPA's Environmental Health Committee convened to consider the Agency's assessment of risk from exposure to gasoline vapors. At about the same time, HEI received a statement of concern from the Motor Vehicle Manufacturers Association (MVMA), with a request that HEI review the existing data base on the health effects of unburnt gasoline. HEI's Board of Directors agreed that such a review would be appropriate in this instance even though the HEI normally evaluates only its own research. The Board also asked that EPA support HEI involvement in this issue. In February 1985, the agency formally requested that HEI consider unburnt gasoline vapors among its research priorities.

### STATUTORY CONTEXT

Gasoline vapor emissions occur along the chain of fuel transfer operations, which begin at the refinery and end at the local service station. Atmospheric emissions resulting from bulk loading and unloading of gasoline (*i.e.*, from refinery to service station underground storage tanks) are referred to as "Stage I" emissions, and vapor recovery systems designed to limit airborne releases at these fuel transfer points are called "Stage I" controls. Gasoline vapor releases which occur at service stations during vehicle refueling are called "Stage II" emissions, and systems for the recovery of vapors at the pump nozzle are designated "Stage II" controls. Both Stage I and Stage II control systems transfer the vapors displaced from the receiving container back to the dispensing container and are generally known as "vapor balance" systems. In addition, installation of a carbon canister and fill-pipe seal on motor vehicles can be used to capture and absorb vapors that would otherwise be released during the refueling process. This approach to gasoline vapor recovery is known as "onboard control."

Regulations that are currently in place to control gasoline vapor emissions focus on reducing non-methane hydrocarbon emissions that participate in ozone-generating reactions. Under Section 3(b)(1)(B) of the Clean Air Act, the EPA has promulgated two new source performance standards for volatile organic compounds (VOCs). One applies to emissions from loading operations at all bulk terminals constructed after December 1980, and the other, to storage tank emissions at new bulk terminals. EPA has also mandated VOC emission reductions in regions that have not attained primary ozone standards by requiring 90 percent control of Stage I emissions.

A number of strategies are available to control vapor emissions in the gasoline marketing system. The exercise of recommending specific regulatory actions or optimal control strategies falls outside the purview of the Health Effects Institute. However, Appendix A, which briefly describes the available strategies, is included for the interested reader.

## EVALUATION OF THE HEALTH EFFECTS OF GASOLINE VAPORS

In June 1984, the EPA released a risk assessment that it conducted on exposure to unburnt unleaded gasoline vapor. The EPA's risk assessment relies heavily on a single two-year animal carcinogen bioassay (the PS-6 study) sponsored by the American Petroleum Institute (API). In the API study, Fischer 344 rats and B6C3F1 mice of both sexes were exposed to wholly vaporized unleaded gasoline for approximately two years. The major findings were increased kidney (or renal) tumors in male rats and increased liver tumors in female mice (Tables 1, 2, 3).

The International Agency for Research on Cancer (IARC) operates under the auspices of the World Health Organization (WHO) and in 1971 established a program to evaluate potential human cancer risks. As a part of this program, IARC established criteria with which to classify the carcinogenicity of chemicals, groups of chemicals, industrial processes, or occupational exposures. These criteria (Appendix B) have generally gained wide acceptance. According to IARC criteria, gasoline vapors, as generated in this study, are designated animal carcinogens and must be presumed to be human carcinogens in the absence of specific evidence to the contrary.

Although the chronic study was well conducted, its relevance to human risk assessment is uncertain because a number of key issues are yet to be resolved. The issues center on 1) the vapor composition in the experiment as compared to ambient compositions and 2) the applicability to man of the animal models used in the bioassay. In addition to these unresolved questions, uncertainties remain regarding 3) the magnitude and extent of human exposures to gasoline vapors in occupational and nonoccupational settings and 4) the interpretation of the epidemiologic studies currently available.

This paper discusses these unresolved issues and places them in the context of the National Research Council's (NRC) framework for risk assessment. The NRC divides the risk assessment process into four steps: Hazard Identification, Dose-Response Assessment, Exposure Assessment, and Risk Characterization. The steps, though identified separately, are not mutually exclusive and are conceived as interactive.

### Gasoline Composition: Liquid and Vapors

In the API chronic study, the gasoline was entirely vaporized for animal exposure, meaning that the inhaled mixture was identical to that in the liquid phase. This mixture is not representative of the evaporative mix found in ambient situations because of the differential volatility of the hydrocarbon compounds present in gasoline. Stated simply, gasoline is a complex mixture composed of a number of different hydrocarbons and other compounds. The larger hydrocarbon molecules are less volatile and will, therefore, be present in lower proportion in ambient vapors than in those generated in the API study.

This distinction is important because certain subsets of the higher molecular weight compounds are the ones that appear most likely to be responsible for toxic effects in the kidneys (nephrotoxicity) of male rats exposed to wholly vaporized gasoline. For example, branched alkanes with eight or more carbons are perhaps the compounds in gasoline that are most nephrotoxic in male rats. The proportion of wholly vaporized gasoline containing these compounds is roughly 20 times the proportion measured in ambient vapors. Thus, for kidney-related endpoints, upon which much of the issue focuses, experiments such as the chronic bioassay described may overstate the toxic potential in ambient environments.

**Animal Models**

Problems remain concerning the applicability of the experimental animal models to humans.

**Mice:** The B6C3F1 mouse strain used in the PS-6 study has a high spontaneous incidence of liver tumors. Furthermore, increased incidences of mouse liver tumors have been noted following treatment with a broad variety of chemicals, many of them non-genotoxic. (The term genotoxic refers to a compound's ability to damage the genetic material in a cell. A substance's genotoxicity is often indicative of its potential carcinogenicity.) Such lability coupled to high spontaneous rates suggests that tumorigenesis in mouse livers may result non-specifically from factors that either stimulate cellular proliferation or alter genetic expression. Some scientists believe that increased numbers of liver tumors in mice such as those observed in the PS-6 study are minimally relevant to humans. Others, while recognizing that there may be differences in susceptibility to specific substances among species, regard the induction of liver tumors in mice as a warning of possible carcinogenic risk to humans.

**Rats:** The uncertainties concerning kidney tumors in exposed Fischer 344 male rats are more focused than those expressed in connection with mouse liver tumors. The difficulty with species extrapolation in this case lies primarily in the male rat's high susceptibility to hydrocarbon-induced renal toxicity. The toxic mechanisms have been postulated to involve $\alpha$-2-$\mu$globulin, a major urinary protein of the male rat, synthesized in the liver. This protein has not heen detected at comparable levels in other species, including humans, or in the female rat. One hypothesis suggests that, following hydrocarbon exposure, $\alpha$-2-$\mu$globulin accumulates in the kidney tubules of the male rat, thereby triggering the pathologic sequelae. These include extensive exposure-related damage to renal tubules and enhanced old rat nephropathy. (Old rat nephropathy is a degenerative condition that frequently occurs in aging rats.) The development of renal tumors may also be related to these sequelae, though this has not

been conclusively demonstrated. If it is true that the rat is, indeed, uniquely susceptible to renal effects from gasoline vapor, then the relevance to humans of the API study is diminished.

In addition to these species-specific issues, a question remains about whether the exposure rate in chronic animal studies is relevant to humans who are typically exposed to gasoline vapor at the self-service pump for only minutes at a time. Also, studies to date on gasoline's genotoxicity are negative, suggesting that gasoline hydrocarbons may not be directly responsible for initiating carcinogenic processes. Finally, benzene, a constituent of the PS-6 blend and a carcinogen in humans and rodents, is not believed to have contributed to increased incidence of tumors in the PS-6 study.

### Exposure Assessment

Gasoline vapor exposure assessment is an area of great interest and increasing activity. This increased focus is in recognition of the limited data base currently available and tbe need to fill existing gaps. The data accumulated thus far yield order-of-magnitude estimates on perhaps the largest sources of population exposures to gasoline vapors, namely refineries and gasoline stations. Ongoing and future work will enhance our understanding of temporal patterns of exposure and of the chemical species reaching the breathing zone; in addition, assessments should broaden to include other populations such as tank truck drivers and residents downwind of refining or marketing sources.

### Epidemiology

Recent analyses of the epidemiologic literature related to petroleum operations concentrate on renal cancer. The available studies may weakly suggest but do not prove a cause-effect association between exposure to petroleum hydrocarbons and increased renal cancers.

One of the major deficiencies of the epidemiologic literature relates to exposure assessment. Although occupational exposure data bases are only now being developed, it is likely that they will have limited value in assessing long-term retrospective health trends, since work practices have changed and ambient vapor concentrations are probably lower than they used to be. Therefore, establishing exposure estimates for previous studies is difficult or not possible, and for most of the available studies, job description must serve as an uncertain surrogate for exposure.

## CONCLUSIONS

The information from the preceding section can be recast into the NRC criteria framework for risk assessment as follows:

### Hazard Identification

- According to IARC criteria, wholly vaporized unleaded gasoline is an animal carcinogen and a presumptive human carcinogen. However,

the vapor composition in the animal studies is significantly different from that of the ambient vapors to which humans are exposed.

- The specific constituents of gasoline responsible for carcinogenicity in animals remain to be identified.

- The epidemiologic literature, although weakly suggesting carcinogenic effects in petroleum workers in several studies, remains inconclusive in demonstrating carcinogenicity.

- Research to date on the genotoxicity of unleaded gasoline is negative. Thus, the carcinogenic effects of gasoline in animals may arise through alternate means such as promotion, a possibility thus far untested.

- Effects unrelated to cancer resulting from exposure to unleaded gasoline vapors at ambient concentrations have not yet been thoroughly researched. At high concentrations, however, wholly vaporized unleaded gasoline causes nephrotoxicity in male rats.

**Dose-response Assessment**

- The key difficulty arises in quantitative extrapolation to humans from the mouse and rat animal models, each of which appears to have characteristics that render it a questionable surrogate for man. The mouse strain used in the PS-6 study is highly susceptible to the induction of liver tumors, and thus may not appropriately represent the sensitivity of the general human population. The relevance of the male rat kidney tumor model is also questionable, since rats appear to metabolize hydrocarbons differently than do other species.

- If the carcinogenic effects of gasoline vapors in animals are not due to tumor initiation, but instead to tumor promotion or cocarcinogenesis, then the multistage model may not apply to the high/low dose extrapolation for gasoline vapors.

- Human exposure to gasoline vapor, such as may occur in the filling of fuel tanks, is typically sporadic and brief, whereas the exposure of laboratory animals in the experiments conducted to date has generally been sub-chronic or chronic. Such differences in the rate of exposure may importantly influence the dose to target tissues.

**Human Exposure Assessment**

- The temporal and chemical characteristics of the exposures of various human sub-populations have not been well defined.

- The relevant data base is expanding, but the absence of historical exposure data complicates interpretation of the available epidemiologic literature.

### Summary of Concluding Statement

The information needed for the adequate characterization of the risk to humans of ambient gasoline vapors is not available. The PS-6 study and others have shown that some components of gasoline can affect rodent species and increase cancer rates in rats and mice. In the absence of other evidence, therefore, the possible carcinogenicity of gasoline vapors to human beings cannot be dismissed. However, it is not possible to draw accurate conclusions concerning the degree of human risk.

## HEI Involvement

In July 1984, the Motor Vehicle Manufacturers Association (MVMA) requested that HEI undertake a review of existing data on the health effects of unburnt gasoline vapors. The MVMA asked that HEI evaluate the need, if any, for a research program designed to address major unresolved issues, especially in regard to the carcinogenicity of gasoline vapors. Because significant differences in interpretation of the available scientific data appeared to exist between the U.S. EPA and the automotive industry, the HEI Board of Directors thought that this was the kind of problem for which HEI was established. The Board therefore accepted the request that HEI act as a neutral, independent third party, but asked that EPA join industry in calling for HEI involvement. In February 1985, the agency formally requested that HEI consider unburnt gasoline vapors among its research priorities.

Acting on recommendations from both the Health Research Committee and Health Review Committee, the Institute contracted with two private consulting firms (Battelle Pacific Northwest Laboratories and Environ Corporation) to provide reviews of the existing toxicological literature on gasoline vapors. Draft reports from these contractors were reviewed at a workshop held at the HEI offices on May 16, 1985, and subsequently were revised on the basis of reviewer comments. In addition, HEI commissioned outside reviews of recent epidemiologic analyses which assessed the existing data on the association between occupational exposure to gasoline vapor and kidney cancer.

Staff members of HEI met with the Health and Environment Subcommittee of the MVMA on June 12, 1985, to discuss results of the May 1985 workshop. On July 12, 1985, HEI staff members met with representatives of the U.S. EPA to discuss the specifics of the EPA gasoline vapor risk assessment and to review HEI activities on the gasoline vapor issue. The Health Review Committee reviewed the initial draft of this report on July 22, 1985, and subsequent drafts were circulated to members of both the Research and Review Committees. On August 27, 1985, the draft final report was transmitted to the Board by the Health Review Committee for approval. On September 3, 1985, the Board approved HEI publication of this report.

## Statutory Context

Gasoline vapor emissions occur along the chain of fuel transfer operations, which begins at the refinery and ends at the local service station (Fig. 1). Atmospheric emissions resulting from bulk loading and unloading of gasoline (i.e., from refinery to service station underground storage tanks) are referred to as "Stage I" emissions, and vapor recovery systems designed to limit airborne releases at these fuel transfer points are called "Stage I" controls. Gasoline vapor releases which occur at service stations during vehicle refueling are called "Stage II" emissions, and vapor recovery systems at the pump nozzle are designated "Stage II" controls. Both Stage I and Stage II control systems transfer the vapors displaced from the receiving container back to the dispensing container, and are generally known as "vapor balance" systems (Fig. 2). In addition, installation of a carbon canister and fill-pipe seal on motor vehicles can be used to capture and absorb vapors that would otherwise be released during the refueling process. This approach to gasoline vapor recovery is known as "onboard control" (Fig. 3).

The EPA estimates that for non-occupational exposures, 80 percent of the potential gasoline vapor-induced cancers are expected to result from exposure during self-service refueling, and, therefore, that Stage II emissions pose the predominant public health risk (15). EPA and various states have already taken certain steps to control refueling emissions. Section 3(h)(1)(B) of the Clean Air Act, 42 U.S.C. §7411(b)(1)(B), requires the EPA Administrator to promulgate regulations establishing federal standards of performance for new sources of air pollutants. The Statute defines "standard of performance" as a standard "establishing allowable emission limitations," and provides that it "shall reflect the degree of emission limitation and the percentage reduction achievable through application of the best technological system of continuous emission reduction...". Pursuant to this authority, EPA has promulgated two new source performance standards for volatile organic compounds (VOC's). One standard applies to emissions from loading operations at all bulk terminals constructed after December 1980; the other, to storage tank emissions at new bulk terminals. In addition, EPA has promulgated for several air quality control regions regulations requiring 90 percent control of VOC's for Stage I emissions. (Requirements for control of Stage II emissions were also imposed, but in 1977 were deferred indefinitely.) State VOC regulations control (i) storage tank emissions at existing bulk terminals, (ii) loading operations at 70 percent of existing terminals, and (iii) loading operations at 50 percent of existing bulk plants and service station storage tanks. Despite the deferral of EPA requirements for Stage II controls, California and the District of Columbia require Stage II vapor balance systems for ozone control.

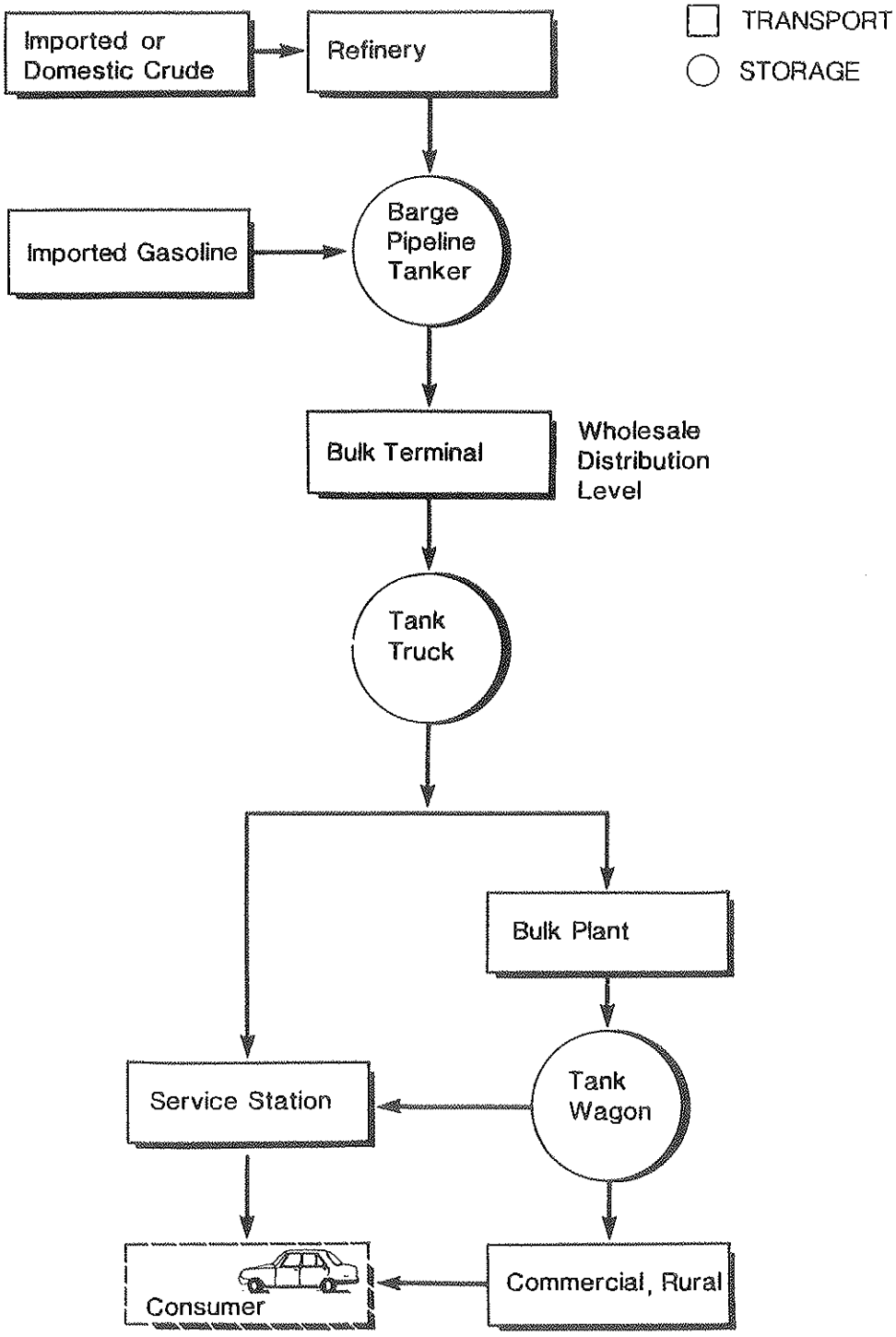



Figure 1. Gasoline Marketing in the U.S. Adapted from: *Evaluation of Air Pollution Regulatory Strategies for Gasoline Marketing Industry*. EPA-450/3-84-012a, July 1984.

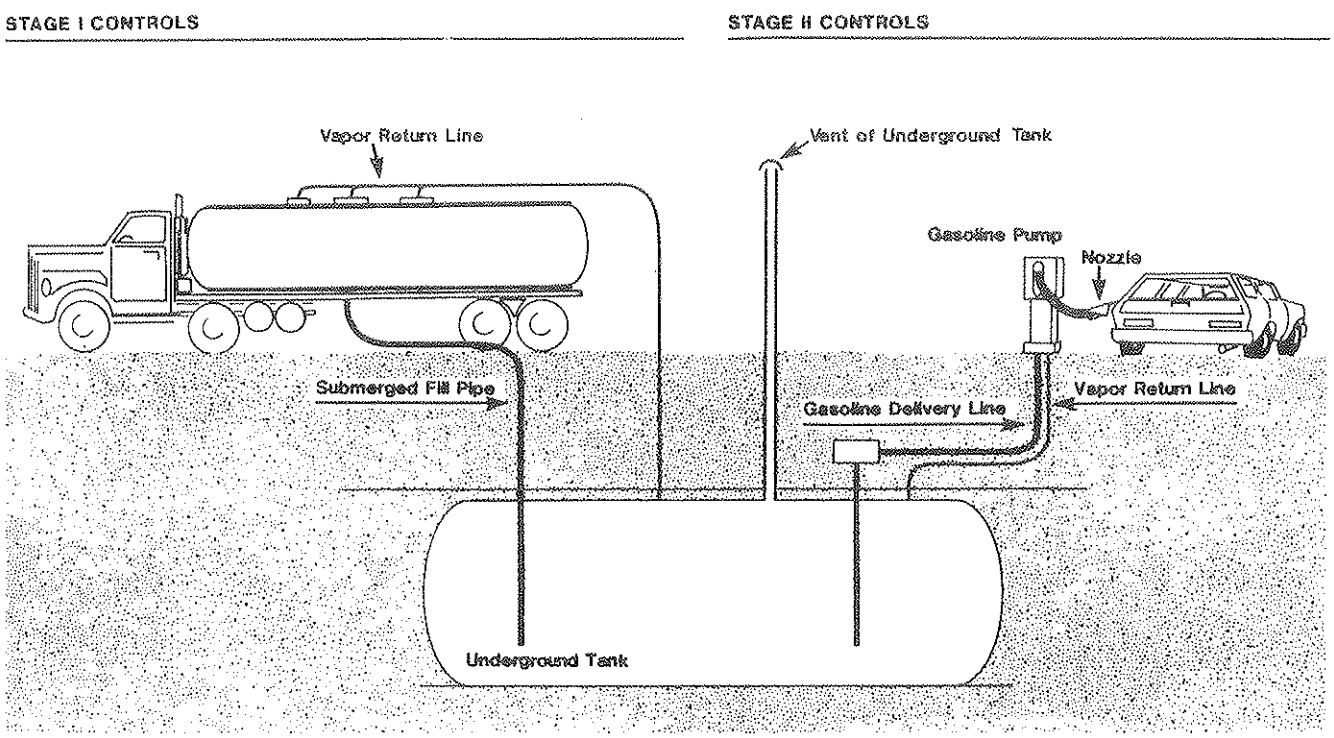
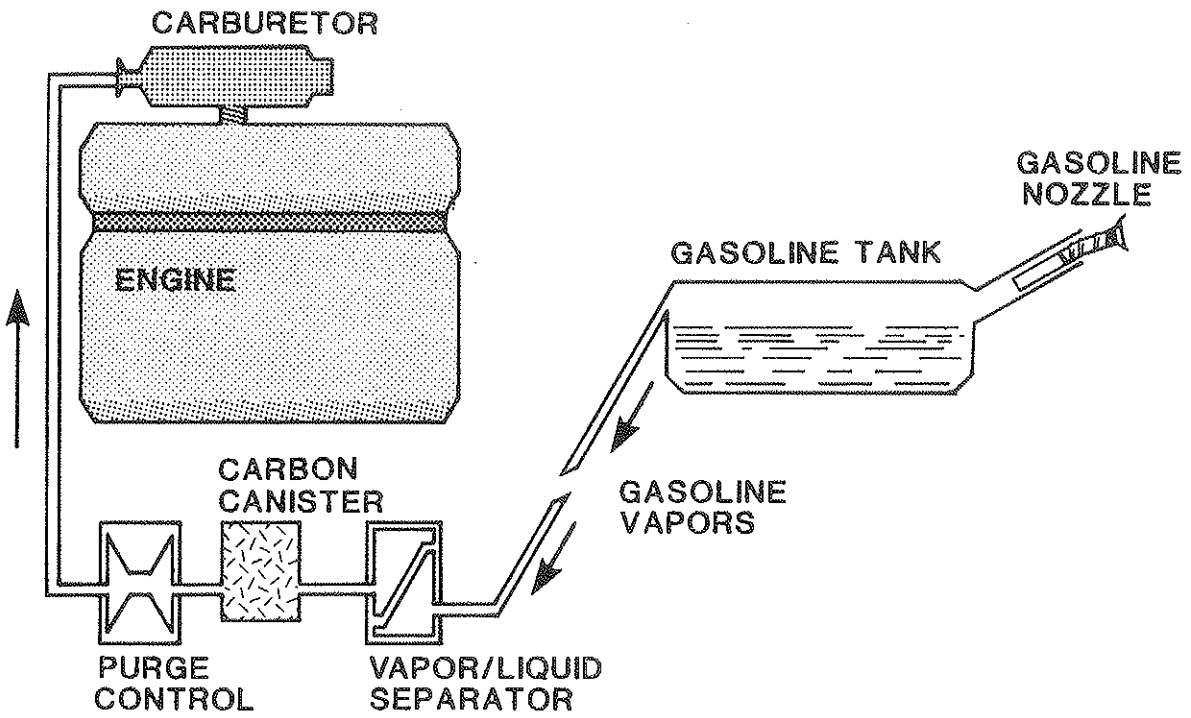



Figure 2. Service Station Vapor Balance System. Adapted from: *Evaluation of Air Pollution Regulatory Strategies for Gasoline Marketing Industry*. EPA-450/3-84-012a, July 1984.

A number of strategies are available to control vapor emissions in the gasoline marketing system. The exercise of recommending specific regulatory actions or optimal control strategies falls outside the purview of the Health Effects Institute. However, Appendix A, which identifies some of the available strategies, is included for the interested reader.

## ONBOARD CONTROLS




## FILL PIPE MODIFICATIONS

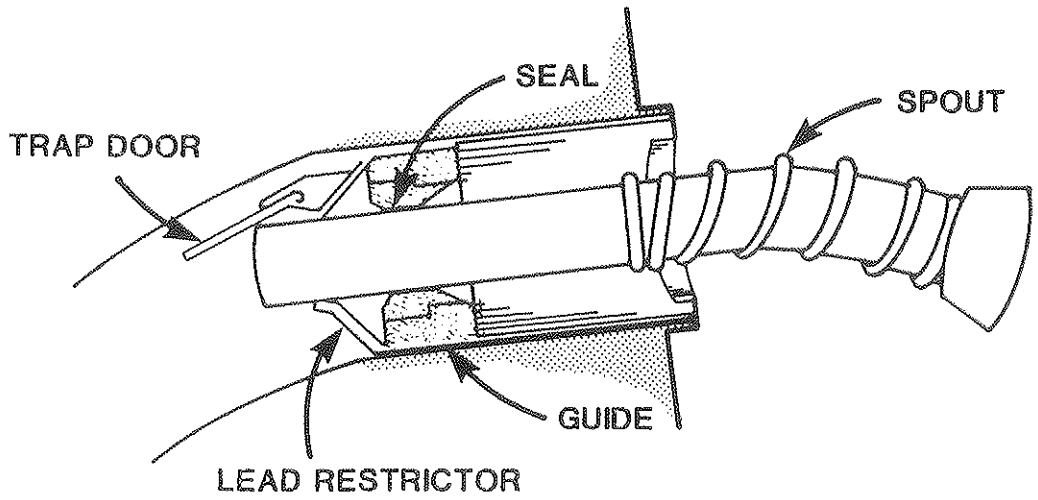



Figure 3. Onboard Controls for Vehicle Refueling Emissions. Adapted from: *Evaluation of Air Pollution Regulatory Strategies for Gasoline Marketing Industry*. EPA-450/3-84-012a, July 1984.

At issue is the level of human health risks associated with exposure to gasoline vapors. The following sections discuss our knowledge of gasoline vapor exposures and effects, and considers whether it is sufficient to support a valid risk characterization.

Populations that are exposed to gasoline vapors include workers in refineries, at bulk terminals, and bulk plants; tank truck drivers; service station attendants; self-service customers; and residents of communities located near fuel transfer operations.

The EPA is currently considering whether potential health effects, primarily carcinogenic, warrant further implementation of gasoline vapor controls (*i.e.*, Stage I, Stage II, onboard) (15). The impetus for EPA's concern comes primarily from the results of a single two-year animal carcinogen bioassay (the PS-6 study) sponsored by the American Petroleum Institute (API) (14).

In the API chronic study (Appendix C), rats (Fischer 344) and mice (B6C3F1) of both sexes were exposed to wholly vaporized unleaded gasoline for approximately two years (31). Exposures were for 6 hours/day, 5 days/week for the entire duration of the experiment. Four different concentrations were employed to test for a dose-response relationship: 0 ppm (control), 67 ppm, 292 ppm, and 2,056 ppm. Animals were sacrificed for interim examination at 3, 6, 12, and 18 months, and for final analyses at the termination of the experiment (Table 1).

**Table 1**
**API Protocol Outline for 2-Year Inhalation Study on Gasoline**

| | Numbers of Test Animals* | | | |
|---|---|---|---|---|
| | Rats | | Mice | |
| **Group and Concentration** | **M** | **F** | **M** | **F** |
| Control | 100 | 100 | 100 | 100 |
| 0.30 mg/Liter (67 ppm) | 100 | 100 | 100 | 100 |
| 1.29 mg/Liter (292 ppm) | 100 | 100 | 100 | 100 |
| 9.15 mg/Liter (2,056 ppm) | 100 | 100 | 100 | 100 |

*Ten animals per sex and species were sacrificed for examination at 3, 6, 12, and 18 months of exposure. All animals surviving to 24 months of exposure were sacrificed for final analyses.

Adapted from: "A Chronic Inhalation Study with Unleaded Gasoline Vapor" in *J. Am. College Tox.* 3:231-248, 1984.

According to the investigators, "The most important findings in this chronic study are the early and progressive renal [or kidney] 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 [Table 2]."

**Table 2**
**API Unleaded Gasoline Blend 2-Year Inhalation Toxicity Study Results**

| | Incidence of Kidney Tumors in Male Rats[a] | |
|---|---|---|
| **Group and Concentration** | **n[b]** | **# tumors** |
| Control | 49 | 0 |
| Low Dose (67 ppm) | 59 | |
| Carcinoma | | 1 |
| Mid Dose (292 ppm) | 56 | |
| Carcinoma | | 2 |
| Adenoma | | 2 |
| Sarcoma | | 1 |
| High Dose (2056 ppm) | 45 | |
| Carcinoma | | 6 |
| Adenoma | | 1 |

(a) Diagnosed at termination or in those rats that died after 18 months. One female in the mid dose group had a renal sarcoma.
(b) Sample sizes from ref. 14, p. 5-65.
Adapted from: "A Chronic Inhalation Study with Unleaded Gasoline Vapor" in *J. Am. College Tox.* 3:231-248, 1984.

Also of interest was the occurrence of a renal sarcoma in a mid-dose female rat and three renal neoplasms in two high-dose female mice. In addition, the study reports a significant increase in hepatocellular carcinomas and adenomas in female mice (Table 3). The chronic exposure did not appear to increase mortality in either species, and the tumors were not immediately life-threatening (31).

Table 3
API Unleaded Gasoline Blend
2-Year Inhalation Toxicity Study Results

| | Mice with Liver Tumors (%)[a] | |
|---|---|---|
| Group and Concentration | M | F |
| Control | 46 | 14 |
| Low Dose (67 ppm) | 36 | 19 |
| Mid Dose (292 ppm) | 45 | 21 |
| High Dose (2056 ppm) | 44 | 48* |

*$p < .05$
(a) Diagnosed from 18 months to final sacrifice
Adapted from: "A Chronic Inhalation Study with Unleaded Gasoline Vapor" in *J. Am. College Tox.* 3:231-248, 1984.

The International Agency for Research on Cancer (IARC) operates under the auspices of the World Health Organization (WHO) and in 1971 established a program to evaluate potential human cancer risks. As a part of this program, IARC established criteria with which to classify the carcinogenicity of chemicals, groups of chemicals, industrial processes, or occupational exposures. These criteria (Appendix B) have generally gained wide acceptance. According to IARC criteria, the research suggests that gasoline vapors, as generated in this study, are animal carcinogens and presumptive human carcinogens.

Despite these designations and the fact that the chronic study was well conducted, a number of key issues require resolution before the relevance of the animal results to man can be established and before the risks encountered in ambient human environments can be quantified with any confidence. The issues center on 1) the vapor composition in the experiment as compared to ambient compositions and 2) the applicability to man of the animal models used in the bioassay. In addition to these unresolved questions, uncertainties remain regarding 3) the magnitude and extent of human exposures to gasoline vapors in occupational and nonoccupational settings and 4) the interpretation of the epidemiologic studies currently available.

## UNRESOLVED ISSUES

### Gasoline Composition: Liquid and Vapors

Motor vehicle gasoline is a complex mixture composed of a blend of various refinery streams (Fig. 4) and consists almost entirely of

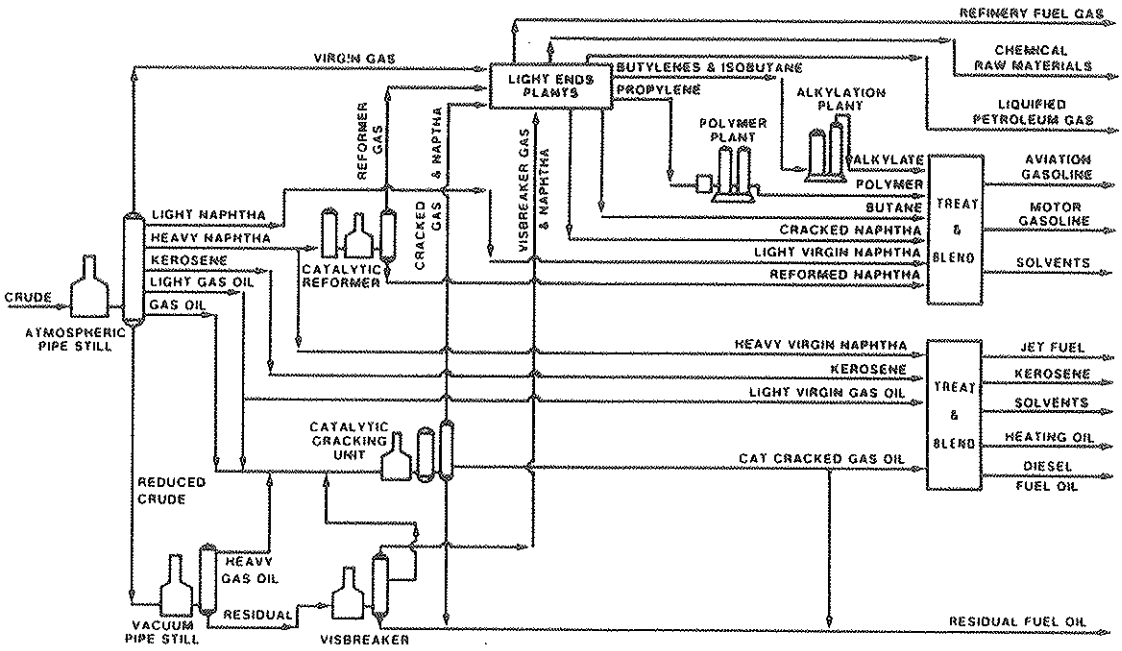



Figure 4. Typical Fuel and Solvent Products Flow Plan. From: *Advances in Modern Environmental Toxicology, Vol. VII Renal Effects of Petroleum Hydrocarbons. (See Reference 11.)*

hydrocarbons: alkanes, alkenes, cycloalkanes, and aromatics (11). Trace amounts of sulfur-, oxygen-, nitrogen-, and metal-containing compounds are also present. Gasolines are not formulated to chemical specification but rather to performance criteria, such as freedom from knock, quick starting, fast warm-up, and combustibility. The fuel blend, termed PS-6, used in the API study and its hydrocarbon breakdown are described on pp. 243-247 of the appended paper (Appendix C). PS-6 is considered to be representative of motor vehicle fuels commercially marketed in the U.S., and contains benzene (1.7 volume percent) but no lead, ethylene dibromide, or ethylene dichloride.

In the API chronic study, also called the "PS-6 study", the gasoline was entirely vaporized for animal exposure, meaning that the inhaled mixture was essentially identical to that in the liquid phase. This mixture is not representative of the evaporative mix found in ambient situations because of the differential volatility of the hydrocarbon compounds present in gasoline. The larger hydrocarbon molecules are less volatile and will, therefore, be present in lower proportion in ambient vapors than in those generated in the API study. This point is reflected in Table 4, which lists the boiling points of selected gasoline compounds, and shows that volatility tends to be inversely related to molecular size.

Table 4
Boiling Points of Selected Compounds in Gasoline

| Carbon # | Alkanes<br>Pt. (°F) | Compound Boiling |
|---|---|---|
| C-4 | n-butane | 31.1 |
| | i-butane | 10.9 |
| C-5 | n-pentane | 96.9 |
| | i-pentane | 82.1 |
| C-6 | n-hexane | 156.1 |
| | 2,3-dimethylbutane | 136.4 |
| | 2-methylpentane | 140.5 |
| | 3-methylpentane | 145.9 |
| C-7 | n-heptane | 209.2 |
| | 2,3-dimethylpentane | 193.6 |
| | 2-methylhexane | 194.0 |
| | 3-methylhexane | 197.6 |
| C-8 | n-octane | 258.2 |
| | 2,2,4-trimethylpentane | 210.6 |
| | 2,2,3-trimethylpentane | 230.0 |
| | 2,3-dimethylhexane | 235.4 |
| C-9 | n-nonane | 303.4 |
| | 2,2,5-trimethylhexane | 255.2 |
| | 2,2,3-trimethylhexane | 269.1 |
| | Aromatics | |
| C-6 | benzene | 176.2 |
| C-7 | toluene | 231.1 |
| C-8 | o-xylene | 291.9 |
| | m-xylene | 282.4 |
| | p-xylene | 281.0 |

Source: *CRC Handbook of Chemistry and Physics*

The differences in volatility are important because branched alkanes with 6 or more carbons (≥C-6) appear to be the compounds most likely responsible for toxic effects in the kidneys (nephrotoxicity) of male rats exposed to wholly vaporized gasoline. (To date, hydrocarbon-induced renal pathology in male rats is the endpoint of most concern for health risk assessment.) Whereas liquid gasoline contains roughly 30-35 volume percent branched alkanes with 6 carbons or more, the ambient

gasoline vapor phase contains less than 10% of these compounds (20, 35, 38). Moreover, for this class of compounds (≥C-6 branched alkanes), the liquid phase is composed of approximately 25% C-6, 14% C-7, and 60% ≥C-8 species; the vapor phase, in contrast, consists of approximately 66% C-6, 25% C-7 and 10% ≥C-8. Extending this simple comparison, 100 ppm of wholly vaporized gasoline is composed of roughly 8 ppm C-6, 5 ppm C-7, and 20 ppm ≥C-8, whereas 100 ppm of ambient gasoline vapors consists of about 7 ppm C-6, 3 ppm C-7, and 1 ppm ≥C-8. These facts raise significant questions about the relevance to human health of gasoline vapors used in the PS-6 study.

Realistic hazard identification requires that compounds in mixtures be considered in terms of their relative toxicities and their relative concentrations in ambient environments. Experiments with wholly vaporized fuels do not accurately represent the gaseous hydrocarbon mixture likely to be encountered in the real world. For the kidney-related endpoints, upon which much of the concern focuses, such experiments may overstate the toxic potential of ambient environments.

**Animal Models**

Problems concerning the applicability of the experimental animal models to humans compound the question discussed in the preceding section. In the PS-6 study, exposure to gasoline vapors increased liver tumors in female mice and renal tumors in male rats. Before considering factors related to individual species extrapolation, it should be borne in mind that the animals in the PS-6 study were exposed for 6 hours/day, 5 days/week, for approximately 2 years, in contrast to human exposures at the self-service gas pump, which occur typically once or twice weekly for a few minutes at a time. The difference in exposure rate is of potential importance when analyzing whether mechanisms of effects in chronically exposed animals are relevant to humans. If, for example, toxic or tumorigenic effects require prolonged exposure, as the rodents received experimentally, then the inference of risk to people whose exposure is usually intermittent or sporadic becomes questionable.

Another issue that deserves attention is the presence of benzene in the PS-6 blend. Benzene is carcinogenic in humans as well as in both sexes of mice and rats. One may, therefore, question whether benzene contributed together with the other gasoline hydrocarbons to the increased tumor burden in exposed animals. However, the sex- and site-specificity for tumorigenesis in the PS-6 study are not consistent with the findings of previous studies in which benzene was administered alone and in high doses. Findings from these studies indicate that benzene induces tumors at multiple sites in mice and rats of both sexes (39). There are no studies showing that benzene causes renal cancers, and the evidence on liver tumorigenesis is ambiguous; a

number of studies indicate increased incidence of liver tumors following benzene exposure (34, 50), while some report no such increase (17, 33, 47, 48, 49). The observations of increased incidence of liver tumors, therefore, are not consistent across studies, and furthermore, fail to show a dose-response relationship (39). By contrast, in the PS-6 study, increased numbers of tumors occurred in a dose-related manner and were restricted to single sites in female mice and male rats. If benzene was partially responsible for tumorigenesis in the PS-6 study, one would expect involvement beyond the female mouse liver and especially beyond the male rat kidney, which is an atypical site for a benzene effect.

Extrapolation issues for mice and rats differ and are considered separately:

Mice: The B6C3F1 mouse strain used in the PS-6 study has a high spontaneous incidence of liver tumors. These rates are highly variable, and tend to be roughly 3-6 times higher in males (7-58%) than females (0-21%) (51, 57). Furthermore, increased incidences of mouse liver tumors have been studied following treatment with a broad variety of chemicals, many of them non-genotoxic (2, 56). (The term genotoxic refers to a compound's ability to damage the genetic material in a cell. A substance's genotoxicity is often indicative of its potential carcinogenicity.) Such lability coupled with high spontaneous rates suggests that tumorigenesis in mouse livers results non-specifically from factors that either stimulate cellular proliferation or alter genetic expression. One opinion holds that increased numbers of liver tumors in mice such as those observed in the PS-6 study are of questionable relevance to humans (40). Recently, Fox and Watanabe reported finding an active cellular oncogene from spontaneous B6C3F1 liver tumors; non-tumorous tissue failed to display such activity (16). The investigators concluded: "These results indicate that the B6C3F1 mouse is dissimilar to the genetically diverse human population in its ability to activate, with a very high frequency, a specific tumor associated oncogene." The late appearance of the liver tumors in the PS-6 study without any other signs of hepatotoxicity compounds the difficulty of extrapolating the mouse data to humans. It is also puzzling that the changes in incidence occurred only in the female mice, and brought the final incidence in the high-dose female group up to the level observed in the non-exposed males.

Despite these problems interpretation of the mouse liver data, the potential relevance to humans of increased mouse liver tumorigenesis following gasoline vapor exposure cannot be completely overlooked. More needs to be known about hydrocarbon-induced carcinogenic processes in the mouse liver.

**Rats:** The key pathological manifestations in rats from the PS-6 study are confined to male kidneys, and consist of the following series of events: progressive hydrocarbon-induced nephropathy, advanced old rat nephropathy, and tumorigenesis (Fig 5).

Hydrocarbon-induced nephropathy originates in the proximal tubule, and in its chronic phase includes regenerative epithelium (indicative of repair processes), tubular dilatation with intratubular protein, and medullary mineralization (6, 55). Separate time course gavage studies demonstrate that hyaline droplet formation, the first sign of hydrocarbon-induced toxicity, appears within one day, peaks at three days, and remains elevated for at least four weeks (the longest duration examined in the gavage time-course studies). Within a week of hyaline droplet formation, cellular regeneration appears and in turn is followed within 2-3 weeks by tubular dilatation at the cortico-medullary junction (20).

"Old-rat nephropathy" is a condition which appears more commonly in the male and appears to be accelerated under the influence of hydrocarbon-induced nephropathy. It is a progressive glomerulonephrosis "characterized by interstitial fibrosis, thickening of tubular basement membranes, interstitial chronic inflammation,

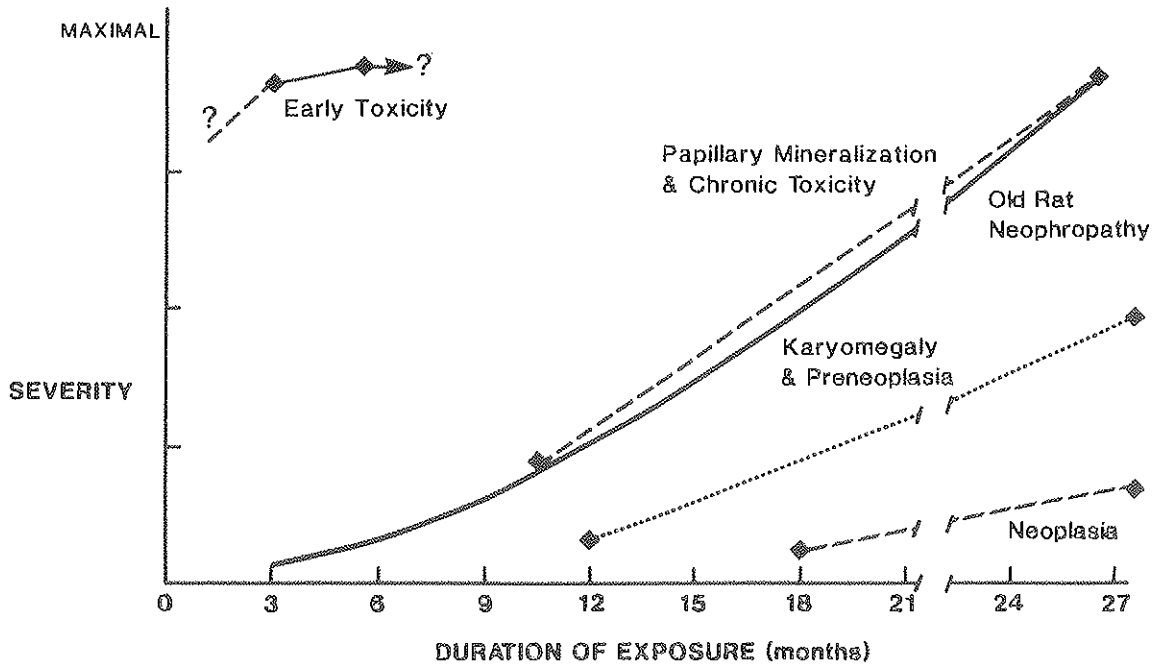



Figure 5. Progression of Unleaded Gasoline-Induced Renal Lesions. Adapted from: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons. (See Reference 55.)*

vascular thickening in the interlobular and afferent arterioles, glomerular hyalinization, and tubular atrophy, particularly in the P1 [*i.e.*, the initial portion of the] proximal tubules" (55). Old-rat nephropathy frequently complicates the assessment of other renal pathologies such as hydrocarbon-induced injury, but fortunately in the PS-6 study did not obscure the detection of preneoplastic changes, which included karyomegaly (enlarged nuclei), hyperplasia, and an early benign neoplasm.

In tbe PS-6 study, primary renal tumors appeared in a total of 13 males, none in the control group, 1 at 67 ppm, 5 at 292 ppm, and 7 at 2,056 ppm. All of these tumors were observed after 18 months or at termination of the experiment. One female at 292 ppm also had a primary renal neoplasm. Sample sizes at termination were roughly 50 rats per dose/sex group. The male tumors consisted of 3 adenomas, 9 carcinomas, and 1 sarcoma; the female tumor was a sarcoma.

Spontaneous rates for renal tumors in Fischer F344 rats are extremely low, usually well below 1% (3, 18). There is no doubt that chronic exposure to wholly vaporized PS-6 fuel caused the elevated renal tumor incidence seen in the PS-6 male rats. The major questions relate to 1) the rat as a species highly, if not uniquely, susceptible to the renal effects of hydrocarbons, 2) the components of gasoline responsible for renal effects, 3) the sequence of events and mechanisms leading to renal tumorigenesis, and 4) their relationship, if any, to the carcinogenic effects on the mouse liver.

To date, all relevant evidence points to the male rat as unusually, if not uniquely, susceptible to the nephrotoxic effects of various hydrocarbons (Tables 5 & 6). Kuna and Ulrich studied rats and squirrel monkeys of both sexes exposed for 90 days (6 hr./day, 5 days/week) to 1,552 and 384 ppm atomized unleaded gasoline and 374 and 103 ppm atomized leaded gasoline (26). Only male rats at the high dose of unleaded gasoline developed renal injury. The U.S. Air Force and U.S. Navy have studied rats, mice, hamsters, and dogs exposed to the vapors of synthetic jet fuels (5, 32). Although various toxic effects were found in these species, only the male rat displayed kidney effects, characterized by enhanced tumor incidence in the presence of degenerative injury. In motor vehicle gasolines, branched alkane compounds appear to be predominantly responsible for nephrotoxicity in male rats. This interpretation is also supported by subchronic inhalation experiments with rats exposed to different individual naphtha streams and by experiments gavaging rats with individual compounds, distillate cuts, or naphtha streams. The inhalation experiments, conducted by Standard of Indiana around the time of the PS-6 study, produced evidence highly suggestive of a structure-activity relationship (Appendix D); and the gavage experiments, conducted

**Table 5**
**A Partial Listing of Chemicals that Affect the Male Rat Kidney in Biological Testing**

| Chemical | Species Tested | Exposure Regimen | Renal Injury |
|---|---|---|---|
| Vapors of Varnish Makers & Painters Naphtha | Rats (male) Harlan Wistar | Subchronic Inhalation Route | + |
| | Dogs | | – |
| Stoddard Solvent | Rats (male) Harlan Wistar | Subchronic Inhalation Route | + |
| | Beagle Dogs (male) | | – |
| 60 Solvent | Rat (male) | Subchronic Inhalation Route | + |
| | Dog (male) | | – |
| JP-5 Shale Jet Fuel | Rat (male/female) | Subchronic Inhalation Route | +/– |
| | Dog (male/female) | | –/– |
| | Mice (female) | | – |
| JP-4 JetFuel | Rat (male/female) | Subchronic Inhalation Route | +/– |
| | Dog (male/female) | | –/– |
| | Mice (female) | | – |
| Methyl Isobutyl Ketone | Rat (male) | Subchronic Inhalation Route | + |
| | Dog (male) | | – |
| | Monkey (male) | | – |

Adapted from: *Advances in Modern Environmental Toxicology, Vol. VII, Renal Effects of Petroleum Hydrocarbons. (See Reference 1.)*

**Table 6**
**Biological Testing of Decalin, a Prototype Volatile Hydrocarbon**

| Species Tested | Renal Injury |
|---|---|
| Rat (male/female) | +/– |
| Mice (female) | – |
| Dog (male/female) | –/– |
| Guinea pig (male/female) | –/– |
| Mice (male) | – |

Adapted from: *Advances in Modern Environmental Toxicology, Vol. VII, Renal Effects of Petroleum Hydrocarbons. (See Reference 1.)*

under API sponsorship as follow-on to the PS-6 study, allowed the analysis of compound-specific toxicity (19, 20). Briefly stated, the gavage studies show that the degree of branching within compounds is an important determinant of nephrotoxicity, perhaps more important than molecular size. Toxicity was observed only in branched alkanes with 6 or more carbons.

Immediately following inhalation or gavage, hydrocarbons reaching the bloodstream probably distribute in the body in relation to the lipid content of tissue. Rodents and humans of both sexes are probably similar with respect to this early passive uptake stage. In the liver, hydrocarbons undergo important oxidative and possibly conjugative processes that increase the compounds' water solubility, and thus facilitate renal excretion. These metabolic-excretory pathways may have features unique to species and sex and of relevance to the issue at hand. Kloss *et al.* examined the disposition of radioactively-labeled 2,2,4-trimethylpentane (TMP) given orally to male and female Fischer-344 rats (25). TMP is among the most nephrotoxic compounds in gasoline. Within 72 hours, no sex differences were seen in disposition of excreted label in expired air, urine, or feces. No differences in organ distribution of TMP appeared, with the exception of the kidneys which, in males, had 10 times as much label as female kidneys, the activity being associated with the renal cortex.

The genotoxicity studies conducted to date are negative for whole unleaded gasoline and TMP (9, 20, 27, 28, 29). The pathologic process in male rats leading from gasoline vapor exposure to neoplasia is not yet clear. The evidence collected to date implies, but does not prove, that pre-existing hydrocarbon nephropathy is a necessary condition for renal tumorigenesis and may be its proximal cause. Thus far, renal neoplasia has not been observed in the absence of hydrocarbon-induced injury. The emerging picture suggests that the relatively early-appearing damage/repair cycle in the proximal tubule may "promote" the development of kidney tumors. The potential influence of old rat nephropathy in this process is, thus far, unknown.

The species/sex specificity of this mechanistic model may be linked to a circulating low-molecular weight (16,000-18,000 daltons) protein, α-2-μglobulin, which is synthesized in the liver of mature rats and apparently participates in the initiation of renal injury following hydrocarbon exposure. The protein has not been detected in comparable concentrations in other species, including humans, or in female rats. Normally, α-2-μglobulin is freely filtered in the kidney glomerulus and accounts for over 50% of the excreted urinary protein in adult male rats. A large fraction of filtered α-2-μglobulin, however, is reabsorbed into the epithelia of the proximal tubule and degraded into its amino acids. Degradation occurs within the tubule cells in lysosomes, organelles rich in digestive enzymes. The amino acids are ultimately released into the bloodstream, available once again for protein synthesis at other sites.

In hydrocarbon-exposed male rats, the normal cycling of α-2-μglobulin in the kidney is disrupted. A number of investigators have advanced the working hypothesis that, in the rat liver, certain hydrocarbon metabolites bind covalently with α-2-μglobulin to form complexes that the lysosomal apparatus in the kidney cannot degrade. Lock *et al.* have shown that administration of TMP to male and female rats produced no changes in the level of α-2-μglobulin in the liver, but caused a dramatic increase of α-2-μglobulin in male kidneys (30). Alden *et al.* gavaged Fischer-344 male rats with decalin, a "volatile prototype hydrocarbon", and demonstrated electrophoretically and immunohistochemically that the resulting hyaline droplets in the proximal convoluted tubule contained α-2-μglobulin (1). Thus, on the basis of the disposition studies of Kloss *et al.* (above) and those just discussed, it can be concluded that metabolites of nephrotoxic hydrocarbons (specifically TMP) and α-2-μglobulin accumulate in male rat kidneys.

Kloss and Bus have proposed a metabolic pathway in the liver leading to formation of a TMP-metabolite-α-2-μglobulin complex, which is ultimately trapped in the kidneys (Fig. 6) (25). According to

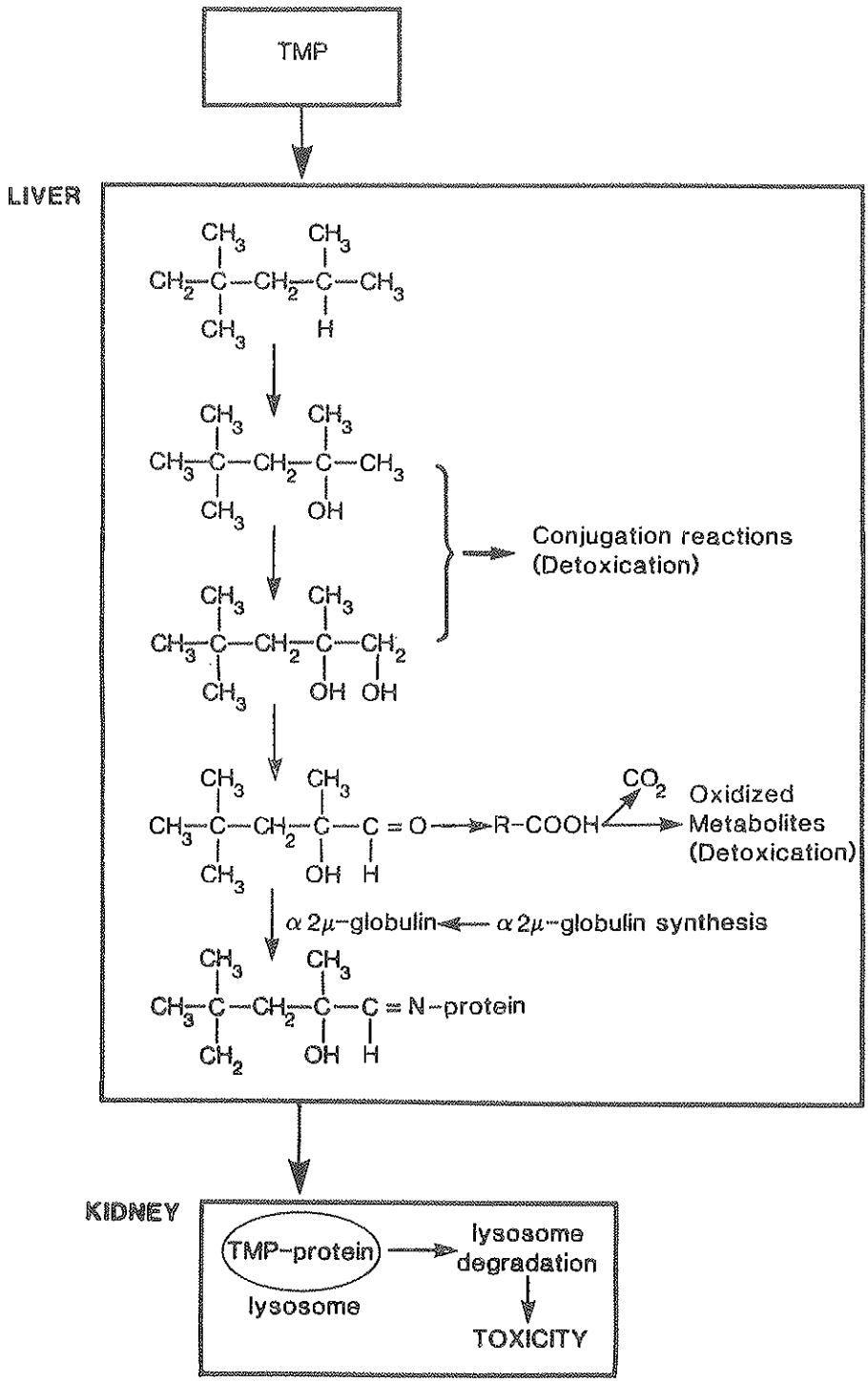



Figure 6. Proposed Metabolism/Bioactivation of 2, 2, 4-trimethylpentane in the Male Rat. From: "Hydrocarbon-Mediated Nephrotoxicity", *CIIT Activities* 5:1, May 1985.

these investigators, "By virtue of its [α-2-μglobulin] covalent association with the TMP metabolite, this 'modified' protein may create conditions such that it is no longer degraded by lysosomal peptidases to its constitutive amino acids. Over time, this lack of protein degradation would manifest itself as an increase in protein deposition or hyaline droplet accumulation within the proximal tubule of the male rat kidney. This phenomenon, of course, is precisely the hallmark of hydrocarbon nephrotoxicity. Continued 'loading' of the lysosomes with altered protein may lead to disruption of the integrity of these organelles, resulting in intracellular release of catabolic peptidases which could cause cell damage or death. Such events could stimulate a wave of cell proliferation to repair the dead and damaged cells; this proliferative response ultimately may be responsible for the hyperplastic and neoplastic kidney lesions seen after chronic exposure to hydrocarbon compounds" (24).

**Summary:** Thus, the extrapolation to humans of the tumor data in the PS-6 study and reliance on the mouse and rat models used in that study are both tenuous. In B6C3F1 mice, statistically significant exposure-related increases in the incidence of liver tumors appeared in high-dose females, with the suggestion of a trend in the other two female exposure groups. Male livers, more prone than females to develop spontaneous tumors, were not detectably affected. Questions related to the sex-specificity of the gasoline vapor effect in mouse liver remain unaddressed, possibly submerged beneath the larger problems of interpreting liver tumorigenesis in this susceptible mouse strain. The problems center on the high spontaneous rate of liver tumorigenesis in B6C3F1 mice and the liver's high propensity to neoplasia following a variety of chemical treatments. Chemically-induced liver tumors in mice have historically presented difficulties with interpretation, to the point that use of the mouse liver tumor model is under review by the National Toxicology Program (40).

The uncertainties concerning kidney tumors in exposed Fischer 344 male rats are more narrowly focused than those in connection with mouse liver tumors. The difficulty with species extrapolation in this case lies primarily in the male rat's high susceptibility to hydrocarbon-induced renal toxicity. The toxic mechanisms may involve α-2-μglobulin, a protein synthesized in the liver of mature rats, which, following hydrocarbon exposure, accumulates in the kidney tubules of male rats with pathologic sequelae. These include extensive exposure-related damage to renal tubules, enhanced old-rat nephropathy, and the development of renal tumors.

Research is currently being conducted to test the hypothesis that the initial reaction in the toxic chain occurs in the liver when specific hydrocarbon metabolites bind α-2-μglobulin to form a complex that the

kidney tubule cannot process. A number of other areas require attention to help explain the pathogenesis of changes in the male rat kidney. These include further comparative studies of gasoline hydrocarbon metabolism in the liver, the role of the kidney's mixed function oxidase system in further metabolism of hydrocarbons, and the role of hydrocarbon-induced renal injury, with the possible interaction of exacerbated old rat nephropathy in promoting tumorigenesis. Future laboratory research should also concentrate on the temporal aspects of tissue injury and recovery to help determine whether chronic toxic effects are relevant to humans who typically receive infrequent and brief exposure.

Though such studies will more fully explain the toxic and tumorigenic mechanisms in rats, extrapolation to man may still be fraught with difficulty. If the ongoing research confirms the hypothesized carcinogenic mechanisms in male rats, further efforts will be needed to determine whether similar mechanisms are operative in humans.

**Exposure Assessment**

Detailed and accurate exposure assessments are essential to risk characterization. The exposure data apply to the analyses of all health endpoints of concern, and help to define the usefulness and applicability of results from animal and *in vitro* studies.

Gasoline vapor exposure assessment is an area of great interest and increasing activity. This increased focus is in recognition of the limited data base currently available and the need to fill existing gaps. Exposures to gasoline vapors occur in both nonoccupational and occupational environments. Nonoccupational populations include self-service customers in gas stations, residents downwind of known sources such as refineries, and occupants of dwellings with attached garages. Occupational cohorts include refinery workers, workers in bulk plants and terminals, tank truck drivers, and service station attendants. Data collected for a number of scenarios provide a feel for the levels of exposure likely to be encountered in some typical situations. Data presented earlier on gasoline and vapor composition suggest that, as a first order estimate, branched alkanes with 6 carbons or more constitute about 10% of the hydrocarbon vapor volatilized in refineries and gas stations. Wen *et al.* studied workers in refineries and marketing terminals (Table 7) (58). For personal sampling periods of 7 hours or more, mean exposure of refinery workers was 5.4 ppm hydrocarbons with 5, 50, and 95 percentile values of 0.15, 1.8, and 20.4 ppm respectively; for marketing terminals, the mean was 11.2, with corresponding percentile values of 0.05, 5.4, and 30.0 ppm. Short-term personal sampling (less than 7 hours) in marketing terminals showed 5% of the exposure exceeding 340 ppm. The Threshold Limit Value for total hydrocarbons is 300 ppm. In the same study, Wen *et al.* reported

8-hour time-weighted average (TWA) exposures of 3.1 ± 2.9 (std. dev.) ppm for service station attendants. Halder reported 8-hour TWA exposures in refineries on the basis of almost 5,000 samples collected by API member companies (20): 97.1% of the exposures were less than 22.5 ppm; 2.4% from 22.5 to 112.3 ppm; 0.27% from 112.3 to 224.6 ppm; and 0.17% greater than 224.6 ppm.

**Table 7**
**Comparison of Measurements of Total Hydrocarbons Between Refineries and Marketing Terminals**

| Types of Samples | N | Mean | (ppm) value at 5% | 50% | 95% |
|---|---|---|---|---|---|
| | | **Refineries** | | | |
| Full | 1201 | 5.4 | 0.15 | 1.8 | 20.4 |
| Partial | 216 | 13.7 | 0.10 | 3.7 | 60.0 |
| Area | 1400 | 15.2 | 0.02 | 2.2 | 50.0 |
| | | **Marketing Terminals** | | | |
| Full | 66 | 11.2 | 0.05 | 5.4 | 30.0 |
| Partial | 1491 | 71.5 | 0.26 | 13.0 | 340.0 |
| Area | 390 | 13.4 | 0.08 | 3.1 | 46.7 |

Adapted from: *Advances in Modern Environmental Toxicology, Vol. VII, Renal Effects of Petroleum Hydrocarbons. (See Reference 58.)*

Clayton Environmental Consultants measured gasoline vapor concentrations during refueling operations at 13 gas stations (7). The average geometric mean during unleaded refueling was 44 ppm, with a range of 14 to 89 ppm; leaded was 52 ppm average (13-120 ppm); and premium was 50 ppm average (18-110 ppm). Clayton's chemical speciation included n-pentane, n-hexane, and a number of aromatics such as benzene, hut did not report on higher or branched aliphatics. The EPA is in the process of publishing a new study on gas station refueling vapors, which should broaden this data base (4).

The data accumulated thus far yield order-of-magnitude estimates with respect to the largest suspected sources of population exposures to gasoline vapors. Ongoing and future work will enhance our understanding of temporal patterns of exposure and of the chemical species reaching the breathing zone; in addition, assessments should

broaden to include other populations such as tank truck drivers and residents downwind of refining or marketing sources.

## Epidemiology

Perhaps nowhere is the deficiency of adequate exposure data more acute than in the epidemiological literature on occupational groups exposed to gasoline vapors. Although occupational exposure data bases are now being developed, it is likely they will have limited value in assessing long-term retrospective health trends, since work practices have changed and ambient vapor concentrations are probably lower than they used to be. For the most part available studies have used job descriptions as surrogates for exposure.

The recent analyses of the epidemiologic literature related to petroleum operations concentrate predominantly on renal cancer, owing in large measure to excess cancers in kidneys of male rats chronically exposed to wholly vaporized gasoline in the PS-6 study (12, 13, 41, 42). Taken collectively, the available studies may weakly suggest, but do not prove, an association between exposure to petroleum hydrocarbons and increased renal cancers. This conclusion is reached after consideration of the six epidemiological canons for causation:

**(1) Strength of association.** In those studies with excess kidney cancers, the overall relative risk was below 1.75, and, therefore, suggests only a weak to moderate association, if any. None of the excesses was statistically significant.

**(2) Consistency.** A large proportion of the available studies, particularly those with short follow-up times, report relative risks below one. For example, in the group of twelve cohort studies[a] reviewed by Enterline and Viren, 6 had standard mortality ratios (SMR) above 100, 5 below 100, and one had no kidney deaths with which to compute an SMR (12).

**(3) Temporal relationship.** Of the six criteria this one is perhaps best satisfied. In the Enterline-Viren review, the authors noted a possible association between occupationally related kidney cancer and an index of age (bladder cancer deaths/kidney cancer deaths). Enterline followed up with an analysis of five studies for which temporal data were available (13); four showed a positive association of kidney cancer with time (36, 44, 54, 58), and one did not (37). Using Wen *et al.*'s exposure data (above), a proportionate adjustment for exposure duration, and various techniques to deduce expected deaths from kidney cancer for each of the four studies, Enterline calculated each study's estimate of the unit risk factor. The factors were similar to the unit risk factor that

[a]ref. 10, 21, 22, 23, 36, 37, 43, 44, 46, 52, 53, 58

EPA calculated from the rat data in the PS-6 study (14). In discussing the suggestiveness of his findings, Enterline also commented that "the close agreement shown here may be only a coincidence." The comment appears apt in view of the clear differences between the PS-6 rats and humans with respect to the specific vapors they were exposed to and their temporal exposure patterns. Moreover, the renal cancers in the rats did not shorten life, but in the humans the renal cancers were the proximal cause of death.

A number of caveats require mention, despite the fact that Enterline's analyses were thoughtful and careful and possibly suggestive. One study of the original five with temporal data was discarded because the renal cancer SMR correlated negatively with time (37); within each of the four used, the calculated unit risk factors correlated positively with estimated exposure duration, but the variation in risk among the four correlated negatively with exposure duration. One of the studies showed no secular trend when latency rather than length of employment was the independent variable (58). Another (54), by Enterline's reckoning, was significantly weakened by difficulties related to its use of proportionate mortality ratios and to ascertainment of death.

**(4) Dose-response relationship.** The scarcity of exposure data prevents a serious attempt to analyze for dose-response in the available literature.

**(5) Biologic plausibility and coherence.** As discussed earlier in some detail, the mechanisms of renal toxicity apparently operative in gasoline vapor-exposed-rats are of questionable relevance to humans. The question remains open whether humans are at increased risk of gasoline vapor-induced kidney effects by alternative biological mechanisms.

**(6) Specificity.** This criterion seeks to determine whether exposure is related to a single disease, possibly at a single site. For example, liver angioscarcoma is relatively specific to vinyl chloride exposure; and mesothelioma, to asbestos inhalation. High specificity in these cases helped prove causality. The lack of disease or site specificity does not necessarily argue against a causal relationship, but may hamper efforts to demonstrate its existence. Renal effects have not been shown consistently in human studies. In a recent review of refinery workers, however, Savitz and Moure show evidence of increased risk for kidney cancer and other malignancies, including melanoma, leukemia, and cancers of stomach, brain, and pancreas (45). Such findings suggest that if the putative hydrocarbon effects lack site specificity, the focus on renal effects may be too narrow.

Therefore, additional study is required to clarify whether there are causal links between human exposure to gasoline vapors and disease- or site-specific morbidity and mortality. Due mostly to the PS-6 study, the endpoint most studied to date in the context of occupational risk from gasoline vapors has been death from kidney cancer. Although hydrocarbon nephropathy is well studied in the rat, information on potential toxic effects of hydrocarbon vapors in humans is scarce. Phillips collected scattered case studies that suggest that a predominant injury may be of the immune complex type at the glomerular basement membrane; hydrocarbon injury in rat kidneys is localized in the proximal convoluted tubule (41). However, renal susceptibility to injury at any site may he as important as the specific site of injury. Usually, renal neoplasms in humans occur in relatively normal appearing kidneys, in contrast to hydrocarbon-exposed rats, in which injury appears to precede tumorigenesis. In connection with such species comparisons, Trump *et al.* note: "The only possible equivalent, which has not been well characterized in humans, is the apparent increase of adenomas and carcinomas in patients with chronic renal disease whose lives have been extended through dialysis. This is a recent finding which needs much more investigation, but it is consistent with the view that any type of chronic renal injury, e.g., old-rat nephropathy, may be conducive to increased neoplasia, possibly acting as promotor and/or cocarcinogen" (55). It may, therefore, be extremely useful in future epidemiologic studies to include renal pathology data in addition to cancer and mortality statistics.

Finally, potential effects at other sites are not ruled out. Comparatively little toxicological research has been conducted to examine specifically for gasoline vapor effects on other organs. The PS-6 study and a number of subchronic animal studies screened for biological effects, but, aside from the effects already described and some high-dose related toxicity (e.g., weight loss, respiratory irritation), were essentially negative. The lung, nervous system, and reproductive outcome have been the subject of a small number of studies. However, no definitive conclusions emerge from them. Savitz and Moure have suggested that sites other than kidney may be at increased cancer risk from chemical exposures in the petroleum occupations (45). They advise that future studies will improve on the past if they 1) incorporate complete exposure assessments, 2) take latency of cancer into account, and 3) eliminate confounding factors such as ethnicity and cigarette smoking.

## FINDINGS AND RECOMMENDATIONS

This paper has examined the adequacy of the existing data base on health effects from gasoline vapor exposure in terms of quantitative risk assessment. The information upon which this paper is hased is derived

mainly from chemical analyses of gasoline, animal toxicology studies, exposure assessments, and epidemiological observations.

The central issue is whether currently available data on gasoline vapors are adequate for quantitative risk characterization. To address this question, the material presented is abstracted and recast into the National Research Council's (NRC) framework for risk assessment (8).

The NRC Committee divides the risk assessment process into four steps. The steps, though identified separately, are not mutually exclusive and are conceived as interactive.

**1) Hazard Identification:** This process focuses on determination of whether exposure to a substance can alter an individual's state of health. This step serves as an alert to potential ambient risk factors and may rely on data collected in human, animal, cellular, or even sub-cellular studies.

**2) Dose-Response Assessment:** This step aims to develop quantitative relationships which describe the probability of a health outcome (e.g., lung cancer) in humans as a function of dose or exposure level for a single agent (e.g., benzo(a)pyrene) or specified mixtures (e.g., cigarette smoke). Data are gathered mainly from experiments on non-human subjects or test systems (e.g., Ames assay) that usually employ exposures in considerable excess of ambient levels. Thus, high-to-low-dose extrapolation and species extrapolation are both necessary to characterize the human dose-response.

**3) Exposure Assessment:** The objective of this step is to characterize actual human exposures to chemical or physical agents that occur during real-life situations. Exposure assessments rely on ambient measurements, personal monitoring data, and modeling approaches to provide data on the pattern and extent of exposures experienced by individuals. In the case of complex mixtures, chemical analyses to elucidate composition should accompany quantitative exposure data to ensure that laboratory exposures represent ambient conditions.

**4) Risk Characterization:** This step derives input from the preceding three steps in order to quantify the magnitude of public health risks (e.g., exposure-related mortality). Risk assessment, the combination of these four steps, obviously requires an extensive and multi-faceted data base that permits integration of data from cell and animal studies, human clinical and epidemiology studies, and exposure evaluations.

In the following discussion, the three steps leading to risk characterization are examined, and recommendations for research to

reduce scientific uncertainties are presented; the analysis does not explicitly deal with risk characterization. It is important to emphasize that the recommendations represent a broad-based research agenda designed to reduce important uncertainties surrounding this issue. The cost to implement all of the recommended research is great and might not realistically be borne by any single agency or organization. The suggestions for research are meant to provide a guideline for future investigations that might be undertaken to examine specific issues. They do not necessarily reflect HEI's own research plans, which must take into account other research needs as well as resource limitations.

**Hazard Identification**

- Based on results of the PS-6 study, and according to IARC criteria, wholly vaporized unleaded gasoline is considered to be an animal carcinogen and a presumptive human carcinogen. However, the PS-6 study used a vapor mix significantly different from the ambient vapors to which humans are exposed, and the question remains as to whether a realistic mix would have produced comparable responses in mice and rats.

  **Recommendation:** A chronic animal study that uses test atmospheres representative of ambient human environments could lessen uncertainties concerning toxicity and carcinogenicity. A prelude to the conduct of such a study would be research to establish a reference gasoline that, when volatilized, represents vapor compositions that have been measured at the breathing zone during self-service refueling.

- The specific constituents of gasoline responsible for toxicity remain to be identified.

  **Recommendation:** Research should continue to identify specifically compounds that lead to tissue toxicity and those that ultimately lead to tumor production. Identification of the specific ambient toxic factor(s) will promote more accurate assessment of the toxic potential of different human environments.

- The epidemiologic literature, though weakly suggestive of carcinogenicity in several studies, remains inconclusive in demonstrating effects from occupational exposures.

  **Recommendation:** A feasibility study is needed to determine whether existing data bases might be used to obtain further information on kidney or other organ pathologies in refinery or distribution personnel. Furthermore, the value of existing epidemiologic data could be enhanced significantly through improved exposure estimates for specific job classifications.

Future epidemiologic studies should incorporate quantitative exposure assessments, account for latency, and control for confounding factors, such as smoking. There is also a critical need to study epidemiologically "elusive" or transient populations, such as gas station attendants.

- The research to date on the genotoxicity of gasoline is negative. Thus, the observed carcinogenic effects of gasoline in rodents may have arisen through promotion, a possibility thus far untested in animal systems.

  **Recommendation:** Mutagenic assays with greater specificity for suspected target organs, especially kidney and liver, need to be developed. Results of such assays will clarify future directions in studying the mechanisms of effects observed in vivo. If such research rules out genotoxicity in target tissue, then other enhancing aspects of hydrocarbon injury might be investigated in more detail.

- Effects unrelated to cancer resulting from exposure to unleaded gasoline vapors at ambient concentrations have not yet been thoroughly researched. At higher concentrations, however, wholly vaporized unleaded gasoline causes nephrotoxic effects in male rats.

  **Recommendation:** Future chronic and subchronic animal studies should examine for toxicity in a variety of tissues, including kidney and liver. The limited literature available suggests that inhaled hydrocarbons possibly cause pulmonary effects and neurotoxicity. An expanded data base on toxicology will aid in designing future epidemiology studies.

**Dose-Response Assessment**

- The key difficulty arises in quantitative extrapolation to humans from the mouse and rat animal models, each of which appears to have characteristics that render it a questionable surrogate for man. The mouse strain used in the PS-6 study is highly susceptible to the induction of liver tumors and thus may not appropriately represent the sensitivity of the general human population. The relevance of the male rat kidney tumor model is also questionable, since rats appear to metabolize hydrocarbons differently than do other species.

  **Recommendation:** Research should continue to elucidate the mechanisms of hydrocarbon-induced nephropathy in male rats and to determine whether there are analogous toxic processes in humans. Hydrocarbon uptake, disposition and metabolism in liver and kidney need to be studied further to help localize the cellular and biochemical targets of injury. Such studies, conducted on individual suspect compounds, may narrow the list of those that are of potential concern to humans. In a more generic sense, research is needed to

elucidate the relationships between hydrocarbon nephropathy and 1) advanced development of old rat nephropathy and 2) kidney tumorigenesis. Finally, the reasons for increased numbers of tumors in female, as opposed to male, mouse livers should be further investigated.

- If gasoline vapors are not tumor initiators, but promoters or cocarcinogens instead, then the multistage model may not be appropriate for high/low dose extrapolation. In such a case, other theoretical models may project risks that differ from those of the multistage model.

  **Recommendation:** Alternate dose-response models which complement proposed biological carcinogenic mechanisms and exposure patterns should be investigated.

- Human exposure to gasoline vapor, such as may occur in the filling of fuel tanks, is typically sporadic and brief, whereas the exposure of laboratory animals in the experiments conducted to date has generally been sub-chronic or chronic. The extent to which such a difference in the duration of exposure may influence the toxicokinetics of inhaled vapors, and hence the effective dose of hydrocarbons to target cells in the liver or kidney cannot be predicted with certainty from the available data.

  **Recommendation:** The influence of the duration and intensity of exposure on the effective dose to target cells calls for further study in efforts to improve the reliability of risk assessments for humans.

**Exposure Assessment**

- Temporal and chemical characteristics of exposures for various sub-populations are lacking, but the data base is expanding.

  **Recommendation:** Efforts to characterize ambient vapors and exposure patterns for various high exposure groups should continue. It is especially important to develop the capability to estimate (model) the distribution of public exposures in gasoline stations and other areas. With such information and better knowledge of toxic mechanisms, improved upper-bound limits for health risks can be developed.

- Absence of historical exposure data complicates interpretation of the epidemiologic literature.

**Recommendation:** As discussed earlier, existing occupational data may contain information that will make possible better partitioning of workers into high- and low-exposure groups. This possibility should be investigated, along with the feasibility of using industrial hygiene techniques for developing retrospective exposure histories.

## Conclusion

Available evidence on the health effects of gasoline vapors suggests the possibility of adverse health consequences (e.g., toxicity, carcinogenicity) for occupationally-exposed individuals, such as refinery workers, tank truck drivers, and service station attendants. Within the general population, regular customers at self-service gasoline stations may be the segment receiving the most frequent, though not necessarily the highest, exposure. The existing data are not adequate, however, to provide the basis for a realistic assessment of risks (i.e., morbidity, mortality) in exposed populations. According to IARC criteria, the API-sponsored PS-6 study has shown wholly vaporized gasoline to be an animal carcinogen and a presumptive human carcinogen. Yet because people rarely, if ever, encounter wholly vaporized gasoline vapors during the course of their daily activities, findings from the API study do not necessarily meet the informational requirements for the first step in quantitative risk assessment, hazard identification. Moreover, given the difficulties discussed earlier in extrapolating from the PS-6 chronic animal experiments to humans, use of the API data to develop dose-response relationships suitable for human risk assessment is questionable. In addition, adequate data are not currently available to characterize accurately the nature (i.e., composition of vapors, pattern of exposures) and extent (i.e., exposure distribution) of human exposures to gasoline vapors. Given these informational deficiencies, development of a meaningful and realistic quantitative risk assessment is very problematic.

This is not to say, however, that existing scientific uncertainties preclude an assumption of risk. Results of the API study might reasonably lead one to suspect that gasoline vapors are a human carcinogen. This suspicion is not dispelled, moreover, when the available epidemiologic studies on kidney cancer are examined. Taken collectively, the epidemiologic studies provide weakly suggestive evidence in support of, but do not prove, an association between exposure to petroleum hydrocarbon vapors and increased kidney cancer. The available epidemiologic evidence, therefore, neither negates nor confirms the interpretation that gasoline vapors are a potential human carcinogen. Thus, the actual magnitude of associated health hazards, if any, cannot be estimated accurately without additional data.

On the basis of the available animal studies and established scientific guidelines, the existing data can be used to calculate a so-called "upper-bound" risk factor for gasoline vapor exposure, and the EPA has done so. The important issue centers on the fact that the actual risk might be anywhere between zero and the upper bound. Whether mandatory controls should be implemented on the basis of available estimates of health risk, despite the uncertainties discussed in this report, or whether additional research should be first undertaken to

reduce scientific uncertainties concerning hazard identification, dose-response relationship, and exposure are policy questions. If it is decided on the basis of policy that further research is needed prior to or concurrent with regulatory action, then specific projects may be developed by the HEI and others from the research recommendations presented above.

## References


1. Alden C.L., Kanerva R.L., Ridder G., and Stone L.C. The pathogenesis of the nephrotoxicity of volatile hydrocarbons in the male rat. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons*. Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 107-120, 1984.

2. Becker, F.F. Morphological classification of mouse liver tumors based on biological characteristics. Cancer Res. 42:3918-3923, 1982.

3. Billups, L.M. In Melby and Altman, eds. Handbook of Laboratory Animal Science, Vol. III. CRC Press, Inc., 1976.

4. Bond A.E., Thompson V.L., and Ortman G.C. Environmental Monitoring Systems Laboratory, U.S. EPA, RTP; and Black F.M. and Sigsby J.E. Jr., Atmospheric Sciences Research Laboratory U.S. EPA, RTP, "Self Service Station Vehicle Refueling Exposure Study" (unpublished).

5. Bruner R.M. Pathologic findings in laboratory animals exposed to hydrocarbon fuels of military interest. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons*. Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Puhlishers, Princeton, 133-140, 1984.

6. Busey W.M., and Cockrell B.Y. Non-neoplastic exposure-related renal lesions in rats following inhalation of unleaded gasoline vapors. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons*. Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 57-64, 1984.

7. Clayton Environmental Consultants, Inc. Gasoline Exposure Study for The American Petroleum Institute. CEC Job No. 18629-15, August 25, 1983.

8. Committee on the Institutional Means for Assessments of Risks to Public Health, Commission on Life Sciences, National Research Council, *Risk Assessment in the Federal Government: Managing the Process*, National Academy Press, Washington, DC., 19-28, 1983.

9. Conaway C.C., Schreiner C.A., and Cragg S.T. Mutagenicity evaluation of petroleum hydrocarbons. In Macfarland C.E., Holdsworth C.E., Macgreggor J.A., Call R.W., and Kane M.L., eds. Advances in Modern Environmental Toxicology, Volume VI Applied Toxicology of Petroleum Hydrocarbons. Princeton Scientific Publishers, 98-107, 1984.

10. Divine B.J., and Kaplan S.D. Unpublished Texaco Mortality Study, Texaco, Inc., 1983.

11. Domask W.G. Introduction to petroleum hydrocarbons: Chemistry and composition in relation to petroleum-derived fuels and solvents. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons*. Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 1-25, 1984.

12. Enterline P.E, and Viren J. The epidemiologic evidence for an association between gasoline and kidney cancer. Environ. Health Perspect. (in press).

13. Enterline P.E. Lifetime risks of kidney cancer caused by gasoline estimated from animal and human studies. Prepared for the Motor Vehicle Manufacturers Association of the United States, Inc., November 30, 1984.

14. Estimation of the Public Health Risk from Exposure to Gasoline via the Gasoline Marketing System. Office of Health and Environmental Assessment, Office of Air Quality Planning and Standards, EPA, June, 1984.

15. Evaluation of Air Pollution Regulatory Strategies for the Gasoline Marketing Industry. EPA — (450/3-84-012a). U.S. EPA, July, 1984.

16. Fox T.R., and Watanabe P.G. Detection of a cellular oncogene in spontaneous liver tumors of B6C3F1 mice. Science 228:596-597, 1985.

17. Goldstein B.D., Snyder C.A., Laskin S., Bromberg I., Albert R.E., and Nelson N. Myelogenous leukemia in rodents inhaling benzene. Toxicology Letters. 13:169-173, 1982.

18. Goodman D.G., Ward J.M., Squire R.A., Chu K.C., and Linhart M.S. Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol. Appl. Pharmacol. 48:237-248, 1979.

19. Halder C.A., Warne T.M., and Hatoum N.S. Renal toxicity of gasoline and related petroleum naphthas in male rats. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons*. Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 73-88, 1984.

20. Halder C.A. Research on the Health Effects of Gasoline. Presented to the U.S. EPA-CAG, Washington, DC., December 11, 1984.

21. Hanis N.M., Shallenberger L.G., Donaleski D.L., and Sales E.A. A retrospective mortality study of workers in three major U.S. refineries and chemical plants. Part I: Comparisons with U.S. population. J. Occup. Med., 27:283-292, 1985.

22. Hanis N.M., Stavraky K.M., and Fowler J.L. Cancer mortality in oil refinery workers. J. Occup. Med. 21:3, 167-174, 1979.

23. Kaplan S.D. Mortality study of petroleum refinery workers: Update of follow-up. Unpublished SRI Project No. 2183, 1982.

24. Kloss M.W., and Bus J.S. Hydrocarbon-mediated nephrotoxicity. CIIT Activities 5:1, May, 1985.

25. Kloss M.W., Cox M.G., Norton R.M., Swenberg J.A., and Bus J.S. Sex-dependent differences in the disposition of [$^{14}$C]-2,2,4-trimethylpentane in Fischer-344 rats. In: *Renal Heterogeneity and Target Cell Toxicity*. P. Bach. ed., John Wiley and Sons, Ltd., Sussex, in press, 1985.

26. Kuna R.A., and Ulrich C.E. Subchronic inhalation toxicity of two motor fuels. J. Am. College Tox. 3:217-229, 1984.

27. Litton Bionetics, Inc. Mutagenicity of Unleaded Gasoline. Final Report. Submitted to American Petroleum Institute, Washington, D.C. March, 1977. LBI Project No. 2697. API Medicine and Biological Science Department Publication No. 28-30173, 1977.

28. Litton Bionetics, Inc. Rat Bone Marrow Cytogenetic Analysis. Unleaded Gasoline. Final Report. Submitted to American Petroleum Institute, Washington, D.C. Revised, November, 1977. LBI Project No. 20847. API Medicine and Biological Science Department Publication No. 26-60099, 1977.

29. Litton Bionetics, Inc. Mutagenicity Evaluation of Gasoline, API PS-6 Fuel in the Mouse Dominant Lethal Assay. Final Report. Submitted to American Petroleum Institute, Washington, D.C. Revised, June, 1980. LBI Project No. 21141-02. API Medicine and Biological Science Department Publication No. 28-31344, 1980.

30. Lock E.A., Stonard M.D., and Elcombe C.R. The selective induction of ω- and β-oxidation of fatty acids in the liver and kidney of male rats administered 2,2',4-trimethylpentane. The Toxicologist 5:59, 1985.

31. MacFarland H.N., Ulrich C.E., Holdsworth C.E., Kitchen D.N., Halliwell W.H., and Blum S.C. A chronic inhalation study with unleaded gasoline vapor. J. Am. College Tox. 3:231-248, 1984.

32. MacNaughton M.G., and Uddin D.E. Toxicology of mixed distillate and high-energy synthetic fuels. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons.* Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 121-132, 1984.

33. Maltoni C. and Scarnato C. First experimental demonstration of the carcinogenic effects of benzene. Long-term bioassays on Sprague-Dawley rats by oral administration. Med. Lav. 70:352-357, 1979.

34. Maltoni C., Cotti G., Valgimigli L., and Mandrioli A. Hepatocarcinomas in Sprague-Dawley rats following exposure to benzene by inhalation: first experimental demonstration. Med. Lavoro 73:446-450, 1982.

35. McDermott H.J., and Killiany S.E. Jr. Quest for a gasoline TLV. Am. Ind. Hyg. Assoc. J. 39:110-117, 1978.

36. Morgan R.W., and Wong O. An epidemiologic analysis of the mortality experience of Mobile Oil Corporation employees at the Beaumont, Texas refinery. Report to the Mobile Oil Corporation, April 10, 1984 (unpublished).

37. Morgan R.W., Wong O., and Claxton K. et al. Cause-specific mortality among employees at the Chevron Richmond and El Segundo refineries. Submitted to Standard Oil Company of California, December, 1983.

38. Myers M.E., Stollsteimer J., and Wims A.M. Determination of hydrocarbon type distribution and hydrogen/carbon ratio of gasolines by nuclear magnetic resonance spectrometry. Anal. Chem. 47:2010-2015, 1975.

39. National Toxicology Program (NTP). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/N Rats and B6C3F1 Mice (gavage studies). NIH Publication No. 84-2545. NTP-84-072, 1984.

40. Office of Science and Technology Policy. Chemical Carcinogens; Notice of Review of the Science and its Associated Principles. Federal Register 49:21632, May, 1984.

41. Phillips S.C. A review of the human kidney effects of hydrocarbon exposure. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons.* Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 185-202, 1984.

42. Raabe G.K. Kidney cancer epidemiology in petroleum related studies. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons.* Mehlman M.A., Hestreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 259-271, 1984.

43. Rushton L., and Alderson M.R. An epidemiological survey of eight oil refineries in Great Britain. Brit. J. Indus. Med. 38:225-234, 1981.

44. Rushton L., and Alderson M.R. An epidemiological survey of oil distribution centers in Great Britain. Unpublished, 1982.

45. Savitz D.A., and Moure R. Cancer risk among oil refinery workers. J. Occup. Med. 26:662-670, 1984.

46. Schottenfeld D., Warshauer M.E., Zauber A.G., Miekle J.G., and Hart B.R. A prospective study of morbidity and mortality in petroleum industry employees in the United States: A preliminary report. In: *Quantification of Occupational Cancer.* Peto R., and Schneiderman M. Cold Spring Harbor Laboratory, 1981.

47. Snyder C.A., Goldstein B.D., Sellakumar A., Wolman S.R., Bromberg I., Erlichman M.N., and Laskin S. Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J. Toxicol. Environ. Health. 4:605-618, 1978.

48. Snyder C.A., Goldstein B.D., Sellakumar A.R., Bromberg I., Laskin S., and Albert R.E. The inhalation toxicology of benzene: incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. Toxicol. App. Pharmacol. 54:323-331, 1980.

49. Snyder C.A., Goldstein B.D., Sellakumar A., Bromberg I., Laskin S., and Albert R.E. Toxicity of chronic benzene inhalation: CD-1 mice exposed to 300 ppm. Bull. Environ. Contam. Toxicol. 29:385-391, 1982.

50. Snyder C.A., Goldstein B.D., Sellakumar A.R., and Albert R.E. Evidence for hematotoxicity and tumorigenesis in rats exposed to 100 ppm benzene. Am. J. Med. 5:429-434, 1984.

51. Tarone R.E., Chu K.C., and Ward J.M. Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N x C3H/HeN)$F_1$(B6C3$F_1$) mice. J. Natl. Cancer Inst. 66:1175-1181, 1981.

52. Theriault G., and Goulet L. A mortality study of oil refinery workers. J. Occup. Med. 21:5, 367-370, 1979.

53. Thomas T.L., Waxweiler R.J., Moure-Earso R., Itaya S., and Fraumeni J.F. Mortality patterns among workers in three Texas oil refineries. J. Occup. Med. 24:2, 135-141, 1982.

54. Thomas T.L., Decoufle P., and Moure-Eraso R. Mortality among workers employed in petroleum refining and petrochemical plants. J. Occup. Med. 22(2):97-103, 1980.

55. Trump B.F., Lipsky M.M., Jones T.W., Heatfield B.M., Higginson J., Endicott K., and Hess H.B. An evaluation of the significance of experimental hydrocarbon toxicity to man. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbons.* Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 273-288, 1984.

56. Ward J.M., Greisemer R.A., and Weisburger E.K.. The mouse liver tumor as an endpoint in carcinogenesis tests. Toxicol. Appl. Pharmacol. 51:389-397, 1979.

57. Ward J.M., Goodman D.G., Squire R.A., Chu K.C., and Linhart M.S. Neoplastic and nonneoplastic lesions in aging (C57BL/6N x C3H/HeN)$F_1$(B6C3$F_1$) mice. J Natl. Cancer Inst. 3:849-854, 1979.

58. Wen C.P., Tsai S.P., Moffitt K.B., Bondy M., and Gibson R.L. Epidemiologic studies of the role of gasoline (bydrocarbon) exposure in kidney cancer risk. In: *Advances in Modern Environmental Toxicology, Volume VII Renal Effects of Petroleum Hydrocarbon.* Mehlman M.A., Hemstreet G.P., Thorpe J.J., and Weaver N.K., eds. Princeton Scientific Publishers, Princeton, 245-257, 1984.

# Appendix A

## SOME GASOLINE EMISSION CONTROL STRATEGIES

There are several strategies available for controlling gasoline vapor emissions. They include the following four:

**1. Nationwide Stage I Controls.** Section 112 of the Clean Air Act ("the Act"), 42 U.S.C. §7412, empowers EPA to establish national emission standards for "hazardous air pollutants" — that is, pollutants that cause or contribute to air pollution that "may reasonably be anticipated to result in an increase in serious, irreversible, or incapacitating reversible, illness." 42 U.S.C. §7412(a). Refueling vapors include benzene, which was listed as a hazardous air pollutant by EPA in 1977. Thus, section 112 authorizes control of Stage I vapor emissions. By setting a national standard for loading and unloading operations, EPA could effectively require universal adoption of vapor balance control systems for bulk terminals, bulk plants, tank trucks, and service station storage tanks. Section 112(d)(1), 42 U.S.C. §7412(d)(1), provides that the States may develop plans for implementing and enforcing emissions standards for hazardous pollutants. The EPA retains authority to enforce the standards itself. 42 U.S.C. §7412(d)(2). Such a standard would reach approximately 50 percent of existing bulk plants and service stations and 30 percent of bulk terminals that are not presently covered. Acting pursuant to section 112, however, EPA would not be able to require the installation of onboard controls for new cars and trucks. Emission standards under section 112 apply only to stationary sources of air pollution.

**2. Nationwide Stage II Controls.** For the reasons stated above, Section 112 also serves as authority to set national emission standards for refueling operations at service stations, which are stationary sources. Such standards could effectively require the installation of vapor balance systems to transfer the displaced vapors from a vehicle's gas tank back to the service station's underground storage tank. Thus, acting pursuant to section 112, EPA could require the use of vapor balance systems at Stage I sources, Stage II sources, or both.

The same result could be achieved indirectly through defining the "best available technology" for controlling refueling emissions. When EPA establishes standards of performance for new sources, the States may submit procedures for implementing and enforcing those standards. 42 U.S.C. §7411(c). But the States are obligated to adopt similar standards of performance for existing sources and to provide for the implementation and enforcement of those standards. 43 U.S.C. §7411(d)(1). By definition, a "standard of performance" reflects the degree of emission reduction that can be achieved through the "best system of continuous emission reduction which... the Administrator determines has been adequately demonstrated for that category of sources." 42 U.S.C. §7411(a)(1)(C). Thus, EPA can influence State-fashioned standards of performance by defining what is the best

available technology for emission reduction. Section 108(f) of the Act, 42 U.S.C. §7408(f), provides that EPA shall publish information regarding methods for controlling various pollutants, specifically including "programs to control vapor emissions from fuel transfer and storage operations and operations using solvents". By defining vapor balance systems as the best available technology for controlling Stage I and/or Stage II emissions, EPA could require the installation of such systems at either Stage I or II (or both) for existing facilities.

3. **Nationwide Onboard Controls:** In addition to, or instead of, proceeding under section 112, EPA could promulgate regulations requiring onboard controls pursuant to section 202(a)(6) of the Act, 42 U.S.C. §7521(a)(6). That section, added by the 1977 amendments to the Act, authorizes the EPA Administrator to "prescribe, by regulation, standards requiring the use of onboard hydrocarbon technology" if he finds it is feasible and desirable to employ such technology. There is apparently no question that the technology is feasible; the only question is its desirability. The legislative history makes clear that that question is to be answered "from a cost/effectiveness viewpoint". H.R. Rep. No. 294, 95th Cong., 1st Sess., reprinted in [1977] U.S. Code Cong. & Ad. News 1077, 1102. Section 202(a)(6) does mandate, however, that the Administrator "compare the costs and effectiveness of such technology to that of implementing and maintaining vapor recovery systems (taking into consideration such factors as fuel economy, economic costs of such technology, administrative burdens, and equitable distribution of costs)." This language means that the Administrator is to conduct a broad inquiry, drawing on a wide range of data and subjecting it to a searching analysis. The courts are likely to interpret this provision as granting EPA substantial discretion to evaluate and make the trade-offs among the factors listed as relevant by the statute. If promulgated, regulations requiring onboard controls would not take effect until the model year when it would be feasible to implement them, taking into account "adequate lead time for design and production".

4. **Stage II Controls in Nonattainment Areas.** Section 110(a)(1) of the Act requires each State to submit a plan for implementing, maintaining, and enforcing national ambient air quality standards. 42 U.S.C. §7410(a)(1). Section 110(c) gives EPA the authority to correct any deficiencies in those plans. The 1977 Clean Air Act Amendments recognized that certain areas of the country had yet to achieve the national standards, and the amendments required (i) the identification of these "nonattainment areas", and (ii) the submission of new State plans. Section 172(b) of the Act, 42 U.S.C. §7502(b), requires that the revised plans "provide for the implementation of all reasonably available control measures as expeditiously as possible". In the past, EPA has defined what are the "reasonably available control measures" for various sources of pollutants by publishing control techniques

guidelines (CTG) documents. EPA has not, however, defined Stage II vapor balance system controls as reasonably available control technology. By doing so in a CTG bulletin, EPA could require Stage II controls in ozone nonattainment areas, of which there were 27 in 1983.

## Appendix B

### INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) CRITERIA FOR CARCINOGENICITY

The following is excerpted from *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*[a], and lists the groups according to which carcinogenicity is classified:

**Group 1**

The chemical, group of chemicals, industrial process or occupational exposure is carcinogenic to humans. This category was used only when there was sufficient evidence from epidemiological studies to support a causal association between the exposure and cancer.

**Group 2**

The chemical, group of chemicals, industrial process or occupational exposure is probably carcinogenic to humans. This category includes exposures for which, at one extreme, the evidence of human carcinogenicity is almost 'sufficient', as well as exposures for which, at the other extreme, it is inadequate. To reflect this range, the category was divided into higher (Group A) and lower (Group B) degrees of evidence. Usually, category 2A was reserved for exposures for which there was at least limited evidence of carcinogenicity to humans. The data from studies in experimental animals played an important role in assigning studies to category 2, and particularly those in Group B; thus, the combination of sufficient evidence in animals and inadequate data in humans usually resulted in a classification of 2B.

In some cases, the Working Group considered that the known chemical properties of a compound and the results from short-term tests allowed its transfer from Group 3 to 2B or from Group 2B to 2A.

**Group 3**

The chemical, group of chemicals, industrial process or occupational exposure cannot be classified as to its carcinogenicity to humans.

[a]from Supplement 4, pp. 13-14, IARC, October 1982.

# Appendix C

JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 3, Number 4, 1984
Mary Ann Liebert, Inc., Publishers
Reprinted by permission of the publisher.

# 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]

## 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 US and contained 2% 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

[1]Consultant in Toxicology, Pittsburgh, Pennsylvania.
[2]International Research and Development Corporation, Mattawan, Michigan.
[3]American Petroleum Institute, Washington, DC.
[4]Biolabs, Inc., Las Cruces, New Mexico.
[5]Westpath, Inc., Ft. Collins, Colorado.
[6]Exxon Research and Engineering Co., Linden, New Jersey.

and unleaded gasoline in rats and monkeys was completed in 1976, and later a paper was written for publication (Kuna and Ulrich, 1984). During the long hiatus between the original 90-day study report and the later paper of Kuna and Ulrich, a careful reevaluation 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 reevaluation 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 24–26 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 specifications of unleaded gasoline in use in the US 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 US gasolines averaged about 1%, with a maximum approaching 2%; therefore, benzene content of the gasoline was adjusted to the upper limit of US gasolines. The specifications are shown in Table 1, but more detailed information on chemical composition is provided in the Appendix.

### *Animals*

Fischer 344 albino rats and B6C3F$_1$ mice, each species equally divided as to sex, were used. 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 No. 5001 up to week 38; thereafter Purina Laboratory Chow No. 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 that 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 $\pm 1.5$ standard deviations of the group mean; $\pm 1.6$ 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. 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 (Figure 1) designed by Leong (Leong, 1976; Drew, 1978). The supply air was filtered and controlled for temperature and

TABLE 1. SPECIFICATIONS OF UNLEADED MOTOR GASOLINE

| | *Sample Used in Study* | *Unleaded Commercial Average*[a] |
|---|---|---|
| Research octane No. | 92.0 | 92.1 |
| Motor octane No. | 84.1 | 83.6 |
| (R + M)/2 | 88.1 | 87.9 |
| Reid vapor pressure, pounds | 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, hours | 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%[b] |

[a]DuPont Road Octane Survey, summer 1976.
[b]Average benzene content typical of US 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 |

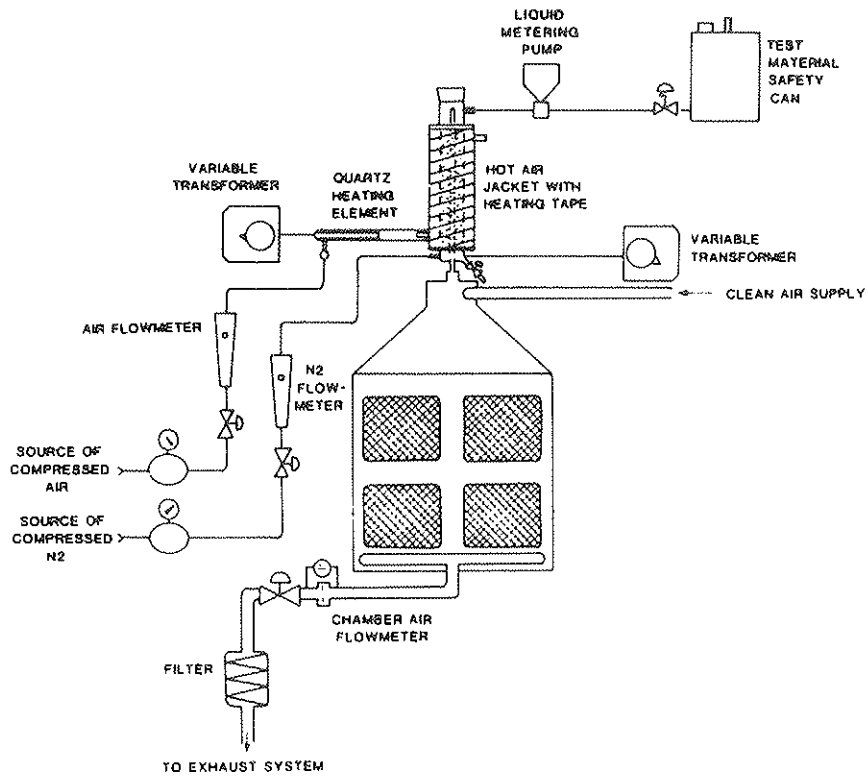



FIG. 1. Schematic diagram of vapor generation and exposure system.

humidity, and flow rates between 900 and 1900 L/min, 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 L/min 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 that 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. These conditions resulted in the appearance of a single peak for the complex hydrocarbon mixture, thereby facilitating expression of results as total hydrocarbon concentration.

TABLE 3. CHROMATOGRAPH OPERATING CONDITIONS

| | |
|---|---|
| Gas chromatograph | Varian 2400 |
| Detector | Flame ionization |
| Column | 5 feet × 1/8 inch OD<br>Stainless Steel<br>1.5% OV-101 on 100/120<br>Mesh Chromosorb GHP |
| Sample loop size | 5 cc |
| Column temperature | 200°C |
| Detector temperature | 270°C |
| Injector temperature | 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<br>8 for 275 ppm<br>64 for 50 ppm |
| Chart speed | 2.5 cm/min for 1500 ppm<br>0.25 cm/min for 275 ppm<br>2.5 cm/min for 50 ppm |

Standard curves for calibration were prepared by injecting a known volume of liquid gasoline into a 25 L 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 7 male and 7 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 was withdrawn from the orbital sinus, and the following enzyme activities were determined as recommended by NCI (1976); alkaline phosphatase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, ornithine carbamyl transferase, and isocitrate dehydrogenase.

Hematological 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 leukocyte 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% survivability criterion was used to terminate each group; this resulted in the termination times shown in Table 4.

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. 109 weeks = 25.2 months.
107 weeks = 24.7 months. 113 weeks = 26.1 months.

At the 3, 6, and 12 month interim sacrifices, 10 rats and 10 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.

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

### *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.

TABLE 5. TISSUES SELECTED FOR WEIGHING

| | |
|---|---|
| Brain | Thyroid/parathyroid complex[a] |
| Heart | Kidneys[b] |
| Liver | Pituitary[a] |
| Testis | Lung with trachea[a] |
| Ovaries[a] | Adrenals[a] |

[a]Tissues weighed after fixation.
[b]Tissues weighed in toto, prior to dissection.

TABLE 6. TISSUES PREPARED FOR MICROSCOPIC EXAMINATION

| *Both Species*[a] | |
|---|---|
| Gross lesions and tissue masses (and regional lymph nodes, if possible) | Nasal cavity |
| Blood smear (as required by pathologist) | Heart |
| Mandibular lymph node | Esophagus |
| Salivary gland | Stomach |
| Sternebrae, femur, or vertebrae including marrow | Uterus |
| Thyroids | Brain (three sections, including frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons) |
| Parathyroids | Thymus |
| Jejunum | Trachea |
| Colon | Pancreas |
| Liver | Spleen |
| Gallbladder (mice) | Kidneys |
| Prostate | Adrenals |
| Testes | Urinary bladder |
| Ovaries | Pituitary |
| Lungs and mainstream bronchi | Spinal Cord |
| Larynx | Eyes |
| *Rats Only* | |
| Optic nerve | Mesenteric lymph node |
| Harderian gland | Skeletal muscle |
| Zymbal gland | Sciatic nerve |
| Oral mucous membrane | Skin |
| Duodenum | Epididymides |
| Ileum | Seminal vesicles |
| Cecum | Cervix |
| Mammary gland | Fallopian tubes |
| | Head |

[a]As recommended by NCI (1976).

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 ±SD 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 4 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 particularly 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 that 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, II, III, and 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 2 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% of the male mice in Groups III and 2% 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% in Group I metastasized to the lungs. In the moribund female mice and those that died on test, tumors in 6% in Group I, 10% in Group III, and 7% 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 morphologically similar to renal tubular epithelium. There was no evidence of peripheral invasion; it had distinct and discrete morphological 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.

*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% in Groups III and IV, respectively. Chronic interstitial inflammatory foci with a predominantly lymphoid cell type were observed at 20 and 70% 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 3–6 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% in Groups I, II, III, and IV, respectively. Proteinaceous casts were observed in 27% of the rats of Group I, 80% in Group III, and 100% in Group IV. The incidence of chronic interstitial inflammation was 18, 20, 100, and 100% in Groups I, II, III, and 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% 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% in Groups I, II, III, and IV, respectively. Mineralization in the renal pelvis occurred in 20% of the male rats of Groups III and in 80% in Group IV. Progressive glomerulonephrosis was diagnosed in 1 male rat from Group IV. Another new finding was karyomegaly, very large nuclei within renal tubular epithelial cells in male rats.

The complexity of morphological 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 lesions 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 Group I, 30% in Group III, and 20% 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% in Groups I, II, III, and IV, respectively. Mineralization in the renal pelvis was seen in 20% of Group III and 80% 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 12–18 month interval.

TABLE 7. PRIMARY RENAL NEOPLASMS IN RATS

| | | Number of Neoplasms | |
|---|---|---|---|
| *Test Group* | *Neoplasm* | *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[a] | 1 | 0 |

[a]Occurred in male rat at 18 months.

At the final sacrifice, nearly all male rats exhibited progressive glomerulonephrosis. The incidence rates were 100, 95, 97, and 100% in Groups I, II, III, and IV, respectively. A slightly lower rate of occurrence was seen in female rats. Mineralization in the renal pelvis occurred in 5, 63, and 91% 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 neoplasmas diagnosed at termination or in those rats that died after 18 months. The total number of these primary renal tumors was 14, with 0, 1, 6, and 7 in Groups I, II, III, and IV, respectively, as shown in Table 7. All but 1 of these primary renal neoplasms occurred in male rats, making the occurrence in males 3 adenomas, 9 carcinomas, and 1 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 and 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 whorllike 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 anatomical 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% in males, and 40, 46, 34, and 67% in females, in Groups I, II, III, and IV, respectively.

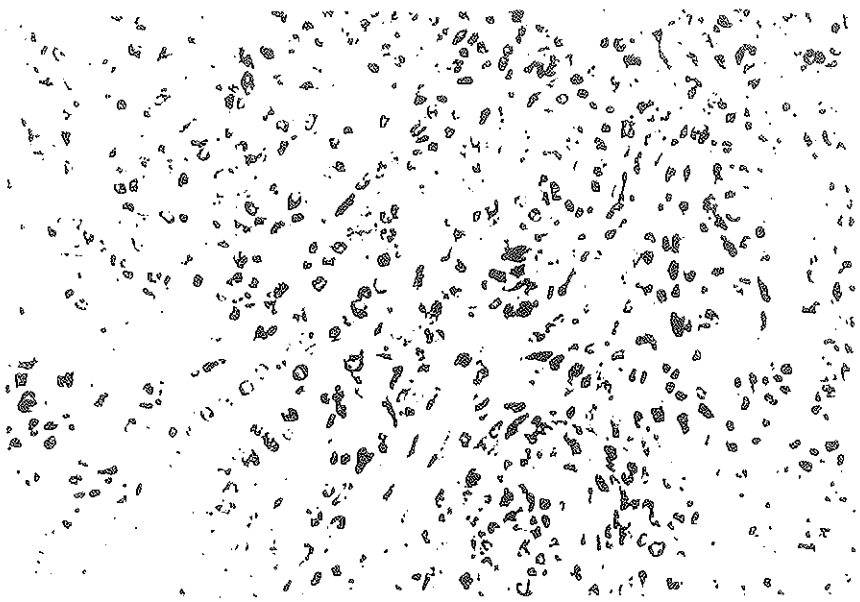


FIG. 2. Histologic appearance of a renal carcinoma composed of epithelial cells arranged in a tubuloacinar pattern. Note cellular pleomorphism and anaplasia.

## 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. (1975a,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 (1984). 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 (ca. 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 1 male rat in the high concentration group at the 12 month interim sacrifice. By 18 months, 20–30% 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, 2 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 1 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, metastases 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 (Appendix.) There are 5 main classes: n-alkanes, isoalkanes, cycloalkanes, alkenes, and aromatics. Some evidence is beginning to accrue that suggests that the renotoxic effect of whole gasoline may be largely due to the presence of 1 or 2 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 5 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 have 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 2 minutes (0.28 ppm based on an 8-hour time-weighted average).

## ACKNOWLEDGMENTS

A number of investigators have contributed to various aspects of this study: its design, performance, and evaluation. We wish especially to thank Drs. B.K.J. Leong, W.R. Richter, and J.F. Hardesty and Mr. N.K. Snyder for their assistance. Drs. R.N. Roth and C.A. Lapin, along with Drs. S.C. Lewis, and J.K. Baldwin, Ms. B.K. Hoover, and Messrs. R.M. Siconolfi and R.C. Anderson, were particularly active in the quality assurance review and evaluation of the detailed final report of the study.

## APPENDIX 1: COMPOSITION OF GASOLINE

The specifications used to define such petroleum products as gasoline are directed toward 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 shown in Table 1 in the text. The gasoline used in the present study was formulated by blending 4 refinery streams, as shown in Table A1.

The antioxidant consisted of 76% 2,6-ditertiary butylphenol, with the remainder about equal parts of 2-tertiary butylphenol and 2,4,6-tritertiary butylphenol. The metal deactivator was a 50% solution of N,N'-disalicylidene-1,2-diaminopropane in commercial xylene. The concentration of 5 pounds/1000 bbl corresponds to approximately 20 ppm wt/wt or 14 ppm wt/vol.

Like gasoline, the 4 refinery streams in Table A1 are specified largely by physical parameters, with only minimal chemical compositional information, as shown in Tables A2, A3, A4, and A5.

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 A6. The specific individual compounds indentified as major contributors in Table A6 are listed in Table A7.

It should be noted that about 75% of the gasoline is comprised of 42 of the compounds deter-

TABLE A1. FORMULATION OF UNLEADED GASOLINE

| *Generic Stream*[a] | *CAS Number* | *Volume %* |
|---|---|---|
| Light catalytic cracked naphtha | 64741-55-5 | 7.6 |
| Heavy catalytic cracked naphtha | 64741-54-4 | 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 pounds/1000 bbl |
| Metal deactivator | | 5 pounds/1000 bbl |

[a]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 A2. 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 predominantly in the range of C-4 through C-11 and boiling in the range of approximately −20°C to 190°C (−4°F to 374°F). It contains a relatively large proportion of unsaturated hydrocarbons.

| *Tests* | *Range of Company Data*[a] |
|---|---|
| Gravity, °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 |
| Naphthenes, % | 10–16 |
| Aromatics, % | 6–28 |
| Saturates, % | — |

[a]Based on data submitted by 11 companies.

TABLE A3. 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 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*[a] |
|---|---|
| Gravity, °API | 36–47.1 |
| Sulfur, weight % | 0.08–0.3 |
| Nitrogen, ppm | 21–110 |
| Reid vapor pressure, psia | 0.3–4.1 |
| Distillation, (ASTM D-86 equiv.), °F | |
| 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 |

[a]Based on data submitted by 6 companies.

TABLE A4. 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°C to 190°C (95 to 374°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*[a] |
|---|---|
| Gravity, °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 |
| Naphthenes, % | 0.5–4.4 |
| Aromatics, % | 30.9–69.9 |
| Saturates, % | — |
| Benzene, vol % | 0.6–3.97 |

[a]Based on data submitted by 9 companies.

TABLE A5. 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*[a] |
|---|---|
| Gravity, °API | 70.4–74.1 |
| Sulfur, weight % | 0.002–0.01 |
| Nitrogen, ppm | 1.1 |
| Flash pt., °F | 122 |
| Aniline pt., °F | 166 |
| RVP, pounds | 4.2–6.9 |
| Distillation, (ASTM D-86), °F | |
| IBP | 104–120 |
| 10% | 154–175 |
| 50% | 208–230 |
| 90% | 235–300 |
| 95% | — |
| EP | 258–335 |
| Paraffins, % | 99+ |
| Olefins, % | 0.01–0.5 |
| Naphthenes, % | 1.0 |
| Aromatics, % | 0.0–1.0 |
| Saturates, % | 98.5 |
| RON (clear) | 93.8–95.2 |
| MON (clear) | 90.5–92.5 |

[a]Based on data submitted by 3 companies.

TABLE A6. 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 through 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 through C-13 | >75 | – | 2.65 | No information | | |
| Total isoalkanes | C-4 through 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 through C-13 | >76 | – | 0.62 | No information | | |
| Total cycloalkanes | C-5 through C-13 | >185 | 49 | 4.68 | 5 | 1.89 | 40% |
| Alkenes | 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 through C-12 | >128 | – | 5.34 | No information | | |
| Total alkenes | C-2 through C-12 | >157 | 29 | 8.96 | 8 | 3.26 | 36% |
| Benzene | C-6 | 1 | 1 | 1.69 | 1 | 1.69 | |
| Alkylbenzenes | 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 through C-12 | ⩾36 | 14 | 26.08 | 9 | 20.84 | 80% |
| Indans/Tetralins | C-9 through C-13 | Large | – | 1.54 | No information | | |
| Naphthalenes | C-10 through C-12 | 15 | – | 0.74 | No information | | |
| Total aromatics | C-6 through C-13 | >51 | 14 | 28.36 | 9 | 20.84 | 73% |
| | | *SUMMARY* | | | | | |
| Alkanes | C-3 through C-10 | 8 | 8 | 11.4 | 3 | 10.2 | 90% |
| Isoalkanes | C-4 through C-13 | >141 | 51 | 46.5 | 17 | 38.0 | 82% |
| Cycloalkanes | C-5 through C-13 | >185 | 49 | 4.7 | 5 | 1.9 | 40% |
| Alkenes | C-2 through C-12 | >157 | 29 | 9.0 | 8 | 3.3 | 36% |
| Aromatics | C-6 through C-13 | >51 | 14 | 28.4 | 9 | 20.8 | 73% |
| TOTAL | | >542 | 151 | 100.0 | 42 | 74.2 | |

TABLE A7. IDENTIFICATION OF MAJOR CONTRIBUTORS

| | |
|---|---|
| Alkanes (3) | |
| n-Butane | |
| n-Pentane | |
| n-Hexane | |
| Isoalkanes (17) | |
| Isobutane | 2,2,4-Trimethylpentane |
| Isopentane | 2,3,4-Trimethylpentane |
| 2-Methylpentane | 2,3,3-Trimethylpentane |
| 3-Methylpentane | 2,2,3-Trimethylpentane |
| 2,3-Dimethylbutane | 2-Methyloctane |
| 2-Methylhexane | 3-Methyloctane |
| 3-Methylhexane | 4-Methyloctane |
| 2,3-Dimethylpentane | 2,2,5-Trimethylpentane |
| 2,4-Dimethylpentane | |
| Cycloalkanes (5) | |
| Methylcyclohexane | Cyclopentane |
| 1,*cis*,-3-Dimethylcyclopentane | Methylcyclopentane |
| 1,*trans*,-3-Dimethylcyclopentane | |
| Alkenes (8) | |
| Propylene | *trans*-Pentene-2 |
| *trans*-Butene-2 | *cis*-Pentene-2 |
| *cis*-Butene-2 | 2-Methylpentene-1 |
| Pentene-1 | 2-Methylpentene-2 |
| Aromatics (9) | |
| Benzene | p-Xylene |
| Toluene | 1-Methyl, 3-ethylbenzene |
| Ethylbenzene | 1-Methyl, 4-ethylbenzene |
| o-Xylene | 1,2,4-Trimethylbenzene |
| m-Xylene | |

mined. In Table A1, benzene adjustment to approach 2% 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 A6, the benzene content was estimated to be 1.69%. More recent reanalyses of the gasoline by an improved method indicate that the actual benzene content was 1.80–1.96%, a satisfactory approximation to 2%.

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

## REFERENCES

CARPENTER, C.P., KINKEAD, E.R., GEARY, D.L., Jr., SULLIVAN, L.J., and KING, J.M. (1975a). Petroleum hydrocarbon toxicity studies. III. Animal and human response to vapors of Stoddard solvent. Toxicol. Appl. Pharmacol. **32**, 282–297.

CARPENTER, C.P., KINKEAD, E.R., GEARY, D.L., Jr., SULLIVAN, L.J., and KING, J.M. (1975b). Petroleum hydrocarbon toxicity studies. VI. Animal and human response to vapors of "60 Solvent." Toxicol. Appl. Pharmacol. **34**, 374–394.

CARPENTER, C.P., GEARY, D.L., Jr., MYERS, R.C., NACHREINER, D.J., SULLIVAN, L.J., and KING, J.M. (1977). Petroleum hydrocarbon toxicity studies. XV. Animal response to vapors of "high naphthenic solvent." Toxicol. Appl. Pharmacol. **41**, 251–260.

COCKRELL, B.Y., IVERSON, W.O., and PHILLIPS, R.D. (1983). Anatomical kidney changes in rats following inhalation exposure to $C_{10}$–$C_{11}$ isoparaffinic solvent. Toxicologist **3**, 25.

COLEMAN, G.L., BARTHOLD, S.W., OSBALDISTON, G.W., FOSTER, S.J. and JONAS, A.M. (1977). Pathological changes during aging in barrier-reared Fischer 344 male rats. J. Gerontol. **32**, 258–278.

DREW, R.T., ed. (1978). *Proceedings, Workshop on Inhalation Chamber Technology.* Springfield, VA: Brookhaven National Laboratory, US Department of Energy, US Department of Commerce, pp. 14, 15.

DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics **20**, 482–491.

DuPONT ROAD OCTANE SURVEY, summer 1976.

GOODMAN, D.G., WARD, J.M., SQUIRE, R.A., CHU, K.C., and LINHART, M.S. (1979). Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol. Appl. Pharmacol. **48**, 237–248.

HANIS, N.M., STAVRAKY, K.M., and FOWLER, J.L. (1979). Cancer mortality in oil refinery workers. J. Occup. Med. **21**, 167–174.

HANIS, N.M., HOLMES, T.M., SHELLENBERGER, L.G., and JONES, K.E. (1982). Epidemiological study of refinery and chemical plant workers. J. Occup. Med **24**, 203–212.

KUNA, R.A., and ULRICH, C.E. (1984). Subchronic inhalation toxicity of two motor fuels. J. Am. Coll. Toxicol. **3**, 4.

LEONG, B.K.J. (1976). *Proceedings, 7th Annual Conference on Environmental Toxicology.* Springfield, VA: National Technical Information Service, pp. 141–149.

LYKKE, A.W.J., and STEWART, B.W. (1978). Fibrosing alveolitis (pulmonary interstitial fibrosis) evoked by experimental inhalation of gasoline vapors. Experientia **34**, 498.

NATIONAL CANCER INSTITUTE (NCI) (1976). *Guidelines for Carcinogen Bioassay in Small Rodents.* DHEW Pub. (NIH) 76–801.

PITTS, L.L., BRUNER, R.H., D'ADDARIO, A.P., and UDDIN, D.E. (1983). Induction of renal lesions following oral dosing with hydrocarbon fuels. Toxcologist 3, 70.

SIEGEL, S., (1956). *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill.

STEEL, R.G.D., and TORRIE, J.H. (1960). *Principles and Procedures of Statistics.* New York: McGraw-Hill.

TARONE, R.E., CHU, K.C., and WARD, J.M. (1981). Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N × C3H/HeN) $F_1$ ($B6C3F_1$) mice. J. Natl. Cancer Inst. **66**, 1175–1181.

THOMAS, D.G., BRESLOW, N., and GART, J.J. (1977). Trend and homogeneity analyses of proportions and life table data. Computer Biomed. Res. **10**, 373–381.

Address reprint requests to:
*H.N. MacFarland*
*232 O'Hara Manor Drive*
*Pittsburgh, PA 15238*

Submitted September 28, 1983
Accepted March 21, 1984

# Appendix D

*Advances in Modern Environmental Toxicology*
*Volume VII. Renal Effects of Petroleum Hydrocarbons.*
Mehlman, M.A., Hemstreet, G.P., Thorpe, J.J., and Weaver, N.K., ed.
Princeton Scientific Publishers, 1984.
Reprinted by permission of the publisher.

CHAPTER VI

# RENAL TOXICITY OF GASOLINE AND RELATED PETROLEUM NAPHTHAS IN MALE RATS

**Clive A. Halder**
*Standard Oil Company (Indiana)*
*200 East Randolph Drive, Chicago, Illinios 60601*

**Thomas M. Warne**
*Amoco Oil Company, Amoco Research Center*
*Warrenville Road and Mill Street, Naperville, Illinois 60566*

**Nabil S. Hatoum**
*IIT Research Institute, Life Sciences Research Division*
*10 West 35th Street, Chicago, Illinois 60616*

## ABSTRACT

Subchronic inhalation toxicity of seven petroleum naphtha streams and an unleaded gasoline blend was studied in Sprague-Dawley rats. The gasoline blend and five of the naphthas produced severe renal toxicity in male rats, whereas the two remaining naphthas induced only mild or no renal toxicity. The lesions consisted of excessive hyalin droplet formation in the epithelium of the proximal convoluted tubules, degenerative changes of the proximal convoluted tubules in the renal cortex, and tubular dilatation and necrosis at the corticomedullary junction. These effects appeared to be associated with exposure to petroleum-derived hydrocarbons and have been reported in inhalation studies conducted on a number of other petroleum materials. A review of the chemical composition indicated that chemical class was a major factor in the nephrotoxic potential of the tested materials and that, as a class, the alkanes (paraffins) appeared to be most effective in producing nephrotoxicity. The alkenes (olefins), which in these materials were structurally similar to the alkanes, also produced nephrotoxicity. Aromatic hydrocarbons, on the other hand, did not produce a nephrotoxic effect.

## Structure-Activity Relationship

The naphtha materials, by virtue of their different processing treatments, are composed of different classes of compounds and/or proportions of these classes of compounds. The predominant chemical classes found in these materials include the normal and branched alkanes (paraffins), cycloalkanes (naphthenes), alkenes (olefins), and aromatic compounds. The fact that some naphtha materials did not cause renal toxicity suggests that certain classes of compounds, or combinations of compounds from one or more chemical classes, in sufficient quantities, were responsible for the production of these renal lesions.

The compositions of the test materials were reviewed in an effort to draw some correlations between nephrotoxicity and chemical composition (Table XI). Full-range alkylate naphtha, which produced the most severe nephrotoxic response, consisted of 98% normal and branched alkanes (paraffins), whereas heavy catalytic-reformed naphtha, which produced no effect, was composed of about 93% aromatics and only 7% alkanes. Both materials had minimal amounts of alkenes (olefins). These findings suggested that the alkanes contributed most to the nephrotoxicity, while the aromatics did not appear to be involved. The fact that both materials contained only minimal amounts of alkenes suggested that alkenes may not be necessary for inducing nephrotoxicity. However, since polymerization naphtha, which is comprised of approximately 92% alkenes, also produced nephrotoxicity, it is evident that alkenes are also involved. It is important to realize, however, that the alkenes contained in the polymerization naphtha are structurally very similar to the normal and branched alkanes comprising full-range alkylate naphtha, and, therefore, it is possible that structural conformation plays an important part in the nephropathy rather than functional group, at least

TABLE XI
Summary of Composition and Nephrotoxic Effects

| Material | Composition [%] | | | Renaltoxicity |
|---|---|---|---|---|
| | Alkanes[a] | Alkenes | Aromatics | |
| Light Straight-Run | 96 | 0 | 4 | + + |
| Light Catalytic-Cracked Naphtha | 39 | 32 | 29 | + |
| Light Catalytic-Reformed Naphtha | 67 | 2 | 31 | + + |
| Heavy Catalytic-Reformed Naphtha | 7 | 0 | 93 | – |
| Full Rangs Alkylate Naphtha | 98 | 2 | 0 | + + + + + |
| Polymerization Naphtha | 8 | 92 | < 1 | + + + |
| Thermal-Cracked Naphtha | 58 | 30 | 12 | + + + + |
| Unleaded Gasoline Blend | 45[b] | 12[b] | 43[b] | + + |

[a]Includes cyclo, normal, and branched; [b]Estimated

as far as the alkanes and alkenes are concerned. Also, the fact that materials that contained high proportions of alkanes also induced renal toxicity in male rats further supports the hypothesis that the alkanes were primarily responsible for the induction of nephrotoxicity in rats exposed to these petroleum materials. Lastly, the unleaded gasoline was a blend of some of the naphtha materials described in this report. The exact composition is proprietary, but it did contain a certain proportion of a strongly nephrotoxic naphtha, that is, full-range alkylate naphtha. Thus, the nephrotoxic responses noted in the 21-day and 90-day studies of unleaded gasoline probably reflect the compositional makeup of the blend, with the different proportions of naphthas each contributing to the overall nephrotoxicity on the basis of their individual potencies.

It is interesting to note the almost 100-fold difference in nephrotoxic potency between full-range alkylate naphtha and light straight-run naphtha, even though both materials are comprised almost entirely of alkanes. However, by virtue of different processing treatments, the composition of the alkane components of these two streams differs substantially. For example, full-range alkylate naphtha is produced by alkylating 3- through 5-carbon alkenes with isobutane to form a predominance of highly branched alkanes, particularly 8-carbon isomers such as the trimethylpentanes. On the other hand, light straight-run naphtha, by virtue of being derived from unprocessed distillation cuts of crude oil, contains a much larger variety of alkanes in varying proportions than does full-range alkylate naphtha. The predominating alkanes in this material consist of straight-chained and/or lightly branched alkanes. These facts, in conjunction with the variance in relative potency between the two streams, further suggest that there exists a range of potencies among the different normal and branched alkanes within the alkane class as a whole and that some of the most potent are concentrated in the full-range alkylate naphtha material.

SUPPLEMENT

# AN UPDATE ON GASOLINE VAPOR EXPOSURE AND HUMAN CANCER: AN EVALUATION OF SCIENTIFIC INFORMATION PUBLISHED BETWEEN 1985 AND 1987

January 6, 1988

# AN UPDATE ON GASOLINE VAPOR EXPOSURE AND HUMAN CANCER: AN EVALUATION OF SCIENTIFIC INFORMATION PUBLISHED BETWEEN 1985 AND 1987

## Table of Contents

## ABSTRACT

At the request of the U.S. Environmental Protection Agency and the motor vehicle manufacturers, the Health Effects Institute (HEI) published a report in 1985 that analyzed the scientific evidence for an association between exposure to gasoline vapors and cancer. Concern at that time arose from the results of a chronic two-year bioassay (the PS-6 study) that reported increased kidney tumors in male Fischer-344 rats and increased liver tumors in female $B6C3F_1$ mice exposed to wholly vaporized gasoline. The 1985 HEI Report concluded that "the information needed for the adequate characterization of the risk to humans of ambient gasoline vapors is not available. The PS-6 study and others have shown that some components of gasoline can affect rodent species and increase cancer rates in rats and mice. In the absence of other evidence therefore, the possible carcinogenicity of gasoline vapors to human beings cannot be dismissed. However, it is not possible to draw accurate conclusions concerning the degree of human risk."

The purpose of this supplement is to describe the research findings that have emerged since the publication of the 1985 HEI report that are relevant to the health effects of gasoline refueling vapors. Research on the renal effects of petroleum hydrocarbons reinforces the earlier conclusion that the kidney of the male rat is particularly susceptible to the toxic effects of petroleum hydrocarbons, and that the observed renal neoplasms may develop by a mechanism that appears to be unique to that species and sex. In contrast, little attention has been directed to the understanding of the mechanism responsible for increased liver tumors in female $B6C3F_1$ mice exposed to wholly vaporized gasoline. During the past two years, a number of reports have been published that provide useful data on the composition of gasoline vapors that are generated during refueling, and on the uptake of specific gasoline compounds by the respiratory tract. Epidemiologic studies are currently in progress to determine if exposure to petroleum vapors is a risk factor for kidney cancer in occupationally exposed cohorts.

# AN UPDATE ON GASOLINE VAPOR EXPOSURE AND HUMAN CANCER: AN EVALUATION OF SCIENTIFIC INFORMATION PUBLISHED BETWEEN 1985 AND 1987

Prepared by the
Health Effects Institute

January 6, 1988

## PURPOSE

In September 1985, the Health Effects Institute (HEI) published an analysis that it had conducted of the informational basis for quantifying cancer risks from exposure to unleaded gasoline vapors (HEI, 1985). For the general public, this exposure occurs most frequently at the self-service gasoline pump. Using the National Research Council's framework for risk assessment (NRC, 1983), the HEI analysis concluded that important information was lacking in the three areas that lead to the actual quantification of risk, namely, hazard identification, dose-response assessment, and exposure assessment. The purpose of this review is to describe research findings that have emerged since the publication of the 1985 HEI report that are relevant to the issue of the health effects of unleaded gasoline vapors.

## BACKGROUND

In July 1984, the HEI industrial sponsors, who are members of the Motor Vehicle Manufacturers Association, asked the Health Effects Institute to "immediately undertake a review of the issue concerning potential adverse health effects from refueling vapor." The U.S. Environmental Protection Agency (EPA) followed with a similar request in February 1985, when Dr. Bernard Goldstein, Assistant Administrator for Research and Development, asked HEI to "complete an examination of available and ongoing research on adverse health effects from exposure to gasoline vapors" and to "identify important gaps that should be addressed." These requests were stimulated by EPA consideration of regulations for enactment of controls that would limit exposure of the public to refueling vapors (EPA, 1984). The technology that is available to limit human exposures to gasoline vapors include Stage II pump-nozzle controls, onboard absorptive canisters, and limitations on fuel volatility.

Three concerns related to air quality and human health provided the impetus for the EPA to consider establishing regulations to control exposure to refueling vapors (EPA, 1984): (1) these vapors contain benzene, which is recognized as a human

carcinogen, and is regulated under Section 112 of the Clean Air Act (CAA); (2) hydrocarbons in gasoline vapor contribute to the formation of ozone, a criteria pollutant regulated under Section 110 of the CAA; and (3) the results of a chronic bioassay conducted in rats and mice (discussed below) indicated that exposure to wholly vaporized gasoline resulted in increased kidney (or renal) tumors in male rats and increased liver tumors in female mice.

When HEI became involved (in 1984) with the question of the potential human health effects that may result from exposure to refueling vapors, the major concern driving the issue was the third factor cited above, the possibility that the vapors inhaled during refueling (exclusive of benzene) are human carcinogens. The basic question that HEI posed was: Is the scientific database sufficient to accurately and appropriately quantify the public health impact, specifically increased cancer incidence, from exposure to gasoline vapor during self-service gasoline refueling?

## THE HEALTH EFFECTS INSTITUTE 1985 ANALYSIS

Concern that gasoline vapors may be carcinogenic arose primarily from the results of an animal carcinogen bioassay (the PS-6 study) sponsored by the American Petroleum Institute (API) (MacFarland et al., 1984). This study was designed and implemented for hazard identification, and not to define a dose-response relationship. Rats (Fischer-344) and mice ($B6C3F_1$) of both sexes were exposed to wholly vaporized unleaded gasoline for approximately two years. Exposures were for six hours a day, five days a week for the entire duration of the experiment. Four different concentrations of unleaded gasoline vapors were employed: 0 $mg/m^3$ (control), 300 $mg/m^3$ (67 ppm), 1,290 $mg/m^3$ (292 ppm), and 9,150 $mg/m^3$ (2,056 ppm). (During a typical self-service refueling operation, a patron is exposed for several minutes to between 50 and 150 $mg/m^3$ of gasoline vapor.) Animals were sacrificed for interim examination at three, six, twelve, and eighteen months, and for final analysis at the termination of the experiment.

The most important findings of this study were (1) the appearance of early (three months) and progressive renal tubular disease in exposed male rats; no such effects were seen in female rats or mice of either sex; (2) significantly increased incidence of renal tumors, including carcinomas, in male rats exposed to the middle and high exposure levels; and (3) significantly increased incidence of hepatocellular carcinomas in female mice in the high exposure group. Chronic exposure did not appear to increase mortality in either species.

The 1985 HEI analysis, which was titled "Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research," examined the scientific uncertainties surrounding the question of gasoline

vapor carcinogenicity. The report identified the following key issues that required resolution before an accurate assessment of carcinogenic risk from ambient refueling vapors could be conducted (HEI Report, 1985):

Gasoline composition: The wholly vaporized fuel used in the PS-6 study contains a greater proportion of higher molecular weight paraffin compounds believed to be responsible for hydrocarbon-induced nephropathy in male rats, than do the vapors at self-service gasoline pumps. Exposure to wholly vaporized fuels was not considered representative of ambient gaseous hydrocarbon mixtures.

Animal model: Questions were raised regarding the applicability of the animal models used in the PS-6 study (the Fischer-344 rat and the $B6C3F_1$ mouse) for direct extrapolation to humans. The male rat is unusually, if not uniquely, susceptible to the nephrotoxic effects of various hydrocarbons. The $B6C3F_1$ mouse is highly susceptible to the induction of liver tumors.

Exposure monitoring: The magnitude and extent of human exposures to gasoline vapors in occupational and non-occupational settings were not well quantified.

Epidemiology: The epidemiologic literature was, at most, weakly suggestive of increased renal cancers in workers exposed to the vapors of petroleum products. The results were statistically weak, were inconsistent across studies, and lacked acceptable estimates or measures of exposure.

The 1985 HEI report reached the following conclusion:

> The information needed for the adequate characterization of the risk to humans of ambient gasoline vapors is not available. The PS-6 study and others have shown that some components of gasoline can affect rodent species and increase cancer rates in rats and mice. In the absence of other evidence, therefore, the possible carcinogenicity of gasoline vapors to human beings cannot be dismissed. However, it is not possible to draw accurate conclusions concerning the degree of human risk (HEI Report, 1985, p. 11).

## RESEARCH ACTIVITY IN THE PAST TWO YEARS

The issues noted above have been addressed, to varying degrees, in the research conducted and/or published subsequent to 1985, but focus has been mainly on the first and second categories. Consequently most of the remainder of this report addresses progress in these two areas.

### Gasoline Composition: Liquid and Vapors

The issue of the sensitivity of animal models to gasoline vapor exposure cannot be separated from the distinction that must

be drawn between the composition of wholly vaporized gasoline and the composition of ambient gasoline vapors. This distinction is illustrated in the graphs shown in Figure 1, taken from a study conducted by Bond and co-workers (Bond et al., 1986). Gasoline vapors consist mainly of C-4 and C-5 compounds, "light-end" hydrocarbons, while the higher weight compounds are the most abundant species in liquid whole blends. In a second study, gasoline vapors collected at five distribution terminals contained 67 to 74 percent by weight C-4 and C-5 compounds, 13 percent C-6, 6 percent C-7, and 6 percent C-8+ compounds; 90 percent of the C-4 and C-5 compounds were n-butane, isobutane, n-pentane, and isopentane (Halder et al., 1986).

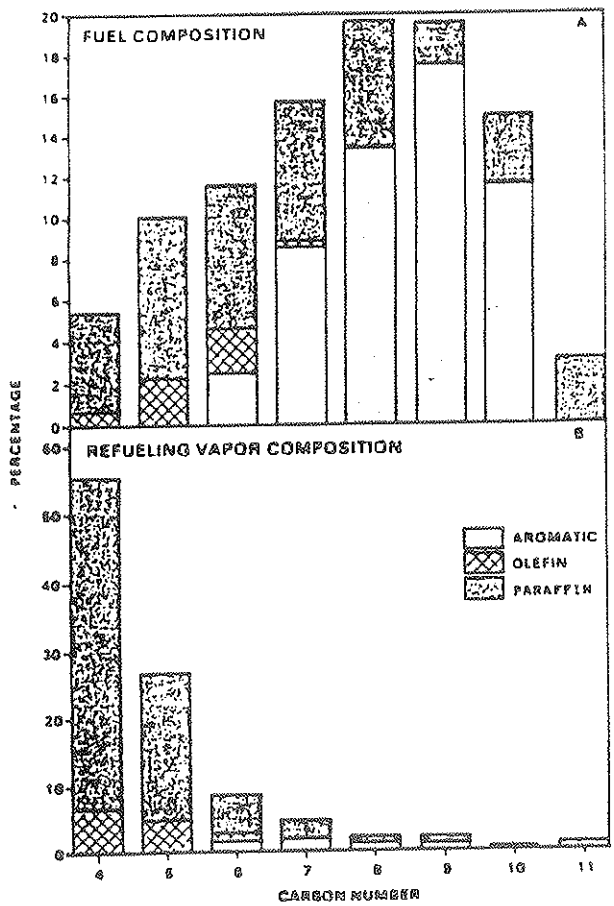



Fig. 1. Hydrocarbon composition of liquid fuel vapor at the breathing zone position.
Source: Bond et al., 1986

Dahl and co-workers (Dahl et al., 1987) characterized the respiratory uptake of the different classes of compounds found in gasoline vapor. The 19 compounds selected for this study contain

between three and nine carbons, and include hydrocarbons that are saturated, unsaturated, branched, straight chain, and cyclic. Fischer-344/N rats were exposed (nose-only) for 80-minute periods to vapor concentration that ranged from 1 to 5,000 ppm. Uptake rates were linearly related to exposure level over the dose range tested. These investigators found that: "(1) highly volatile hydrocarbons are less well absorbed than less volatile hydrocarbons; (2) unsaturated compounds are better absorbed than saturated ones; and (3) branched hydrocarbons are less well absorbed than unbranched ones." This type of information is helpful in better understanding the ultimate dose-to-target tissues for individual gasoline compounds under ambient exposure conditions.

In 1985, it was recognized that differences exist between the composition of liquid and volatilized gasoline. More recent examination of the hydrocarbon composition of ambient gasoline vapors has provided further evidence to support the position that wholly vaporized fuels are not representative of the composition of refueling gasoline vapors. This difference presents a difficulty in extrapolating from the PS-6 chronic animal experiments to humans. The hydrocarbons responsible for renal nephropathy in the rat (higher molecular weight branched alkanes) are much less abundant in ambient gasoline vapors than in the vapors of wholly volatilized gasoline (discussion to follow).

## Animal Models

### A. Rat Kidney Tumors

In the past two years, virtually all of the biological research conducted to evaluate the toxicity and potential carcinogenicity of the hydrocarbons in gasoline vapors has focused on furthering our understanding of the mechanisms of hydrocarbon nephrotoxicity in the male rat. The main purpose of this research has been to determine the extent to which male rat kidney tumor induction after exposure to wholly volatilized unleaded gasoline, found in the PS-6 study, is relevant to humans. At the time HEI issued its report, there was suggestive evidence that the renal lesions that develop in the male rat in response to hydrocarbon exposure may be unique to that species and sex, but several relevant characteristics of their development, the mechanisms of injury and repair, and their potential relationship to renal carcinogenesis had not been investigated.

The descriptive features of hydrocarbon nephropathy in the male rat have been summarized, in a review of a 1983 Workshop on the Kidney Effects of Hydrocarbons, as including the following lesions: "(1) the accumulation of hyaline droplets in the cells of the proximal convoluted tubule, (2) focal degeneration of cells throughout the proximal tubule, (3) proximal tubular regeneration, (4) granular casts and dilated tubular lumina at what appears to be the junction between the proximal tubule and

the descending thin limb, (5) degeneration of epithelial cells adjacent to the casts, and (6) mineralization of tubules in the renal medulla" (Trump et al., 1984).

In the PS-6 study, many of these lesions were observed at 90 days in the kidneys of male rats exposed to unleaded gasoline. Similar findings have been reported for male rats exposed to paraffins (Halder et al., 1984; Phillips and Cockrell, 1984; Viau et al., 1986) decalin (Alden et al., 1984; Kanerva et al., 1987a), petroleum-based and synthetic aviation fuels (MacNaughton and Uddin, 1984), military propellants (Bruner, 1984), d-limonene (Kanerva et al., 1987b), 2,2,4-trimethylpentane (TMP) (Halder et al., 1985) and 1,4-dichlorobenzene (Charbonneau et al., 1988). Some of these compounds also cause a sex-specific increase in renal adenomas and/or carcinomas in rats; others have not been tested in chronic studies. Three compounds, decalin, d-limonene, and TMP, have been used as model compounds for studying the mechanisms of renal toxicity because of their ability to produce the characteristic lesions described above.

The 1985 HEI report discussed the structure-activity relationship believed responsible for hydrocarbon-induced nephrotoxicity in the male rat (see page 25-27 of HEI Report, 1985). Briefly, branched alkane compounds with six or more carbons were suspected of being responsible for the nephrotoxicity attributable to whole fuel blends. This interpretation was based on subchronic inhalation experiments (21 days) in which rats were exposed to different fuel-blending streams; those streams enriched with alkanes conferred the greatest renal toxicity (Halder et al., 1984).

In more recent studies, gavage exposure was utilized to test the nephrotoxicity of individual compounds, distillation fractions, and blending streams (Halder et al., 1985). Rats were gavaged with either 0.5 or 2.0 mg/kg of the test substance once daily, five days per week, for four weeks. The results of these experiments provide confirmatory evidence that the renal toxicity of wholly vaporized gasoline resides almost entirely with branched alkane compounds with six or more carbons; the lighter compounds and those with no branching did not produce renal effects. No dose-effect relationship was observed for the two levels administered, suggesting possible saturation of the metabolic systems involved in the toxicity.

In addition to these gavage experiments, two other studies have been published that have evaluated the renal toxicity associated with the light hydrocarbons, i.e., those that constitute the major fraction of ambient gasoline vapors (Halder et al, 1986; Aranyi et al., 1986). Because of the extreme volatility of these light-end components, these experiments were conducted using inhalation exposure rather than gavage. Exposure of male and female Sprague-Dawley rats to a vapor mixture consisting of 25 percent each (by weight) of n-butane, n-pentane, isobutane, and isopentane, at levels ranging from 120 mg/$m^3$ (44

ppm) to 11,800 mg/m$^3$ (4,437 ppm), for six hours a day, five days a week, over a three-week period, showed no evidence of nephrotoxicity in rats of either sex (Halder et al., 1986).

Aranyi and co-workers (Aranyi et al., 1986) conducted subchronic inhalation experiments in which male and female rats were exposed to three different test atmospheres for six hours a day, five days a week, for thirteen weeks. The test atmospheres consisted of (1) 50 percent (by weight) n-butane and 50 percent n-pentane at two exposure levels: 1,000 and 4,500 ppm (equivalent to 2,660 and 11,970 mg/m$^3$, respectively); (2) 50 percent isobutane and 50 percent isopentane at the same two exposure levels used for the n-compounds; and (3) a 0 to 145$^{\circ}$ F gasoline distillation fraction at 1,200 and 5,200 ppm (equivalent to about 3,300 and 14,300 mg/m$^3$, respectively). The gasoline distillation fraction mixture is representative of the mix of compounds found in ambient gasoline vapors. No increased nephropathy was observed at the end of the 13-week exposure period in rats of either sex exposed to the three test atmospheres. No positive control group was included in the experimental design; therefore, it was not possible to compare the kidney lesion scores observed at the time of the final sacrifice with those induced by a hydrocarbon of known toxicity.

The basis for the species and sex differences in hydrocarbon nephropathy are still not fully understood. However, recent research has implied that two factors, which may be unique to the male rat, have a role in the acute renal response to hydrocarbon exposure and may be related to the production of renal adenomas and carcinomas. These factors are: (1) the accumulation of hyaline droplets and $\alpha_{2u}$-globulin[1] in the area in or surrounding the proximal tubule of the kidney, and (2) the increased renal retention of hydrocarbons or hydrocarbon metabolites.

The earliest histological lesion observed in the kidney of the male rat after exposure to unleaded gasoline or other volatile hydrocarbons is accumulation of hyaline droplets, which appear under the light microscope as abnormal secondary lysosomes within the cytoplasm of epithelial cells that line the renal proximal tubules (Alden et al., 1984; Busey and Cockrell, 1984; Phillips and Cockrell, 1984). In most species, these protein-containing hyaline droplets form in response either to increased protein reabsorption in the proximal tubule or to renal cell injury. They are found in small numbers in the kidneys of normal rats of both sexes, but they increase in number and size in male rats after exposure to hydrocarbons and other toxic compounds.

[1] Alpha$_{2u}$-globulin is the principal urinary protein of the mature male rat. The nomenclature used for this protein in the 1985 HEI report was α-2-μglobulin .

In humans and in experimental animals, protein-containing hyaline droplets form in response to a renal overload of small molecular weight proteins such as lysozyme in myelomonocytic leukemia or light chain immunoglobulins in light chain disease(Cotran et al., 1986).

Kanerva and co-workers (1987a) reported that hyaline droplet accumulation was the first morphological alteration in male Fischer-344 rats exposed to decalin, followed by the appearance of granular casts and chronic nephrosis. A number of investigators have advanced the hypothesis that the hyaline droplets that accumulate in hydrocarbon-treated male rats contain high concentrations of the male rat-specific protein $\alpha_{2u}$-globulin. This protein was recently detected by immunohistochemical procedures in the P-2 segment of the proximal tubule and was localized in some, but not all, hyaline droplets (Stonard et al., 1986b; Olson et al., 1987).

Alpha$_{2u}$-globulin, the major urinary protein in the mature male rat, is synthesized in the liver under multi-hormonal control. It has not been detected in comparable concentrations in the urine of other species, including humans, or in female rats. Normally, $\alpha_{2u}$-globulin , a low-molecular-weight protein (18,700 daltons), is freely filtered in the kidney glomerulus, and accounts for over 50 percent of the excreted urinary protein in adult male rats (Neuhaus, 1981). A large fraction of filtered $\alpha_{2u}$-globulin, however, is reabsorbed into the epithelium of the proximal tubule of the kidney and degraded within lysosomes.

In hydrocarbon-exposed male rats, the normal cycling of $\alpha_{2u}$-globulin is apparently disrupted, resulting in an accumulation of the protein. When HEI issued its 1985 report, a limited data base suggested that hydrocarbon-induced renal injury in male rats was associated with a breakdown in the renal processing of $\alpha_{2u}$-globulin after hydrocarbon exposure, and that $\alpha_{2u}$-globulin and a hydrocarbon metabolite were possibly covalently associated with one another. The leading hypothesis on the mechanism of injury at that time was as follows. In the rat liver, specific hydrocarbon metabolites form covalent bonds (Schiff base adducts) with $\alpha_{2u}$-globulin. The molecular complexes are reabsorbed into the proximal tubules in a form that the epithelial lysosomes cannot degrade. This accumulation of ingested material leads to the nephrotoxic syndrome described earlier, and, with prolonged exposure and progressive injury, may lead to renal tumorigenesis. (See pages 28-30 of HEI 1985 report.)

Investigations conducted within the past two years provide additional evidence in support of the hypothesis that $\alpha_{2u}$-globulin is a key factor in the male rat's susceptibility to hydrocarbon-induced nephropathy. The nature of the bond between hydrocarbon metabolite(s) and $\alpha_{2u}$-globulin appears to differ, however, from the covalent Schiff adduct that was initially postulated. The compound that has been used as a prototypical

hydrocarbon to study this interaction is 2,2,4-trimethylpentane (TMP), a highly toxic component of both commercial unleaded gasoline and the PS-6 fuel. TMP has been shown to induce renal lesions, including hyaline droplet formation in the male rat (MacFarland et al., 1984; Halder et al., 1985; Stonard et al., 1986a), but has not been tested for renal carcinogenicity in a chronic bioassay.

It was established in 1985 (Kloss et al., 1985), that there were sex-dependent differences in the disposition of orally administered radiolabeled TMP in Fischer-344 rats. Female rats rapidly metabolize TMP and retain ten times less label in their kidneys than their male counterparts. Autoradiographic studies showed that the label retained in the kidney of the male rats was confined to the renal cortex.

Table 1. The effect of 2,2,4-trimethylpentane on hepatic and renal $\alpha_{2u}$-globulin in male and female rats.

| | Liver | | Kidney | |
|---|---|---|---|---|
| Fraction | Control | Treated | Control | Treated |
| | | (mg $\alpha_{2u}$-globulin g protein) | | |
| Male | | | | |
| Heavy pellet | 2·0 ± 0·5 | 1·5 ± 1·0 | 9·3 ± 1·5 | 34·2 ± 8·0* |
| Microsomes | 4·6 ± 1·7 | 3·4 ± 1·1 | 2·4 ± 0·8 | 24·9 ± 6·2* |
| Cytosol | < 0·4 | < 0·4 | 30·0 ± 18·2 | 287·4 ± 41·3* |
| Female | | | | |
| Whole homogenate | < 0·4 | < 0·4 | < 0·4 | < 0·4 |

Results are mean values ± S.D. Statistical difference between control and treated *$P < 0·05$ using Students *t* test.

Source: Lock et al., 1987b

It was also known that administration of TMP to male and female rats caused a dramatic increase in the content of $\alpha_{2u}$-globulin in male (but not female) rat kidneys, but produced no change in hepatic levels of $\alpha_{2u}$-globulin (Lock et al., 1985). The data also suggested that the TMP-induced

$\alpha_{2u}$-globulin accumulation in the male rat kidney is not related to stimulation of hepatic synthesis of this protein. These results, now published, are shown in Table 1 (Lock et al 1987b). Almost all of the renal increase in $\alpha_{2u}$-globulin was associated with the cytosolic fraction of the kidney.

In a third study available for review in 1985, Alden and co-workers (1984), demonstrated that in decalin-treated rats the hyaline droplets that accumulated in the proximal convoluted tubule of exposed male rats were associated with $\alpha_{2u}$-globulin. Thus, a basis had been established for pursuing research on the metabolism and disposition of gasoline hydrocarbons, and on the associated role of $\alpha_{2u}$-globulin in male rat hydrocarbon-induced nephropathy.

A number of investigators have examined $\alpha_{2u}$-globulin accumulation after acute or subacute exposure to hydrocarbons or hydrocarbon prototypes. Alpha$_{2u}$-globulin accumulates in the kidneys of male (but not female) adult rats after treatment with TMP (Stonard et al., 1986a; Charbonneau et al., 1987a), saturated aliphatic hydrocarbons (Viau et al., 1986), 1,4-dichlorobenzene (Charbonneau et al., 1988), decalin (Kanerva et al., 1987b, 1987c), d-limonene (Kanerva et al., 1987b), and unleaded gasoline (Olson et al., 1987). As illustrated in Figure 2, the renal concentration of $\alpha_{2u}$-globulin starts to increase four to eight hours after administration of a single dose of TMP, peaks at 24-48 hours, and then slowly declines (Stonard et al., 1986a; Charbonneau et al., 1987a). After five weeks of exposure to saturated aliphatic hydrocarbons, plasma levels of $\alpha_{2u}$-globulin were elevated, but the urinary clearance and net fractional reabsorption of the protein were similar in exposed and non-exposed animals (Viau et al., 1986). Exposed rats, however, accumulated a ten-fold excess of $\alpha_{2u}$-globulin in their kidneys. Hepatic levels of $\alpha_{2u}$-globulin were not increased in TMP-treated animals, suggesting that the accumulation of the protein in the kidney results from deficient renal processing of $\alpha_{2u}$-globulin rather than increased synthesis of this protein in the liver. This hypothesis is supported by the fact that unleaded gasoline treatment had no effect on the hepatic content of $\alpha_{2u}$-globulin nor its mRNA (Olson et al., 1987). In a subsequent study, treatment with estradiol (which inhibits hepatic $\alpha_{2u}$-globulin synthesis) decreased hepatic and renal $\alpha_{2u}$-globulin content in unleaded gasoline-treated male rats by 95 percent and 50 percent respectively, compared to the levels in control animals that did not receive the hormone, suggesting that renal accumulation of $\alpha_{2u}$-globulin is dependent on the bioavailability of the compound (Garg et al., 1987).

When the disposition of $^{14}$C-labeled TMP, a branched alkane with eight carbons, was compared with $^{14}$C-labeled n-octane, a straight-chain compound with eight carbons which is not nephrotoxic in male rats, it was found that at 72 hours, the kidneys of TMP-treated rats had six times the label of those

treated with n-octane, and the kidney-to-liver ratio of label was 17.6 for TMP and 0.35 for n-octane (Peterson et al., 1986a). This study confirms the preferential renal retention of hydrocarbons that cause nephrotoxicity in male rats.

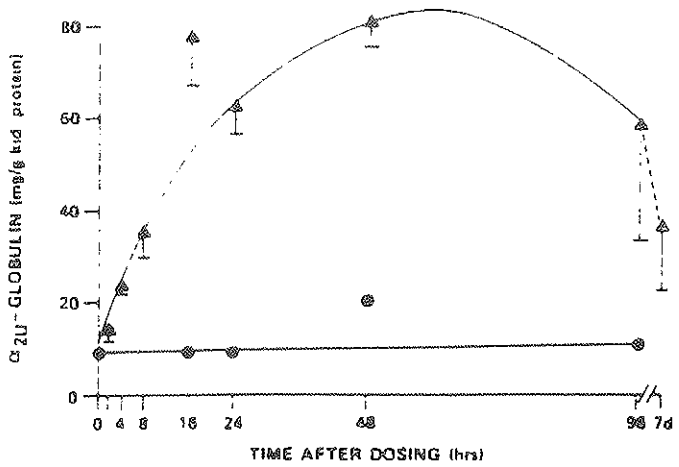



Fig. 2. Renal concentration of $\alpha_{2u}$-globulin in post-puberty male rats at different time periods after a single dose of TMP. (▲) = TMP-treated; (●) = control.

Source: Stonard et al., 1986

The association of hyaline droplet accumulation with increased renal concentrations of $\alpha_{2u}$-globulin was examined in the kidneys of adult male, adult female, and immature male rats given a single oral dose of TMP (Stonard et al., 1986a, 1986b). (Immature male rats do not synthesize $\alpha_{2u}$-globulin.) In the adult males, a dose-dependent increase in the renal concentration of $\alpha_{2u}$-globulin corresponded with the accumulation of hyaline droplets in the proximal tubules. Immunohistochemical procedures localized $\alpha_{2u}$-globulin in the P-2 segment of the renal proximal tubule. The staining intensity increased after exposure to TMP. Neither female rats nor immature males accumulated hyaline droplets or $\alpha_{2u}$-globulin in their kidneys after exposure to TMP. In a related study, castration diminished, but did not abolish, TMP-induced nephrotoxicity in the male rat (Hobson et al., 1986).

Increases in renal $\alpha_{2u}$-globulin have also been associated with hyaline droplet accumulation in male Fischer-344 rats treated with decalin (Kanerva et al., 1987c), d-limonene (Kanerva

et al., 1987b) and unleaded gasoline (Olson et al., 1987). In unleaded gasoline-treated animals, the renal concentration of $\alpha_{2u}$-globulin increased over a dose range of 0.04 to 1.0 ml/kg to a level five times higher than that observed in saline-treated controls. The $\alpha_{2u}$-globulin levels returned to control levels within three days after gasoline exposure was terminated (Garg et al., 1987). Localization of the $\alpha_{2u}$-globulin by immunohistochemical techniques showed that, while many hyaline droplets stained positively for the protein, there were also unstained droplets, indicating the presence of other proteins in the hyaline droplets (Olson et al., 1987). Other immunohistochemical studies have confirmed treatment-related increases in the levels of $\alpha_{2u}$-globulin in the renal cortex of hydrocarbon-treated rats, but the resolution of the microscopic techniques has not been adequate to detemine if the protein is confined to the droplets or what fraction of the droplets contain the protein (Kanerva et al., 1987c). Additional studies are required to establish the protein composition of hyaline droplets from normal and gasoline-exposed rats.

The second factor that has been shown to be important in hydrocarbon-induced nephropathy is the elevated retention of hydrocarbons or hydrocarbon metabolites in male rats (Kloss et al., 1985; Peterson, 1986a). Earlier studies, using TMP as a protoype compound, had indicated that TMP is metabolized primarily to trimethylpentanols, pentanoic acids, and hydroxypentanoic acids (Olson et al., 1985). Accordingly, it was logical to suggest that an aldehyde precursor of the carboxylic acid derivatives may form Schiff base complexes with $\alpha_{2u}$-globulin (Gibson and Bus, 1987).

Loury and associates (1987b) tested this hypothesis in primary hepatocyte cultures from male and female Fischer-344 rats. Cells cultured from males demonstrated the ability to produce $\alpha_{2u}$-globulin in vitro, whereas cells from females were incompetent. Analyses of hepatocyte cultures after incubation with radiolabeled TMP revealed no evidence to suggest that Schiff base complexes had been formed. In a second experiment, adult male rats were gavaged with three 300 mg/kg doses of labeled TMP one day prior to sacrifice. Analyses of liver, blood, kidney, and urine showed no signs of covalent interaction between $\alpha_{2u}$-globulin and TMP metabolites.

Recently, Lock and co-workers (1987a) presented evidence for the first time for an association between a metabolite of TMP and $\alpha_{2u}$-globulin , but the binding was reversible rather than covalent. Male and female Fischer-344 rats were treated with radiolabeled TMP and sacrificed 24 hours later. Radiolabeled material was localized in the cytosolic fraction and resolved into two peaks (on Sephadex G-75 columns), one of which contained 25 percent of the cytosol activity and co-eluted with $\alpha_{2u}$-globulin. The second peak contained activity that eluted in the low-molecular-weight range (<1,000 daltons). Cytosol from the kidneys of female rats contained only the low molecular

weight peak. Dialysis of cytosol from the males led to removal of the low-molecular-weight components and, after treatment with sodium dodecyl sulfate (SDS), loss of all of the radioactivity previously associated with $\alpha_{2u}$-globulin. (SDS is a mild detergent that destroys the secondary and tertiary structure of proteins. SDS does not break covalent bonds.)

Further analyses demonstrated that the TMP metabolite associated with $\alpha_{2u}$-globulin is 2,4,4-trimethyl-2-pentanol (2,4,4-TM-2-P) (Charbonneau et al., 1987a). It is puzzling that the radiolabeled metabolite of TMP and its associated $\alpha_{2u}$-globulin were found in the cytosol fraction, rather than in the fraction containing the lysosomes that are the postulated sites for $\alpha_{2u}$-globulin accumulation and degradation. This result may be an artifact of homogenization and extraction techniques, but it emphasizes the need for further work to localize the subcellular site(s) associated with hydrocarbon metabolism and $\alpha_{2u}$-globulin accumulation. Male and female rats metabolize TMP at comparable rates, but the females excrete more conjugates of 2,4,4-TM-2-P in urine than males, and they retain none of this metabolite in their kidneys. In contrast, 2,4,4-TM-2-P is the major metabolite found in the kidney of TMP-treated male rats (Charbonneau et al., 1987a).

Recent research has been directed toward understanding the long-term consequences of hyaline droplet formation on the kidney tubule. In an experiment designed to examine the dose-effect response for TMP and to identify the sites in the kidney where TMP-induced cell proliferation occurs, male Fischer-344 rats were gavaged with 50 to 2,000 mg/kg TMP for 21 days (Short et al., 1986). The most prominent histopathological change was the increased fraction of proximal tubules with accumulated protein (or hyaline) droplets in treated groups (50 percent) compared to control animals (25 percent). Pulse labeling with (methyl-$^3$H) thymidine [($^3$H)TdR] indicated that the greatest amount of ($^3$H)TdR incorporation occurred in epithelial cells lining the P-2 segment of the proximal tubule, the same region that exhibits hyaline droplet accumulation, degeneration, and necrosis. The labeling indices, however, were low and did not permit accurate quantification.

In a subsequent study, the same group utilized a continuous infusion technique to administer the ($^3$H)TdR, thereby increasing the amount of radiolabel incorporated into the renal tissue (Short et al., 1987). They examined cell proliferation in the kidneys of male F-344 rats after inhalation exposure to 0 to 2000 ppm wholly vaporized unleaded gasoline and 0.20 to 50.0 mg/kg TMP. After a three-week exposure to TMP, the protein droplet score and the number of hyperplastic foci increased in a dose-dependent manner. It should be noted that in a chronic study, Viau and co-workers observed regenerative foci in kidney tubules of rats exposed to hydrocarbons for 5.5 weeks, but not in animals treated for 46 or 68 weeks (Viau et al., 1986). Autoradiographic analysis demonstrated a significant increase in labeling index in

the P-2 segment of the proximal tubule after exposure to both TMP and unleaded gasoline (Figure 3), the same region where hyaline droplets accumulate (Figure 4). The authors point out a similarity in dose response to unleaded gasoline between P-2 cell replication rates in their study and the renal tumor response in the earlier PS-6 study.

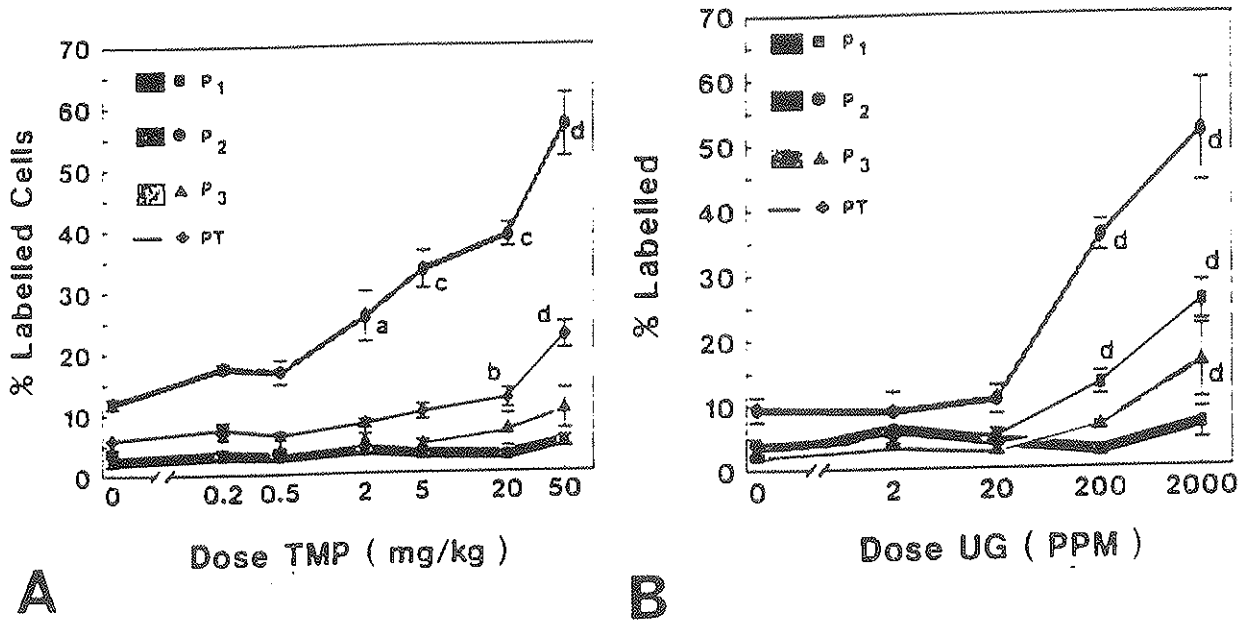



Fig. 3. TMP-induced (A) and unleaded gasoline (UG)-induced (B) cell proliferation in the P-1, P-2, and P-3 segments of the proximal tubule of the kidney.
Source: Short et al., 1987

When the animals were allowed to recover for a week after TMP exposure there were no signs of increased droplet formation or cellular proliferation in the kidney tubules (Short et al., 1987). The reversible nature of acute hydrocarbon-induced renal lesions in male rats has been observed three days after nine daily gavage treatments of Fischer-344 rats with 2.0 ml gasoline/kg (Garg et al., 1986), and a week after a single dose of TMP to Alderley Park rats (Stonard et al., 1986b).

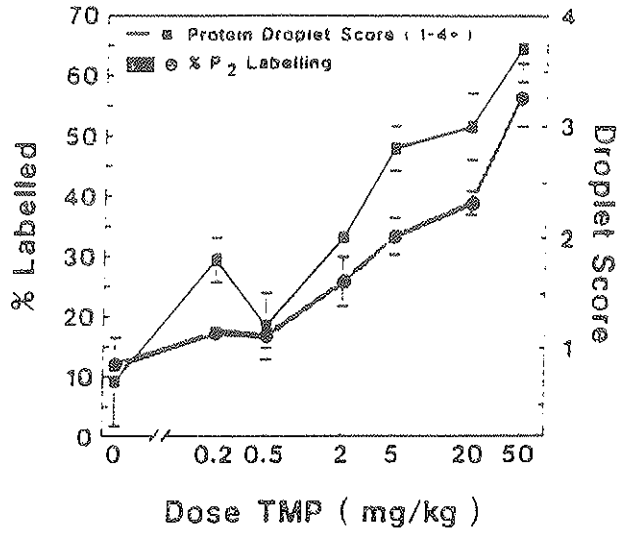



Fig. 4. Dose-responsive relationship between protein droplet score and P-2 cell proliferation in TMP-treated male rats.
Source: Short et al., 1987

The effect of the metabolites of TMP on cell proliferation in the kidney has been examined by Charbonneau and co-workers (1987b). All the major metabolites of TMP that were examined stimulated an increase in protein droplet accumulation and renal $\alpha_{2u}$-globulin concentration, but only TMP and 2,2,4-TMP acid increased the incorporation of ($^3$H)TdR into renal DNA.

Although the research discussed in this section has not resolved all of the uncertainties regarding the relevance of hydrocarbon-induced kidney tumors in male rats to human cancer, it has improved our understanding of the reasons why the male rat is particularly susceptible to the nephrotoxic effects of the branched alkane compounds found in unleaded gasoline. The research conducted in the last two years strengthens the hypothesis that unleaded gasoline (or its active components) belongs to a class of compounds that induce lesions in a specific region of the kidney (in or near the P-2 segment of the proximal tubule) that are associated with a common sequence of interrelated events. This sequence is characterized by (1) the accumulation of $\alpha_{2u}$-globulin and hyaline droplets, (2) tubular dilation and mineralization, (3) focal areas of cell proliferation, and ultimately, (4) lesions associated with chronic nephrosis. Although more research is required to understand the relationship of these events to one another and to

the carcinogenic process, it is clear at present that these $\alpha_{2u}$-globulin-induced events do not occur in female rats or in mice of either sex; nor have they been described in other species, including humans. Current knowledge does not allow us to exclude the possiblity, however, that humans have proteins, as yet unidentified, that may have the capacity to form complexes with hydrocarbon metabolites and produce toxic effects.

B. Genotoxicity

The results discussed thus far demonstrate that inhalation or gavage exposure of male rats to whole fuel blends, or to specific constituents of whole fuel, produces renal tubular injury that, among other things, is characterized by increased incorporation of ($^3$H)TdR into renal tissue. Also, with cessation of exposure, the injury to renal tissue reverses, and ($^3$H)TdR incorporation returns to background levels.

Thymidine, a pyrimidine nucleoside, is one of the building blocks of DNA. Incorporation of thymidine into DNA occurs most commonly as a cell prepares to enter mitosis. Division may occur as part of the normal process of cell turnover within tissue, or it may occur as a repair process in response to tissue injury. DNA synthesis that occurs with the aim of regenerating injured tissue is referred to as replicative DNA synthesis (RDS). Thymidine incorporation may also occur after DNA damage (e.g., strand break, adduct formation), as various repair enzymes in the cell nucleus remove the damaged section of the DNA molecule and reinstate the correct molecular sequence. This process is referred to as unscheduled DNA synthesis (UDS) because it is not linked to the cell cycle.

Given the level of hydrocarbon-induced tubular injury observed at the histologic level, there is little doubt that renal thymidine incorporation, discussed above, represents cells that are proliferating to repair that injury. When HEI issued its 1985 report, no study published up to that date had indicated that gasoline caused enhanced UDS or was genotoxic (see page 28 of HEI 1985 report).

Loury and co-workers (1987a) conducted an experiment to determine the extent to which radioactive thymidine incorporation in the exposed male rat kidney may also represent repair of damaged DNA. In other words, is there evidence for genotoxic activity in the kidney of exposed animals that may be linked with the renal carcinogenicity that follows chronic exposure to whole gasoline vapors? To address this question, an in vivo/in vitro system was designed to quantify UDS and RDS in kidney cells. Male and female Fischer-344 rats were administered whole unleaded gasoline by either inhalation or gavage. At various times after exposure the animals were sacrificed, and the kidney cells were cultured in the presence of ($^3$H)TdR for subsequent autoradiographic analysis. Several other chemicals (e.g., dimethylnitrosamine, N-nitrosomorpholine) with known genotoxic

properties were used as positive controls. Under no condition did exposure to unleaded gasoline at concentrations of up to 2000 ppm stimulate UDS. In contrast, both gavage and inhalation of unleaded gasoline were effective in stimulating RDS in cells from male rats. No effects of unleaded gasoline on RDS were observed in cells from female rats. No evidence of UDS was observed in primary cultures of male Fischer-344 rat kidney cells exposed to unleaded gasoline in agreement with the absence of a direct genotoxic effect of unleaded gasoline on kidney cells in the in vivo assay.

Additional studies have been conducted to determine if either unleaded gasoline or TMP produces gene locus mutations or sister chromatid exchange in the TK6 human lymphoblastoid cell line (Richardson et al., 1986). Assays were carried out both in the presence and absence of S9 activation. Unleaded gasoline was administered either in saturated medium to maximize exposure to water soluble compounds, or as vapor to maximize exposure to volatile components. Under neither condition did unleaded gasoline or TMP induce mutations or sister chromatid exchange in TK6 cells.

In contrast to the results in kidney cells, unleaded gasoline and its metabolites have been reported to induce UDS and RDS in liver cells (Loury et al., 1986). Unleaded gasoline produced a dose-related increase in UDS in cultured male rat hepatocytes treated in vitro while TMP had no effect (Table 2). Positive effects on in vitro UDS were also noted for liver cells from male mice and from a human. In the in vivo/in vitro system, both male and female $B6C3F_1$ mice displayed a small increase in hepatic UDS following gavage with unleaded gasoline but only males showed an increased RDS response to unleaded gasoline. In mice, both sexes showed a positive RDS response to TMP. In male rats, neither UG nor TMP increased UDS in the in vivo/in vitro assay, but TMP did cause a significant increase in RDS. These results differ from the predominantly negative results reported for genotoxic assays of unleaded gasoline in the kidney, and are inconsistent with the results of the PS-6 study where unleaded gasoline did not produce tumors in rat liver. The negative UDS results in rat liver are consistent with the liver tumor results of the UG inhalation study, but the UDS and RDS hepatic responses in mice do not correspond to the finding of increased liver tumors in female mice.

Table 2. Summary of results of Loury et al., (1986) on Unscheduled DNA Synthesis (UDS) in cultured hepatocytes, and UDS and Replicative DNA Synthesis (RDS) in the in vivo/in vitro assay.

| | | UG | TMP |
|---|---|---|---|
| In vitro UDS | | | |
| Rat | M | +(dose-related) | 0 |
| | F | NT | NT |
| Mouse | M | + | NT |
| | F | NT | NT |
| Human (no sex given) | | - | NT |
| In vivo/in vitro UDS | | | |
| Rat | M | 0 | 0 |
| | F | NT | NT |
| Mouse | M | +(small but sig) | NT |
| | F | +(small but sig) | NT |
| In vivo/in vitro RDS | | | |
| Rat | M | +(not sig) | ++ |
| | F | NT | NT |
| Mouse | M | + | ++ |
| | F | 0 | + |

++ = strong effect; - = moderate effect; 0 = no effect; NT = not tested

Source: Loury et al., 1986

C. Mouse Liver Tumors

Most of the biological research on gasoline vapors reported in the past two years has focused on the nephrotoxicity of gasoline. In the PS-6 study, however, female mice in the high-dose group (2,056 ppm or 9,150 $mg/m^3$) had a significantly increased incidence of liver tumors. In contrast to the attention given to the rat findings, little research has been directed toward better understanding the response of mouse liver to hydrocarbons, nor has progress been made in identifying the active compounds or fractions of unleaded gasoline associated with liver tumor production in female $B6C3F_1$ mice.

The 1985 HEI report acknowledged that the relevance to humans of increased liver tumors in mice is not clear. The uncertainties surrounding the extrapolation of mouse liver tumors to humans, and their implication for carcinogenesis risk assessment, have long been a subject of scientific controversy that has not come closer to resolution in the last two years (USISGC, 1986; Maronpot et al., 1987). The $B6C3F_1$ strain of mouse used in the PS-6 study develops liver tumors spontaneously, and exhibits a relatively high frequency of hepatic tumors following treatment with many compounds, some of which are negative in bacterial mutagenicity assays (Pereira, 1985). According to current thinking, non-genotoxic chemicals may stimulate the growth of, or confer a growth and survival advantage on precursor (i.e., initiated) cells in the liver, and thus could serve as tumor promoters (Pereira, 1985; Farber and Sarma, 1987). Our current knowledge does not allow a distinction to be made among the chemicals that induce mouse liver tumors on a mechanistic basis.

The application of molecular biology techniques to the analysis of mouse liver tumors has shown promise for increasing our understanding of the neoplastic process. Recently, activated oncogenes have been detected in spontaneous (Fox and Watanabe, 1985; Reynolds et al., 1987) and chemically-induced (Wiseman et al., 1986) liver tumors. Reynolds and co-workers (Reynolds et al., 1987) described a strategy for comparing patterns of oncogene activation in chemically-induced and spontaneous mouse liver tumors. Oncogene expression was evaluated in $B6C3F_1$ mice treated with furan and furfural, both of which are mouse liver carcinogens in two-year bioassays, but neither of which are mutagenic in the Ames Salmonella assay. All of the oncogenes found in spontaneous tumors from control mice were H-ras. The liver tumors from furan- and furfural-treated mice were heavily represented by H-ras, but also contained K-ras and -raf, as well as non-ras transforming genes of unknown identity. Even within the H-ras gene, carcinogen-treated animals exhibited point mutations at specific locations that contained no detectable mutations in the vehicle-treated controls.

The authors argue that finding a different spectrum of activated oncogenes in chemically-induced tumors, compared to those present in spontaneous tumors, suggests that the carcinogen activated the proto-oncogene by a genotoxic mechanism, whereas if similar oncogenes had been detected, the chemical may have acted as a promoter, possibly via a cytotoxic mechanism. While these findings are of interest in understanding the mechanism of tumor induction, the assays are still in the developmental stages and the interpretation of these results in relation to risk assessment is uncertain. Integration of information obtained at the molecular level with long-term bioassay data should improve our understanding of the mechanism of unleaded gasoline and other chemically-induced liver tumors in $B6C3F_1$ mice.

## Exposure Monitoring

In its 1985 report, HEI noted a paucity of information regarding gasoline vapor exposure assessment. In the past two years, several studies have been conducted to characterize the hydrocarbon content of the breathing zone during refueling at a service station (Bond et al., 1986; Tironi et al., 1986; Kearney and Dunham, 1986; Halder et al., 1986), and in other environments where petroleum vapors are present (Halder et al., 1986). Additional studies have been undertaken to understand the many factors that can affect the concentration and composition of ambient gas vapors (EPA, 1985; Braddock et al., 1986; Furey and Nagel, 1986). These factors include temperature, the temperature difference between dispensed fuel and fuel already in the tank, fuel volatility, and the age of the fuel in the tank.

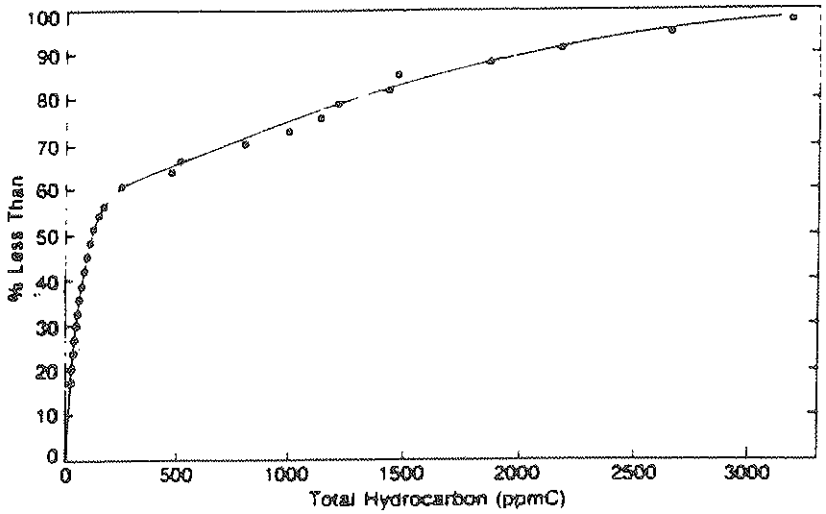



Fig. 5. Cumulative distribution of total hydrocarbon concentrations from combined winter and summer studies.
Source: Tironi et al., 1986

Given the potential for large inter-study variability attributable to fuel and vehicle factors, as well as to local meteorological conditions such as temperature and winds, the available data on the average level and composition of refueling vapors are surprisingly consistent across studies. This may be attributable in part to the fact that certain sources of variability counterbalance each other. For example, in summer, fuels have lower volatility ratings than in winter, which offsets the effects that seasonal changes in temperature would have on evaporative emissions during refueling.

Table 3. Adjustment of reported exposures to tank filling-time only.

| Source | Sampling Situation | No. Samples | Sampling Time | Reported Exposure | Actual Filling Time | Other Adjustment | mg/m³ Adjusted Exposure Range | mg/m³ Geom. Mean |
|---|---|---|---|---|---|---|---|---|
| Kearney & Dunham (11) | Self service | 12 | 10 min | 3.9-63.5 mg/m³ | 1.25 min | None | 31.2-508 | 102 |
| | Full service attendant | 8 | 400 min | 1.9-14.3 mg/m³ | 21.7 min | | 35.0-265 | 85 |
| Balder, et al. (10) | Service plaza attendant | 21 | 432 min | 1.1-130.3 mg/m³ | 27.0 min | Diffusion badge x 1/2 $C_6$° reported x 4 | 35.0-4170 | 128 |
| | Self service simulation | 1 | 27 min | 24.3 mg/m³ | 3.1 min | Diffusion badge x 1/2 | --- | 106 |
| | | 1 | 354 min | 6.5 mg/m³ | 22.0 min | Diffusion badge x 1/2 | --- | 52 |
| Bond, et al. (13) | Single car with sampling rack | 4 parallel | 1.5 min | 84.0-157 ppmC | 1.5 min | Units x 0.56 | 47-88 | 59 |
| | | 4 perpendicular | 1.5 min | 0.0-18 ppmC | 1.5 min | Units x 0.56 | 0-10 | |
| Tironi, et al. (4) | Single car (winter) | 15 | 0.5-2.0 min | 4.0-3210 ppmC | 0.5-2.0 min | Units x 0.56 | 2.2-1750 | 148 |
| | Single car (summer) | 18 | 0.5-2.0 min | 5.0-1220 ppmC | 0.5-2.0 min | Units x 0.56 | 2.8-680 | 47 |
| Clayton Environ. (12) | 2-7 cars in self-service | 288 | 8-10 min | 2.4-690 ppm$C_6$ | 8-10 min | Units x 3.6 | 8.7-2500 | 140 |
| McDermott & Vos (14) | Attendant filling only | 6 | 15 min | 12.1-99.2 ppm gasoline | 15 min | Units x 2.75 | 33.2-273 | 112 |
| | Full service attendant during busy period | 2 | 15 min | 1.81, 2.31 ppm gasoline | 1.5 min | Units x 2.75 | 50 64 | 56 |
| | Full service attendant | 84 | 480 min | 0.42-40.4 ppm gasoline | 26 min | Units x 2.75 | 21.0-2050 | not available |

Source: Williams et al., 1987

Williams (1987) has summarized the data from the literature concerned with gasoline vapor concentrations during refueling (Table 3). The usefulness of Williams' analysis is that the data from all the available studies were adjusted to reflect the exposure level only during filling, and the results have been converted to a common unit of exposure level ($mg/m^3$ total hydrocarbon). For all studies, the geometric mean exposure during refueling ranges from 47 $mg/m^3$ to 148 $mg/m^3$, both extremes reported by Tironi et al. (1986). Despite this consistency in the average exposure levels across studies, the data indicate that, within any specific study, the vapor concentrations during refueling cover a wide range. When assessing ambient concentrations of any substance for the purpose of a health evaluation, the high-end tail of the concentration distribution curve is important to consider, as well as the measure of central tendency.

The distribution of total hydrocarbons in refueling vapors collected in summer and winter in the Detroit, Michigan, area is shown in Figure 5 (Tironi et al., 1986). Though the median level appears to be about 100 ppmC (equivalent to 56 $mg/m^3$), the extremes ranged to 20 to 30 times this value. Much of this variability was due to wind conditions, such as eddy currents in the immediate vicinity of the pump operator. Acknowledging the importance of characterizing the high-end tail of the distribution, the authors listed the 90th percentile exposure concentrations for selected compounds (Table 4). The compounds in the table account for 88 percent and 84 percent of the total carbon found in winter and summer vapors, respectively. The table indicates that, although total hydrocarbon content is fairly stable across seasons (about 400 ppmV for the compounds listed at the 90th percentile), summer vapors are more heavily weighted with the higher molecular weight compounds.

Table 4. 90th percentile concentration of selected hydrocarbons in refueling vapors

| Component | Winter (ppmV) | Summer (ppmV) |
|---|---|---|
| n-Butane | 253 | 163 |
| i-Pentane | 57 | 72 |
| i-Butane | 40 | 47 |
| n-Pentane | 20 | 26 |
| Propane | 25 | 40 |
| trans-2-Butene | 5.9 | 9.7 |
| 2-Methyl-2-butene | 5.8 | 8.4 |
| 2-Methylpentane | 5.7 | 8.6 |
| 2-Methylhexane | 0.91 | 2.4 |
| 2,2,4-Trimethylpentane | 0.47 | 1.1 |
| Benzene | 2.1 | 3.7 |
| Toluene | 1.3 | 3.5 |

Source: Tironi et al., 1986

Table 5. Hydrocarbon composition of refueling vapor and dispensed gasoline

| Hydrocarbon | Mass (Carbon) Percent of Total HC | | | | | |
|---|---|---|---|---|---|---|
| | Winter Study | | | Summer Study | | |
| | Vapor[a] | Gasoline | V/L[c] | Vapor[b] | Gasoline | V/L[c] |
| Propane | 4.12 | 0.14 | 28.6 | 6.32 | 0.09 | 70.2 |
| Isobutane | 7.76 | 0.69 | 11.2 | 9.83 | 0.75 | 13.1 |
| Isobutylene + 1-Butene | 1.35 | 0.14 | 9.78 | 0.98 | 0.27 | 3.63 |
| n-Butane | 51.19 | 8.27 | 6.19 | 30.95 | 4.06 | 7.62 |
| trans-2-Butene | 1.29 | 0.28 | 4.67 | 2.02 | 0.45 | 4.49 |
| cis-2-Butene | 1.30 | 0.29 | 4.55 | 2.07 | 0.42 | 4.93 |
| 3-Methyl-1-butene | 0.16 | 0.12 | 1.28 | 0.56 | 0.17 | 3.29 |
| Isopentane | 14.26 | 7.31 | 1.95 | 18.45 | 7.55 | 2.44 |
| 1-Pentene | 0.71 | 0.51 | 1.38 | 1.05 | 0.55 | 1.91 |
| 2-Methyl-1-butene | 1.05 | 0.76 | 1.38 | 1.69 | 0.94 | 1.80 |
| n-Pentane | 4.90 | 4.51 | 1.09 | 6.40 | 3.41 | 1.88 |
| trans-2-Pentene | 1.27 | 1.22 | 1.04 | 1.89 | 1.24 | 1.52 |
| cis-2-Pentene | 0.63 | 0.66 | 0.95 | 0.90 | 0.69 | 1.30 |
| 2-Methyl-2-butene | 1.48 | 1.74 | 0.85 | 2.44 | 1.75 | 1.39 |
| 2,2-Dimethylbutane | 0.19 | 0.32 | 0.60 | 0.61 | 0.50 | 1.22 |
| Cyclopentene | | | | 0.31 | 0.28 | 1.11 |
| 4-Methyl-1-pentene | | | | | 0.20 | |
| Cyclopentane | 0.27 | 0.45 | 0.60 | 0.26 | 0.35 | 0.74 |
| 2,3-Dimethylbutane | 0.63 | 1.16 | 0.55 | 0.74 | 1.03 | 0.72 |
| $C_6$ Olefin | | | | | 0.12 | |
| 2-Methylpentane | 1.77 | 3.83 | 0.46 | 2.53 | 3.38 | 0.75 |
| $C_6$ Olefin | | | | 0.21 | 0.21 | 1.00 |
| 3-Methylpentane | 1.00 | 2.86 | 0.35 | 1.46 | 2.25 | 0.65 |
| $C_6$ Olefins | 0.17 | 0.47 | 0.37 | 0.33 | 0.62 | 0.53 |
| n-Hexane | 0.82 | 3.20 | 0.26 | 1.07 | 2.11 | 0.51 |
| $C_6$ Olefins | | | | 1.34 | 2.88 | 0.47 |
| Methylcyclopentane | 0.51 | 1.99 | 0.26 | 0.64 | 1.48 | 0.43 |
| 2,2-Dimethylpentane + 2,4-Dimethylpentane | | | | 0.31 | 0.90 | 0.34 |
| Benzene | 0.66 | 3.16 | 0.21 | 1.07 | 3.18 | 0.34 |
| 1,3-Dimethylpentane | | | | | 0.21 | |
| Cyclohexane | 0.10 | 0.70 | 0.15 | | 0.12 | |
| 2,3-Dimethylpentane | | | | | 0.15 | |
| 2-Methylhexane | 0.37 | 2.74 | 0.13 | 0.77 | 3.46 | 0.21 |
| Cyclohexene | | | | | 0.09 | |
| 3-Methylhexane | 0.18 | 1.93 | 0.10 | 0.31 | 1.57 | 0.20 |
| $C_7$ Olefin | | | | | 0.39 | |
| 3-Ethylpentane | | | | | 0.22 | |
| 2,2,4-Trimethylpentane | 0.20 | 1.98 | 0.10 | 0.28 | 1.57 | 0.16 |
| n-Heptane | 0.13 | 1.66 | 0.078 | 0.25 | 1.01 | 0.25 |
| $C_7$ Olefins | | | | | 0.94 | |
| Methylcyclohexane | 0.07 | 1.17 | 0.056 | 0.16 | 0.66 | 0.24 |
| 2,5-Dimethylhexane | | | | | 0.34 | |
| 2,4-Dimethylhexane | | | | | 0.33 | |
| 2,3,4-Trimethylpentane | | | | 0.11 | 1.06 | 0.10 |
| Toluene | 0.40 | 7.82 | 0.052 | 1.23 | 12.93 | 0.095 |
| $C_8$ Paraffins | | | | | 0.46 | |
| $C_8$ Paraffins | | | | | 1.02 | |
| 3-Methylheptane | | | | | 0.81 | |
| 2,2,5-Trimethylhexane | | | | | 0.29 | |
| n-Octane | | | | | 0.52 | |
| Ethylbenzene | 0.01 | 1.13 | 0.008 | 0.08 | 1.36 | 0.059 |
| p-Xylene | | | | | 2.70 | |
| m-Xylene | 0.02 | 3.03 | 0.007 | 0.16 | 1.03 | 0.043 |
| o-Xylene | 0.01 | 1.44 | 0.006 | | 1.56 | |
| Total Percent | 98.98 | 67.68 | | 99.78 | 76.63 | |

[a] Winter sample # 7
[b] Summer sample # 3
[c] Calculated before rounding

Source: Tironi et al., 1986

The vapor-to-liquid (V/L) concentration ratio for a wide range of compounds in gasoline was also calculated (Tironi et al., 1986). This analysis (Table 5) demonstrates, again, the extent to which wholly vaporized gasoline (used in the PS-6 study) contains a significantly higher portion of high molecular weight compounds (i.e., those with suspect toxicity) than are found in ambient gasoline vapors. Figure 1, from Bond et al. (1986) (discussed earlier in this document) reaffirms this point.

Although exposure during refueling is subject to considerable variability, there appears to be sufficient data to estimate population exposures to gasoline vapors during refueling. For example, consider an individual who refuels his or her vehicle twice a week for five minutes per fill, and is exposed to gasoline vapors at a level of approximately 400 ppm (the 90th percentile exposure level stated above) which, according to Williams' conversion factors, would be equivalent to 1,100 $mg/m^3$. For an entire week, average exposure to refueling gasoline vapor would thus be about 1 $mg/m^3$; the composition of this exposure would be similar to that in the "Vapor" column of Table 4. For reference, the average weekly exposure levels in the PS-6 study were 54, 230, and 1,630 $mg/m^3$, with a composition similar to that in the "Gasoline" column of Table 4.

The above considerations apply to exposure due to vapor generated during the refueling process, primarily as a result of vapor displacement from the tank being filled. The exposure level and composition of ambient gasoline vapor would be affected if, for example, fuel spilled and evaporated during refueling. In such a case, the total vapor level would increase, and the increment would probably contain a higher percentage of the heavy compounds than the displaced vapor contains.

Epidemiology

The 1985 HEI report concluded that the epidemiologic literature offered only weakly suggestive evidence of a correlation between exposure to petroleum vapors and an increased incidence of kidney cancer in occupationally-exposed groups (see pp. 33-35 of HEI 1985 Report).

In a recent literature survey, Harrington (Harrington, 1987) reviewed 22 cohort studies and 19 case-control studies of the health experience of workers in the petroleum manufacturing and distribution industry that were published between 1972 and 1986. He observed a wide variability among studies in population size, observation time, person-years at risk, and latency induction time. When Harrington examined mortality due to malignant diseases of all types, he noted that most studies reported a small but insignificant decrease in the standardized mortality ratio (SMR). When specific cancer sites were examined, statistically significant excesses were found for certain sites, but in general these excesses were based on small sample sizes or

were isolated observations. An abbreviated summary of the epidemiological studies published since 1985, as reviewed by Harrington, is presented in Table 6. Some of the papers listed under "cohort studies" are updated analyses of populations described earlier. The SMR for kidney cancer was elevated for exposed workers in two studies, decreased in four studies and unchanged in three studies. In no case was the result statistically significant. Taken collectively, the recently published epidemiological studies do not provide support for an association between exposure to petroleum hydrocarbons and renal cancer.

An additional study, not reviewed by Harrington, examined renal cell carcinoma in relation to employment in petroleum-related industries (McLaughlin et al., 1985). This study was based on data collected in a case-control study of renal carcinoma in the Minneapolis-St. Paul area (McLaughlin et al., 1984), a study that reported a slight (OR=1.4, adjusted for age and smoking), but insignificant, increase in renal carcinomas in petroleum workers. These data were based on a total study population of 495 cases and 697 controls. The follow-up analysis by McLaughlin et al. (1985) considered only the male portion of this population because few women work in petroleum-related industries; as a result, 313 cases and 428 controls were included. The study produced no evidence of a positive association of kidney cancer with any length of employment in petroleum-related occupations. For gas station attendants, kidney cancer was increased slightly as a function of employment duration; however, the data were not significant.

A recent proportionate mortality ratio (PMR) analysis of autombile mechanics and gasoline service station workers in New Hampshire was reported by Schwartz (1987). The analysis revealed a significant association between workplace exposure and mortality from suicide (automobile mechanics PMR=177; $P<0.05$) and leukemia (PMR=328; $P<0.05$). Elevations were seen for other types of neoplasms, but the increases were not significant.

Two major efforts in this area currently underway are being sponsored by API, and are still in progress. One is a case-control study of petroleum company employees, the majority of whom are refinery workers, and the second is a cohort study of "downstream" workers. The term "downstream" means downstream of the refinery, and refers to terminal and petroleum distribution workers. The case-control study is being conducted by Epidemiology Resources Inc. of Chestnut Hill, MA, and the cohort study is being conducted by Environmental Health Associates of Oakland, CA. No data are yet available from either of these studies.

Table 6.

Recent epidemiologic studies of the effects of gasoline exposure on malignant disease

Cohort Studies

| Reference | Population | Number of Deaths | Observation Periods | All Cancer | Kidney | Significant Effects |
|---|---|---|---|---|---|---|
| Divine and Barron, 1986 | 18,798 | 1,771 | 1947-1977 | 0.74 | 0.83 | None |
| | | 2,358 | 1947-1977 | 0.86 | 1.29 | None |
| Kaplan, 1986 | 19,991 | 3,349 | 1930-1980 | 0.87 | 0.68 | None |
| Wen et al., 1986 | 4,080 | 2,213 | 1937-1978 | 0.99 | 0.94 | None |
| | 3,330 | 980 | 1937-1978 | 0.97 | 0.82 | None |
| | 5,117 | 249 | 1937-1978 | 0.75 | 2.51 | None |
| Wong et al., 1986 | 14,179 | 2,292 | 1950-1980 | 0.76 | 0.88 | Non-Hodgkins lymphoma (SMR=1.27) |
| McGraw et al., 1985 | 3,976 | 640 | 1973-1983 | 0.41 | - | Leukemia (SMR=2.13) |
| Nelson, 1985 | 9,137 | 921 | 1970-1982 | 0.84 | 0.99 | Skin/melanoma (SMR=2.01) |
| Divine et al., 1985 | 19,077 | 4,028 | 1947-1947 | 0.75 | 0.96 | None |

Case Control Studies

| Reference | Cases | Population Controls | Observation |
|---|---|---|---|
| Austin et al., 1986 | 14 | 50 Refinery workers | No significant effects of job or benzene exposure on RR[a] of leukemia |
| Thomas et al., 1986 | 718 | Other causes | OR[a] brain cancer = 1.3 (N.S.)[a] |
| Baxter and McDowall, 1986 | 1,080 | All causes | RR bladder cancer = 1.7 drivers; 1.9 lorry/van (N.S.) |
| Dominiano et al., 1985 | 92 | 1,588 Hospital, non-cancer | RR renal cancer = 0.59 (N.S.) |
| McLaughlin et al., 1985 | 506 | 714 Population base | OR renal cancer = 1.0 |

a. Abbreviations: SMR = standardized mortality ratio; RR = relative risk; OR = odds ratio; N.S. = not significant

Adapted from Harrington, 1987

Other Relevant Issues: Benzene

Between 1 and 3 percent (by weight) of typical commercial gasoline is benzene, and benzene may account for up to 1 percent of the mass of ambient gasoline vapors (Tironi et al., 1986; Furey and Nagel, 1986). The 1985 HEI report considered whether benzene was partially responsible for the tumorigenesis that occurred in the PS-6 study, and reached the conclusion that it probably was not: "If benzene was partially responsible for tumorigenesis in the PS-6 study, one would expect involvement beyond the female mouse liver and especially beyond the male rat kidney, which is an atypical site for a benzene effect " (HEI Report, 1985, p.23).

Benzene, however, is a human leukemogen, and has been implicated as a human carcinogen in the findings of several recent epidemiological studies (McGraw et al., 1985; Rinsky et al., 1987; Schwartz, 1987). Rinsky and associates (1987) included retrospective exposure assessments in their study of rubber workers, and estimated that leukemia risks from 40 years of occupational exposure 40 hours a week to 0.1 ppm (about 0.32 $mg/m^3$) of benzene would be essentially no different than background. For an entire week, this 40-hour exposure level averages to about 0.08 $mg/m^3$. The individual in the refueling example above was exposed for a total of 10 minutes per week to the 90th percentile level of 1,100 $mg/m^3$ of gasoline vapor. If 1 percent of the vapor is benzene, the average weekly exposure of this individual to benzene is 0.01 $mg/m^3$, eight times lower than that of the worker exposed to 0.1 ppm during the entire work week. Thus, if (1) the overall average exposure is an appropriate exposure estimate for benzene, (2) the risk estimates of Rinsky et al. are valid, and (3) benzene is not biologically interactive with other gasoline hydrocarbons, then the increased risk from exposure of the general public to benzene during refueling is likely to be very small, and not detectable by epidemiologic means.

## EPA 1987 EVALUATION OF THE CARCINOGENICITY OF UNLEADED GASOLINE

Since the publication of HEI's 1985 report, the EPA has completed its evaluation of the carcinogenicity of unleaded gasoline (EPA, 1987). The EPA reached the following conclusions: "(1) Although employment in the petroleum refineries is possibly associated with cancers of the stomach, respiratory system, and lymphopoietic and hematopoietic tissues, exposure to gasoline cannot be implicated as a causative agent because of confounding exposure to other chemicals and inadequate information on gasoline exposure; (2) the occurrence of liver cancer in female mice and kidney cancer in male rats provides 'sufficient' evidence in animals that inhalation of wholly aerosolized gasoline is carcinogenic; and (3) gasoline vapors from vehicle refueling might be less carcinogenic than indicated

by animal experiments using wholly aerosolized gasoline, if the less volatile components, which are apparently responsible for acute kidney toxicity, also contribute to the observed carcinogenic response" (EPA, 1987).

## SUMMARY AND CONCLUSION

This paper has reviewed recent developments in research directed toward evaluating the potential carcinogenicity of gasoline vapors. Gasoline vapors were identified as potentially hazardous to humans on the basis of a long-term carcinogenesis study in rodents conducted by the API, designated as the PS-6 study (MacFarland et al., 1984). In 1985, the HEI published an analysis of the adequacy of the scientific data base for quantifying cancer risk from exposure to gasoline vapors (HEI, 1985). This document describes the research findings published subsequent to 1985 that are relevant to the health hazards of unleaded gasoline vapors.

In the PS-6 study, lifetime exposure to wholly volatilized gasoline caused renal tumors in male rats (Fischer-344) and liver tumors in female mice ($B6C3F_1$). Interpretation of the results was complicated by uncertainties regarding the utility of these animal models, particularly the male rat, and the representativeness of wholly volatilized gasoline for establishing quantitative exposure-response relationships for humans exposed to ambient gasoline vapors.

The research conducted since 1985 has reduced some of the uncertainty surrounding these issues and lends additional support to the cautious approach adopted at that time: that the information is not available to draw accurate conclusions concerning the degree of human risk that results from exposure to gasoline vapors.

Gasoline Composition: Liquid and Vapors. In the PS-6 study, animals were exposed to gasoline that was entirely vaporized. There were differences, therefore, between the vapor composition to which the animals were exposed and that found in ambient situations. A recent comparison of the hydrocarbon composition of unleaded gasoline liquid with that of the vapors indicates that the vapors consist mainly of C-4 and C-5 compounds, while the liquid phase contains the higher molecular weight hydrocarbons. Analysis of gasoline vapors in a workplace environment confirmed the relatively high proportion (67-74 percent) of C-4 and C-5 compounds in typical vapors. Since the nephrotoxicity of gasoline appears to reside in higher molecular weight hydrocarbons, this difference presents a difficulty in extrapolating from the results of the PS-6 study to humans.

Animal models. In 1985, the HEI noted that the major difficulty with direct species extrapolation from the kidney tumor data in the PS-6 study lay in the male rat's high susceptibility to hydrocarbon-induced renal toxicity. A number of investigators

had suggested that the toxic mechanisms involved $\alpha_{2u}$-globulin, a major urinary protein of the male rat, which had not been detected in comparable levels in female rats or other species including humans. Accumulation of $\alpha_{2u}$-globulin was postulated to initiate a sequence of events in male rats that resulted in renal tubular damage; this in turn might be related to the subsequent development of tumors. It was speculated that if kidney tumors were found to develop by a mechanism unique to the male rat, then the relevance to humans of the PS-6 study would be diminished.

The biological research reported since HEI issued its report has focused almost exclusively on elucidating the nature of hydrocarbon injury in the male rat, and the applicability of this animal model to assessing the risks of human exposure to gasoline vapors.

The chemical component(s) of wholly volatilized gasoline responsible for tumor induction in the PS-6 study have not yet been identified. Short-term studies have shown that branched alkane compounds with six or more carbons are responsible for the acute renal toxicity observed in male rats. C-4 and C-5 compounds, which are the most abundant species in ambient gasoline vapors, did not produce kidney lesions in short-term toxicity studies. In contrast to the progress that has been made in characterizing the components of unleaded gasoline responsible for renal toxicity, no progress has been made in identifying the active compounds or fractions associated with liver tumor production in female mice.

The mechanistic basis for the species and sex differences in hydrocarbon-induced nephropathy has not been fully elucidated. Recent research however has indicated that two factors, unique to the male rat, have a role in the acute renal response to hydrocarbon exposure and may be related to the production of kidney tumors. These factors are (1) the accumulation of hyaline droplets and $\alpha_{2u}$-globulin in or surrounding the proximal tubule of the kidney, and (2) the increased renal retention of hydrocarbons or hydrocarbon metabolites.

The research conducted in the last two years strengthens the hypothesis that gasoline vapors belong to a class of compounds that induces characteristic lesions in or near the P-2 segment of the proximal tubule of the male rat kidney associated with a common pathologic sequence that includes (1) the accumulation of $\alpha_{2u}$-globulin and hyaline droplets, (2) tubular dilation and mineralization, (3) focal areas of cell proliferation, and ultimately, (4) chronic nephrosis. Long-term studies are required to determine the relationship of these acute toxic responses to the carcinogenic process. It is clear, however, that these events do not occur in the female rat of the strains examined thus far nor in other species of laboratory animals studied to date, nor have they been observed in humans. The possibility cannot be excluded that humans have proteins, as yet

unidentified, that respond to hydrocarbon exposure in a manner similar to $\alpha_{2u}$-globulin and produce toxic effects.

Recent investigations of the mechanism of kidney tumor formation support the suggestion that chemically-induced kidney tumors in rodents fall into two categories: tumors that form in the male rat in or near the P-2 segment of the proximal tubule associated with the pathologic sequelae previously described, and tumors that appear in other regions of the kidney not associated with $\alpha_{2u}$-globulin or hyaline droplets and not specific to male rats. The two types of renal tumor may differ in their relevance for human risk assessment.

Exposure monitoring. Efforts to characterize the concentration and composition of refueling vapors have continued. The data collected show that, while exposures during refueling are usually less than 150 $mg/m^3$, they can range higher by an order of magnitude or more. The compositional analyses, however, indicate that the high molecular weight compounds that are responsible for acute toxic effects in the rat are present in ambient vapors in very low quantities. It should also be kept in mind that typical human exposures during fueling of private vehicles occur for only brief periods of time.

Epidemiology. Recently published epidemiological studies provide no evidence suggesting a link between occupational exposure to petroleum products and kidney cancer. Two major studies are currently underway and are expected to provide additional data on petroleum workers in refineries and terminals as well as petroleum distribution personnel.

## References


Alden CL, Kanerva RL, Ridder G, and Stone LC, "The pathogenesis of the nephrotoxicity of volatile hydrocarbons in the male rat," In: Advances in Modern Environmental Toxicology, Volume VII, Renal Effects of Petroleum Hydrocarbons. MA Mehlman, CP Hemstreet III, JJ Thorpe and NK Weaver, eds., Princeton Scientific Publishers, Princeton, pp. 107-120, 1984.

Aranyi C, O'Shea WJ, Halder CA, Holdsworth CE, and Cockrell BY. Absence of hydrocarbon-induced nephropathy in rats exposed subchronically to volatile hydrocarbon mixtures pertinent to gasoline, Toxicol. Ind. Health 2:85-98, 1986.

Austin H, Cole P, and McGraw DS. A case control study of leukemia at an oil refinery, J. Occup. Med. 28: 1169-1173, 1986.

Baxter PJ and McDowall ME. Occupation and cancer in London. An investigation into nasal and bladder cancer using the cancer atlas, BR. J. Indust. Med. 43:44-49, 1986.

Bond AE, Thompson VL, Ortman GC, Black FM, and Sigsby JE Jr. Self service station vehicle refueling exposure study, Proceedings of the 1986 EPA/APCA Symposium of Measurement of Toxic Air Pollutants, Raleigh, NC, pp. 458-466, 1986.

Braddock, JN, Gabele PA, and Lemmons TJ. Factors influencing the composition and quantity of passenger car refueling emissions - Part I, SAE Technical Paper Series, Paper #861558, 1986.

Bruner RH, Pathologic findings in laboratory animals exposed to hydrocarbon fuels of military interest, In: Advances in Modern Environmental Toxicology, Volume VII, Renal Effects of Petroleum Hydrocarbons. MA Melman, CP Hemstreet III, JJ Thorpe and NK Weaver, eds., Princeton Scientific Publishers, Princeton, pp. 133-140, 1984.

Busey WM and Cockrell BY. Non-neoplastic exposure-related renal lesions following inhalation of unleaded gasoline vapors. In: Advances in Modern Environmental Toxicology, Volume VII, Renal Effects of Petroleum Hydrocarbons. MA Mehlman, CP Hemstreet III, JJ Thorpe, and NK Weaver, eds., Princeton Scientific Publishers, Princeton, pp. 57-64, 1984.

Charbonneau M, Lock EA, Strasser J, Cox MG, Turner MJ, and Bus JS. 2,2,4-Trimethylpentane-induced nephrotoxicity, 1, Metabolic disposition of TMP in male and female Fischer 344 rats, Toxicol. Appl. Pharmacol. 91: 171-181, 1987a.

Charbonneau M, Lock EA, Strasser J, Short BG, and Bus JS. Nephrotoxity of 2,2,4-Trimethylpentane (TMP) metabolites in male Fischer 344 Rats. (abs), The Toxicologist 7:89 1987b.

Charbonneau M, Strasser J, Lock EA, Turner MJ, and Swenberg JA. 1,4-Dichlorobenzene-induced nephrotoxicity: similarity with unleaded gasoline (UG)-induced renal effects. In: Nephrotoxicity: Extrapolation from in vitro to in vivo and from animals to man, P Bach and EA Lock ed., in press 1988.

Cotran RS, Rubin RH and Tolkoff-Rubin NE. Tubulointerstitial Disease. In: The Kidney (Third Edition) Vol II. BM Brenner and FC Rector, Jr. eds., WM Saunders Co., Philadelphia PA, 1986, pp 1162-1164.

Dahl, AR, Damon EG, Mauderly JL, Rothenberg SJ, Seiler FA, and McClellan RO. Uptake of 19 hydrocarbon vapors inhaled by F 344 rats, Fund. Appl. Toxicol., in press, 1987.

Divine BJ and Barron V. Texaco mortality study: II. Patterns of mortality among white males by specific job groups, Am. J. Indust. Med. 10:371-381, 1986.

Divine BJ, Barron V, and Kaplan SD. Texaco mortality study: I. Mortality among refinery, petrochemical and research workers, J. Occup. Med. 27:445-447, 1985.

Dominiano SF, Vena JE, and Swanson MK. Gasoline exposure, smoking and kidney cancer, J. Occup. Med: 398-399, 1985.

EPA. Environmental Protection Agency, "Regulatory Strategies for the Gasoline Marketing Industry, "Federal Register 49 (154): 31706-31716, August 8, 1984.

EPA. "Refueling Emissions From Uncontrolled Vehicles," EPA Report No. EPA-AA-SDSB-85-6, 1985.

EPA. Evaluation of the Carcinogenicity of Unleaded Gasoline. EPA Report No. 600/6-87/001, April 1987.

Farber E and Sarma DSR. Hepatocarcinogenesis: A dynamic cellular perspective, Lab. Invest. 56:4-22, 1987.

Fox TR and Watanabe PG. Detection of a cellular oncogene in spontaneous liver tumors of B6C3F1 mice, Science 228:596-597, 1985.

Furey RL and Nagel BE. Composition of vapor emitted from a vehicle gasoline tank during refueling, SAE Technical Paper Series, Paper #860086, 1986.

Garg BD, Olson MJ, Demyan WF, and Roy AK. Rapid post-exposure decay of gasoline-induced hyalin droplets and $\alpha_{2u}$-globulin accumulated in the male rat kidney (abs), The Toxicologist 7:27, 1987.

Gibson JE and Bus JS. Current perspectives on gasoline light hydrocarbon induced male rat nephropathy, Ann. N.Y. Acad. Sci. in press, 1987.

Halder CA, Warne TM, and Hatoum NS. Renal toxicity of gasoline and related petroleum napthhas in male rats, In: Advances in Modern Environmental Toxicology, Volume VII, Renal Effects of Petroleum Hydrocarbons. MA Mehlman, CP Hemstreet III, JJ Thorpe, and NK Weaver, eds., Princeon Scientific Publishers, Princeton, pp. 73-88., 1984.

Halder CA, Holdsworth CE, Cockrell BY, and Piccirillo VJ. Hydrocarbon nephropathy in male rats: Identification of the nephrotoxic components of unleaded gasoline, Toxicol. Ind. Health 1:67-87, 1985.

Halder CE, Van Gorp GS, Hatoum NS, and Warne TM. Gasoline vapor exposures. Part II. Evaluation of the nephrotoxicity of the major $C_4/C_5$ hydrocarbon components, Am. Ind. Hyg. Assoc. 47:173-175, 1986.

Harrington JM. Health experience of workers in the petroleum manufacturing and distribution industry: a review of the literature, Am. J. Indust. Med. 12:475-497, 1987.

HEI Report, Health Effects Institute, "Gasoline vapor exposure and human cancer: evaluation of existing scientific information and recommendations for future research," Report of the Institute's Health Review Committee, Cambridge, MA September 1985.

Hobson DW, Mattie DR, Bruner RH, Uddin DE, Eurell T, and Olson CT. Effect of castration on the development of 2,3,4-trimethylpentane-induced hydrocarbon nephropathy in the male F-344 rat (abs), The Toxicologist 6:173, 1986.

Kanerva, RL, McCracken MS, Alden CL and Stone LC. Morphogenesis of decalin-induced renal alterations in the male rat, Fd. Chem. Toxic. 25:53-61, 1987a.

Kanerva RL, Ridder GM, Lefever FR, and Alden CL. Comparison of short-term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer-344 rats. Fd. Chem. Toxicol. 25:345-353, 1987b.

Kanerva RL, Ridder GM, Stone LC and Alden CL. Characterization of spontaneous and decalin-induced hyaline droplets in kidneys of adult male rats, Fd. Chem. Toxic. 25:63-82, 1987c.

Kaplan SD, Update of a mortality study of workers in petrochemical refineries, J. Occup. Med. 28:514-516, 1986.

Kearney CA and Dunham DB. Gasoline vapor exposures at a high volume service station, Am. Ind. Hyg. Assoc. 47:535-539, 1986.

Kloss MW, Cox MG, Norton RM. Swenberg JA, and Bus JS, Sex-dependent differences in the disposition of [$^{14}$C]-2,2,4-trimethylpentane in Fischer-344 rats, In: Renal Heterogeneity and Target Cell Toxicity, Bach PH and Lock EA, eds., John Wiley and Sons, Ltd., Sussex, pp. 489-492, 1985.

Lock EA, Charbonneau M, Strasser J, Swenberg JA, and Bus JS, 2,2,4-Trimethylpentane (TMP)-induced nephrotixicity II. The reversible binding of a TMP metabolite to a renal protein fraction containing $\alpha_{2u}$-globulin. Toxicol. Appl. Pharmacol. 182-192, 1987a.

Lock EA, Stonard MD, and Elcombe CR. The selective inhibition of ω-and β-oxidation of fatty acids in the liver and kidney of male rats administered 2,2,4-trimethylpentane (abs), The Toxicologist 5:59 1985.

Lock EA, Stonard MD, and Elcombe CR, The induction of ω and β-oxidation of fatty acids and effect on $\alpha_{2u}$-globulin content in the liver and kidney of rats administered 2,2,4-trimethylpentane, Xenobiotica 17: 513-522, 1987b.

Loury DJ, Smith-Oliver T, and Butterworth BE. Assessment of unscheduled and replicative DNA synthesis in rat kidney cells exposed in vitro or in vivo to unleaded gasoline, Toxicol. Appl. Pharmacol. 87:127-140, 1987a.

Loury DJ, Smith-Oliver T, and Butterworth BE. Assessment of the covalent binding potential of 2,2,4-trimethylpentane to rat $\alpha_{2u}$-globulin , Toxicol. Appl. Pharmacol. 88:44-56, 1987b.

Loury DJ, Smith-Oliver T, Strom S, Jirtle R, Michalopoulos G, and Butterworth BE. Assessment of unscheduled and replicative DNA synthesis in hepatocytes treated in vivo and in vitro with unleaded gasoline or 2,2,4-trimethylpentane, Toxicol. Appl. Pharmacol. 85:11-23, 1986.

MacFarland HN, Ulrich CE, Holdsworth CE, Kitchen DN, Halliwell WH, and Blum SC. A chronic inhalation study with unleaded gasoline vapor, J. Am. Coll. Toxicol. 3:231-248, 1984.

MacNaughton MG and Uddin DE. Toxicology of mixed distillate and high-energy synthetic fuels, In: Advances in Modern Environmental Toxicology, Volume VII, Renal Effects of Petroleum Hydrocarbons. MA Mehlman, CP Hemstreet III, JJ Thorpe, and NK Weaver eds., Princeton Scientific Publishers, Princton, 1984, pp. 121-132.

Maronpot RR, Haseman JK, Boorman GA, Eustis SE, Rao GN, and Huff JE. Liver lesions in B6C3F1 mice: The National Toxicology Program experience and position, Arch Toxicol (suppl) 10:10-26, 1987.

McGraw DS, Joyner RE, and Cole P. Excess leukemia in a refinery population, J. Occup. Med. 27:220-222, 1985.

McLaughlin JK, Blot WJ, Mehl FS, Stewart PA, Venable FS, and Fraumeni JF. Petroleum related employment and renal cell cancer, J. Occup. Med. 27:672-674, 1985.

McLaughlin JK, Mandel JS, Blot WJ, Schuman LM, Mehl ES, and Fraumeni JF Jr. A population-based case-control study of renal cell carcinoma, J. Nat. Can. Inst. 72:725-284, 1984.

Nelson NA. Morbidity in AMOCO oil refinery employees - Phase I. Report to AMOCO

Neuhaus OW, Flory W, Biswas N, and Hollerman CE. Urinary excretion of $\alpha_{2u}$-globulin and albumin in adult male rats following treatment with nephrotoxic agents, Nephron 28:133-140, 1981.

NRC. Committee on the Institutional Means for Assessments of Risks to Public Health, Commission on Life Sciences, National Research Council, Risk Assessment in the Federal Government: Managing the Process, National Academy Press, Washington, DC, 1983, pp. 19-28.

Olson CT. Yu KO, Hobson DW, and Serve MP. Identification of urinary metabolites of the nephrotoxic hydrocarbon 2,2,4-trimethylpentane in male rats, Biochem. Biophys. Res. Comm. 130:313-316, 1985.

Olson CT. Yu KO Hobson DW, and Serve MP. The metabolism of n-octane in Fischer 344 rats, Toxicol. Lett. 31:147-150, 1986.

Olson MJ. Garg BD, Murty CUR, and Roy AK. Accumulation of $\alpha_{2u}$-globulin in the renal proximal tubules of male rats exposed to unleaded gasoline, Toxicol Appl. Pharmacol. 90:43-51, 1987.

Pereira MA. Mouse liver tumor data: Assessment of carcinogenic activity, Toxicol. Ind. Health 1:311-333, 1985.

Peterson DR, DeLapp LG, Lington AW, and Phillips RD. Comparison of disposition of n-octane and 2,2,4-trimethylpentane in male Fischer 344 rats (abs), The Toxicologist 6:253, 1986a.

Peterson DR, Halleck RD, and Phillips RD. Effect of 2,2,4-trimethylpentane on protein and DNA synthesis in male Fischer 344 rats (abs) The Toxicologist 6:173, 1986b.

Phillips RD and Cockrell BY. Effect of certain light hydrocarbons on kidney structure and function in male rats, In: Advances in Modern Environmental Toxicology, Volume VII, Renal Effects of Petroleum Hydrocarbons. MA Mehlman, CO Hemstreet III, JJ Thorpe, and NK Weaver, eds., Princeton Scientific Publishers, 1984, Princeton, pp. 89-105.

Reynolds SH, Stowers SJ, Maronpot RR, Anderson MW, and Aaronson SA. Detection and identification of activated oncogenes in spontaneously occurring benign and malignant hepatocellular tumors of the B6C3F1 mouse, Proc. Natl. Acad. Sci. 83:33-37, 1986.

Reynolds SH, Stowers SJ, Patterson RM, Maronpot RR, Aaronson SA, and Anderson MW. Activated oncogenes in B6C3F1 mouse liver tumors: Implications for risk assessment, Science 237:1309-1316, 1987.

Richardson KA, Wilmer JL, Smith-Simpson D, and Skopek TR. Assessment of the genotoxic potential of unleaded gasoline and 2,2,4-trimethylpentane in human lymphoblasts in vitro, Toxicol. Appl. Pharmacol. 82:361-322, 1986.

Rinsky RA, Smith AB, Hornung R, Filloon TG, Young RF, Okun AH, and Landrigan PJ. Benzene and leukemia: An epidemiologic risk assessment, New Eng. J. Med. 316:1044-1050, 1987.

Schwartz E. Proportionate mortality ratio analysis of automobile mechanics and gasoline service station workers in New Hampshire, Am. J. Ind. Med. 12:91-99, 1987.

Short BG, Burnett VL, Cox MG, Bus JS and Swenberg JA. Site specific renal cytoxicity and cell proliferation in male rats exposed to petroleum hydrocarbons, Lab Invest, 57:564-577, 1987.

Short BG, Burnett VL, and Swenberg JA. Histopathology and cell proliferation induced by 2,2,4-trimethylpentane in the male rat kidney, Toxicol. Pathol. 14:194-203, 1986.

Stonard MD, Phillips PGN, Foster JR, Simpson MG, and Lock EA. $\alpha_{2u}$-globulin: measurement in rat kidney following administration of 2,2,4-trimethylpentane, Toxicol. 41:161-168, 1986a.

Stonard MD, Phillips PGN, Foster JR, Simpson MG, and Lock EA. $\alpha_{2u}$-globulin : measurement in rat kidney and relationship to hyaline droplets, Clinica Chimica Acta 160:197-203, 1986b.

Thomas TL, Fontham ETH, Norman SA, Stempagen A, and Hoover RN. Ocupational risk factors for brain tumors. A case referent death certificate analysis, Scand. J. Work. Environ. Health 12:121-127, 1986.

Tironi G. Nebel GJ, and Williams RL. Measurement of vapor exposure during gasoline refueling, SAE Technical Paper Series, Paper #860087, 1986.

Trump BF, Lipsky MM, Jones TW, Heatfield BM, Higginson J, Endicott K, and Hess HB. An evaluation of the significance of experimental hydrocarbon toxicity to man, In: Advances in Modern Environmental Toxicology, Volume VII, Renal Effects of Petroleum Hydrocarbons. MA Mehlman, CP Hemstreet III, JJ Thorpe, and NK Weaver, eds., Princeton Scientific Publishers, Princeton, 1984, pp. 273-288.

USISGC, 1986. U.S. Interagency Staff Group on Carcinogens, Chemical carcinogens: A review of the science and its associated principles, Environ. Health Perspect. 67:201-282, 1986.

Viau C, Bernard A, Gueret F, Maldague P, Gengoux P, and Lauwerys R. Isoparaffinic solvent-induced nephrotoxicity in the rat, Toxicology 38:227-240, 1986.

Wen CP, Tsai SP, Weiss NS, and Gibson RL. Long-term mortality study of oil refinery workers. V. Comparison of workers hired before, during and after World War II (1940-1945), with a discussion of the impact of the study design on cohort results. Am. J. Indust. Med. 9:171-180, 1986.

Williams RL. Breathing zone levels of gasoline vapors during automobile refueling, Presented at Air Pollution Control Association 80th Annual Meeting, New York, NY, Paper #87-31.1, 1987.

Wiseman RW, Stowers SJ, Miller EC, Anderson MW, and Miller JA. Activating mutations of the c-Ha-ras protooncogene in chemically induced hepatomas of the male B6C3F$_1$ mouse, Proc. Natl. Acad. Sci. USA 83:5825-5829, 1986.

Wong O, Morgan RW, Bailey WJ, Swenicki RE, Claxton K and Kheifeto L. An epidemiological study of petroleum refinery employees, Br. J. Indust. Med. 43:6-17, 1986.

HEI HEALTH EFFECTS INSTITUTE 215 First Street, Cambridge, Massachusetts 02142 (617) 491-2926

SPECIAL REPORT
SUPPLEMENT

September 1985
January 1988