Review of the Carcinogenic Potential of Gasoline

by Gerhard K. Raabe

This review examines the animal, human, and mechanistic studies that precede the new studies reported in this volume. Wholly vaporized unleaded gasoline was found to produce a dose-dependent increase in renal carcinoma in male rats and an excess above background incidence of hepatocellular tumors in female mice in the high-dose group. Mechanistic studies suggest that gasoline is not mutagenic and that the probable mechanism for the male rat renal tumors involves a rat-specific protein, α_{2u}-globulin, whose binding with highly branched aliphatic compounds results in renal tubule cell death and, in turn, a proliferative sequence that increases renal tubule tumors. Human evidence generated predominantly from studies of refinery workers does not support a kidney or liver cancer risk in humans. The current epidemiologic database is inadequate to access leukemia risk from low-level benzene exposure from gasoline. Studies of gasoline-exposed workers that incorporate quantitative exposure information are needed.

Introduction

The reports that follow this review represent scientific inquiry into the carcinogenic potential of gasoline and its mechanism of action begun after 1983. To better understand these new studies and the direction of their line of inquiry, it is important to at least briefly review the scientific developments that preceded this work.

Because of the generally low acute toxicity of gasoline, it was not until the mid-1970s that the petroleum industry, through the American Petroleum Institute, began to consider the design of a chronic study of gasoline (1). Concern over the benzene content of gasoline was only beginning to be discussed in scientific literature. However, by this time, it was clear that leaded gasoline was to be phased out and that a chronic study of unleaded gasoline was needed.

Chronic Study of Gasoline

The fuel finally selected for study was custom blended to reflect the average 1976 unleaded summer-grade sold in the United States. The benzene content of the fuel was increased to 2% to reflect the then-current maximum levels.

Other characteristics of test fuel are presented in Table 1. The experimental design of the two-year study was intended to closely mimic the NCI bioassay (Table 2). The decision was also made to study wholly vaporized gasoline for an overall cancer hazard assessment, although exposure of gasoline distribution workers and the public would be expected to be the more volatile fraction (2).

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Table 1. Study gasoline composition (2).

Property	Test material
RO/MO	92/84
RVP	9.5
Distillation, °F	
°F values for IBP through 100% distillation.	
IBP	93
10%	116
50%	216
90%	340
100%	428
SOA, %	66/8/26
Benzene, %	2
Lead, g/gallon	<0.05

Abbreviations: SOA, saturates, olefins, aromatics; RO, road octane; MO, motor octane; IBP, initial boiling point.

Table 2. Experimental design two-year chronic study (2).

Parameter	Description
Animals	100 per sex per species per dose; Fischer-344 rat and B6C3F$_1$ mouse
Concentration	0, 67, 292, 2056 ppm wholly vaporized unleaded gasoline
Exposure	6 hr/day, 5 days/week, 103–113 weeks in 16-m^3 chambers
Interim kills	10 per sex, species, and group at 3, 6, 12, 18 months

The principal results of the chronic study are summarized in Tables 3 and 4. The first indication of a possible carcinogenic response was observed in female mice. The incidence of hepatocellular tumors was elevated above background incidence at all dose groups and was statistically increased above background in the high-dose group (2).

Table 3. Hepatocellular neoplasms in female mice reported and revised incidence summaries.

Study	Gasoline, ppm			
	0	67	292	2056
MacFarland (2)	8/57 (14)[a]	10/52 (19)	12/57 (21)	27/56 (48)
EPA incidence, summary (4)	8/100 (8)	10/100 (10)	12/100 (12)	27/100 (27)
EPA incidence, cancer potency (4)	8/57 (14)	10/52 (19)	13/57 (23)	28/56 (50)
Revised by Richter and MacGregor (personal communication, 1991)	8/79 (10)	13/76 (17)	12/79 (15)	29/78 (37)

[a]Percent incidence in parentheses.

Table 4. Renal tumor incidence in male rats from chronic exposure to unleaded gasoline vapors (2,3).

Tumor type	Gasoline, ppm				Days to first tumor
	0 (control)	67	292	2056	
Renal adenoma	0/100	0/100	2/100	1/100	546
Renal carcinoma	0/100	1/100	2/100	6/100	692
Renal sarcoma	0/100	0/100	1/100	0/100	748
Total	0/100	1/100	5/100	7/100	

The specific incidence rates have been reported differently by MacFarland and EPA as Table 3 illustrates. Recent reexamination of the slides and the original IRDC report by Richter and MacGregor (personal communication) suggest that previous rates were calculated incorrectly. In their revision, the total number of mice with hepatocellular tumors including neoplastic nodules, adenomas, and carcinomas are counted in the numerator. The denominator consists of all mice considered to be at risk; that is, all animals sacrificed or dying on study after 12 months, the time when the first hepatocellular tumor appeared. This revised incidence supports the main effect being predominantly in the high-dose group, which may provide some insight on mechanism.

The major finding of the study related to the kidneys of male rats. At all interim sacrifices and in animals dying after 3 months, there was a dose-dependent increase in kidney lesions in male rats. These consisted of cortical renal tubular basophilia, protein casts, and chronic inflammation. This evidence for cellular regeneration was distinguishable from progressive glomerulonephrosis or "old rat nephropathy," which by the time of final termination affected almost 100% of the male rats (2). Also observed at final termination was a dose-related incidence of microscopic renal cell neoplasms (Table 4). The cause of the nephropathy and its relationship to the renal neoplasms would become the focus of significant scientific inquiry.

Mechanism

It is unlikely that the carcinogenic response in the chronic study results from a direct mutagenic event. Unleaded gasoline has been studied extensively in mutagenic assays. Studies in Salmonella, yeast, mouse lymphoma *in vivo* cytogenetics, mouse dominant lethal systems, and rat cell DNA repair models all support the general conclusion that unleaded gasoline is not a mutagen (4).

Coincident with the results of the gasoline chronic study, other petroleum compounds were becoming identi-

fied as nephrotoxic in the male rat. By the time of the 1983 Workshop on the Renal Effects of Hydrocarbons, the general nature of this male rat nephrotoxicity was broadly acknowledged (5). This "light hydrocarbon nephropathy," as it was called, exhibited a pattern of degeneration, regeneration, dilation, and hyalin droplet formation in male rats but not in female rats or in male or female mice, cats, dogs, or monkeys. This nephropathy was found to be related to branched-chain aliphatic compounds (Table 5), with more highly branched compounds, particularly the eight carbon (C-8) compounds being more active (American Petroleum Institute, unpublished motor gasoline studies, 1984, 1985). A rat specific protein, α_{2u}-globulin, was determined to bind with these compounds and accumulate in renal tubule cells, resulting in cell death. The relevance of this mechanism of toxicity to the carcinogenic response in male rats and its more important possible relevance to man is reviewed by Swenberg and Rodgers in these proceedings (6,7).

Human Evidence

When examining the human evidence, one must first be reminded that the majority of human exposure to gasoline is from gasoline head space vapors being displaced in tanks during fueling operations. As Figure 1 illustrates,

Table 5. Structure–activity relationships, light hydrocarbon nephropathy.[a]

Active	Inactive
2-Methyl pentane	n-Pentane
2,3-Dimethyl butane	2-Methyl butane
2,2,5-Trimethyl hexane	2-Methyl pentene-2
2-Methyl hexane	Methyl clyclopentane
2,3-Dimethyl pentane	m-Xylene
2,2,4-Trimethyl pentane	n-Hexane
	trans-Pentene-2
	Toluene
	1,2,4-Trimethyl benzene

[a]From American Petroleum Institute (unpublished studies, 1984, 1985).
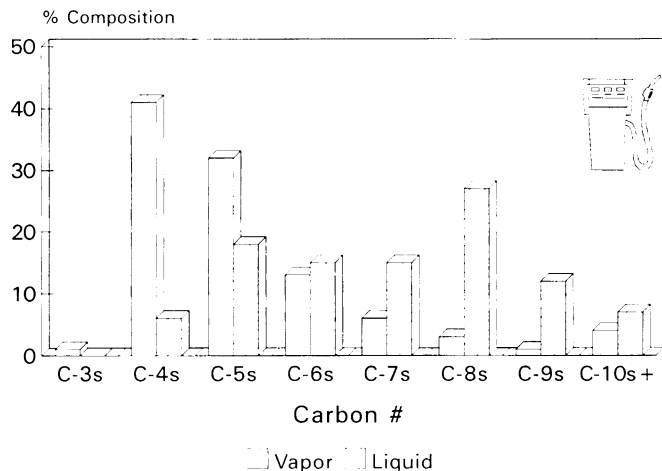
% Composition

FIGURE 1. Typical gasoline vapor versus gasoline liquid composition.

gasoline vapor is different in concentration and chemical species from that of wholly vaporized unleaded gasoline; in particular, C-8 compounds are in lower concentrations in the vapor than the liquid. This suggests that humans are less exposed to the agents most active in the male rat.

A large number of epidemiologic studies of workers in the petroleum industry have been conducted. Systematic reviews of this literature by Wong and Raabe and an IARC monograph work group, both reported in 1989, represent the most comprehensive examination of these studies (8,9). Table 6 lists the major cohort studies reviewed by Wong and Raabe (8). The majority of these studies are of refinery workers who, although exposed to gasolinelike hydrocarbons, are generally not exposed to gasoline. The activities in the refining industry also potentially expose these workers to other materials, some of which are known or suspect human carcinogens (e.g., asbestos, benzene, and polyaromatic hydrocarbons) (9). Unfortunately, none of the studies reviewed provide useful information about exposures. Nevertheless, an examination of liver cancer, kidney cancer, and leukemia in these populations provides some information on the possible risk from exposure to gasoline.

Figure 2 summarizes the standardized mortality ratios (SMR) for liver cancer found in these studies. The meta-SMR reported in this and subsequent figures was calculated by Wong and Raabe as an overall estimate of the industry experience (8). Looking at these studies individually or overall as summarized by the meta-SMR clearly indicates there is no evidence for an increased risk for liver cancer.

Kidney cancer SMRs are summarized in Figure 3. The pattern of SMRs for kidney cancer is less consistent than for liver cancer. Several studies show small excesses, in particular the study by Rushton of UK distribution workers. However, none of these excesses is statistically significant, and the overall meta-SMR is at the expected rate. Cohort studies specific to gasoline-exposed workers are needed before any firm conclusions can be drawn with regard to human kidney cancer risk.

Table 6. Petroleum cohort studies.

Study letter/author[a]	Facility
A. Nelson, 1985	10 Amoco refineries — US
B. Wong et al., 1986	Richmond, El Segundo — California, US Refineries
C. Hanis et al., 1985	Baton Rouge, LA, Baytown, TX, Bayway, NJ Refineries
D. Wen et al., 1983	Port Arthur Refinery — Texas, US
E. Morgan and Wong, 1984	Beaumont Refinery — Texas, US
F. Morgan and Wong, 1985	Paulsboro Refinery — New Jersey, US
G. Enterline and Henderson, 1985	Torrance Refinery — California, US
H. McGray et al., 1985	Wood River Refinery — Illinois, US
I. Joyner, 1983	Deer Park Refinery — Texas, US
J. Divine et al., 1985	13 Texaco refineries — US
K. Divine and Barron, 1987	Production and pipeline — US
L. Rushton and Alderson, 1981	8 UK refineries — United Kingdom
M. Rushton and Alderson, 1983	UK petroleum distribution centers — United Kingdom
N. Theriault and Provencher, 1987	East Montreal Refinery — Canada
O. Christie et al., 1986	Australian petroleum industry — Australia
P. Nakamura, 1987	51 Japanese refineries — Japan
META Wong and Raabe, 1989	Meta-analysis of studies A–P

[a]Study letters correspond to the letters in Figures 2–4. All studies are reviewed in Wong and Raabe (8).
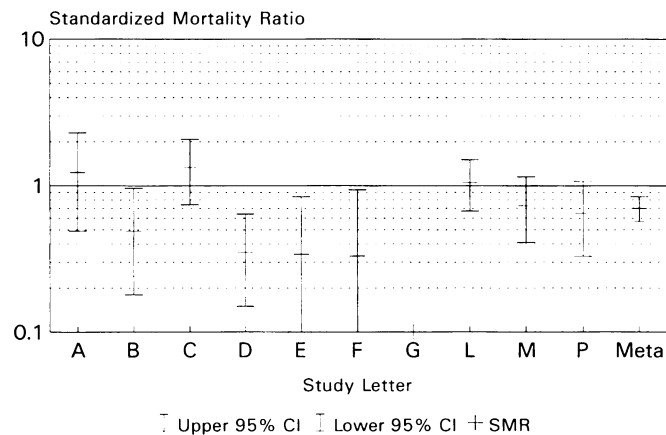

FIGURE 2. Petroleum cohort studies: liver cancer (International Classification of Disease 155–156). See Table 6 for study letter definitions. Adapted from Wong and Raabe (8).
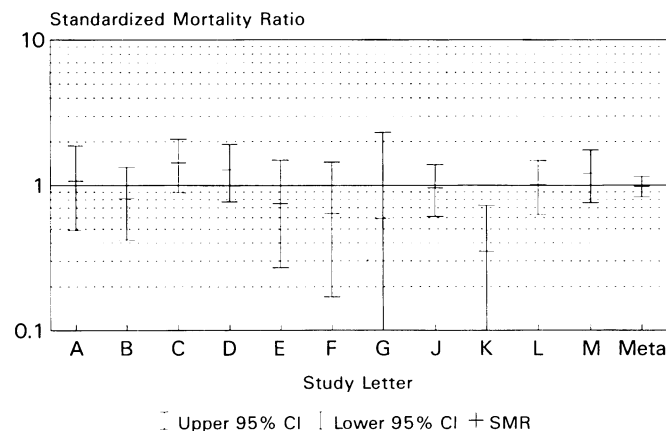

FIGURE 3. Petroleum cohort studies: kidney cancer (International Classification of Disease 189). See Table 6 for study letter definitions. Adapted from Wong and Raabe (8).
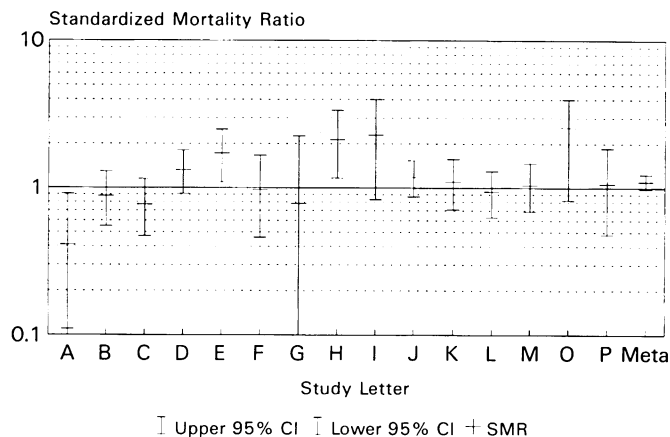
Standardized Mortality Ratio

FIGURE 4. Petroleum cohort studies: leukemia (International Classification of Disease 204–207). See Table 6 for study letter definitions. Adapted from Wong and Raabe (8).

Leukemia risk from gasoline exposure associated with the small percentage of benzene in fuel continues to be of concern. Although it is generally recognized that exposures to benzene from automotive gasoline fueling are low (9), the misuse of gasoline as a solvent and large-scale distribution of gasoline in earlier time periods have not been well studied. Figure 4 shows the leukemia results from occupationally exposed petroleum cohort studies. Although not consistent across studies, there are indications that some refinery workers were historically at increased risk for leukemia. Again, the fact that the SMR in the UK-distribution worker study is elevated is of concern. As with human kidney cancer risk, studies specific to gasoline-exposed workers are needed before any conclusions can be drawn.

Discussion

Overall, the scientific history leading up to this symposium is a complex one. On one hand, we have clear evidence of a carcinogenic risk in animals. On the other hand, we have evolving mechanistic evidence indicating the male rat kidney cancer operates via a mechanism that is not relevant for man. Less is understood about the mouse liver cancer excess, but consistent epidemiologic evidence in petroleum-exposed workers argues against a gasoline-related liver cancer risk for humans. To date, the studies in human populations have generally not provided adequate exposure information to help resolve the uncertainty. Both IARC and EPA determined epidemiology studies as inadequate for making any judgment. It is against this background of scientific uncertainty that the new studies reported in these proceedings were intended to address.

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