Potential Health Effects of Gasoline and Its Constituents: A Review of Current Literature (1990–1997) on Toxicological Data

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We reviewed toxicological studies, both experimental and epidemiological, that appeared in international literature in the period 1990–1997 and included both leaded and unleaded gasolines as well as their components and additives. The aim of this overview was to select, arrange, and present references of scientific papers published during the period under consideration and to summarize the data in order to give a comprehensive picture of the results of toxicological studies performed in laboratory animals (including carcinogenic, teratogenic, or embryotoxic activity), mutagenicity and genotoxic aspects in mammalian and bacterial systems, and epidemiological results obtained in humans in relation to gasoline exposure. This paper draws attention to the inherent difficulties in assessing with precision any potential adverse effects on health, that is, the risk of possible damage to man and his environment from gasoline. The difficulty of risk assessment still exists despite the fact that the studies examined are definitely more technically valid than those of earlier years. The uncertainty in overall risk determination from gasoline exposure also derives from the conflicting results of different studies, from the lack of a correct scientific approach in some studies, from the variable characteristics of the different gasoline mixtures, and from the difficulties of correctly handling potentially confounding variables related to lifestyle (e.g., cigarette smoking, drug use) or to preexisting pathological conditions. In this respect, this paper highlights the need for accurately assessing the conclusive explanations reported in scientific papers so as to avoid the spread of inaccurate or misleading information on gasoline toxicity in nonscientific papers and in mass-media messages. Key words: alkylenzenes, antiknocking agents, benzene, gasoline, gasoline constituents, methanol, MTBE, toxicity. Environ Health Perspect 106:115–125 (1998). [Online 3 February 1998]

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Gasoline is the generic term for petroleum fuel used mainly for internal combustion engines. It is complex, volatile, and inflammable and contains over 500 saturated or unsaturated hydrocarbons having from 3 to 12 carbons. The variable mixture characteristics depend on crude oil origin, differences in process techniques and blends, season to season changes, and the additives required to meet particular performance specifications.

In the strategy for reducing motor vehicle pollution by removing lead and many potentially toxic hydrocarbons in gasoline mixtures, the addition of oxygenates (alcohols and alcohol-derived ethers) has led to the development of reformulated fuels with lower quantities of benzene and aromatics without the use of heavy metal additives. Generally speaking, a common gasoline formulation contains approximately 62% alkanes, 7% alkenes, and 31% aromatics, as well as alcohols, ethers, and additives. The percentage composition may vary significantly according to regulations in force in different countries, not only among the various leaded compositions but also among the unleaded, the reformulated, and the oxygenated.

About 110 million people are exposed to gasoline constituents in the course of refueling at self-service gasoline stations (1), an operation that requires only a few minutes per week, accruing to approximately 100 min/year. During refueling, total hydrocarbon concentrations in the air fall within the range of 20–200 ppm by volume. Major toxic risk comes from breathing exhaust fumes and evaporative and refueling emissions rather than from occasional skin contact from spills. At modern service stations, designed to dispense gasoline quickly and safely, the typical consumer runs the risk of suffering serious negative health effects due to exposure to gasoline is negligible. However, available information on general population exposure is either nonexistent, incomplete, or inconsistent with other data (2).

With increasing frequency over recent years, articles on gasoline appear in scientific and nonscientific printed matter. Among the scientific articles, some refer to new and interesting results, some provide already known information, and others give interesting but incomplete data. In the nonscientific area, some papers make ambiguous or incorrect considerations and provide interpretative explanations capable of distilling deep concern in public opinion on the dangers of gasoline.

In this paper, we examine a series of experimental and epidemiological toxicological studies that have appeared in international literature over the period 1990–1997, in regard to leaded and unleaded gasolines as well as their components or additives. Certain constituents are mentioned that may not be present in all gasoline mixtures, but are mentioned because scientific studies published during the period contribute to a more exhaustive picture of the toxicological profile of gasolines.

Toxicity Studies on Gasoline

Experimental Animal Data

In 1992 the Dutch Directorate-General for Labour carried out an exhaustive review on potential gasoline toxicity (3). The report gives results of toxicity studies in different animal species treated with a gasoline having predetermined quantities of the various constituents. Data on acute oral toxicity, acute and short-term inhalation exposure, intratracheal instillation and long-term toxicity (including carcinogenesis), teratogenic or embryotoxic activity, mutagenicity, and clastogenicity are included. The report refers to a species/sex-specific carcinogenic effect and, even if data are limited, suggests a lack of teratogenic and embryotoxic activity and little evidence of mutagenic and clastogenic effect.

Human and Epidemiological Studies

A relationship between gasoline exposure and the onset of renal and liver cancer, acute myeloid leukemia, myeloma, heart disease, changes in the central nervous system (CNS), skin alterations (including melanoma), and modifications of mucous membranes has been indicated.

Cancer, leukemia, and myeloma. An association between kidney cancer and exposure to gasoline has been suggested in some studies (4–9).

In 1993, Infante (10) referred to two studies in gasoline-exposed workers: the first study suggested an association with kidney cancer, but the second study did not. This apparent contradiction might be explained by other factors such as cigarette smoking, which was not sufficiently considered in the analysis (10). Other epidemiological studies published in 1993, which include a cohort of approximately 100,000 male refinery workers (11)

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and another of 18,135 distribution employees (12), do not support an increased kidney or liver cancer risk when compared with general population. McLaughlin (13), in reviewing epidemiology of renal cell cancer, examined the following risk factors: cigarette smoking, gasoline, obesity, diet, and use of analgesics and diuretics. No link between gasoline exposure and renal cell cancer was found.

A previously unnotified risk of nasal cancer has recently been reported in service station workers in the Nordic countries by Lynge et al. (9). The study also reports an increased incidence of pharyngeal, laryngeal, and lung cancers.

A relationship between the development of acute leukemia and myeloma and exposure to petroleum products such as fuels and exhaust fumes has also been suggested (5,14).

Risk of acute myeloid leukemia has been studied in Sweden, and results indicate that other constituents of gasoline could potentiate the leukemic effect of benzene (15). A mortality study among 6,672 Canadian petroleum marketing and distribution workers and tank truck drivers, conducted by Schnatter et al. in 1993 (6), gave an overall mortality lower than the general Canadian population in marketing and distribution workers; leukemia findings were not evident in the latter category, but were significantly elevated in tank truck drivers. Infante (10) referred to six studies conducted in gasoline-exposed workers: one showed strong associations between gasoline exposure, leukemia, and multiple myeloma; the second suggested a slight association with leukemia, but did not analyze data for multiple myeloma; and the third was inconclusive due to problems linked with study design. In 1996 Collingwood et al. (16) reported that in a cohort study of 4,855 refinery workers in New Jersey, all of whom had been employed for a minimum of 1 year in an arc of time going from 1946 to 1979, mortality from overall leukemia and lymphatic and hematopoietic cancer was at the expected level; mortality from multiple myeloma was, however, lower than expected.

Exposure to gasoline vapors was classified by the International Agency for Research on Cancer (IARC) in 1989 as possibly carcinogenic to humans (Group 2B) (17), mainly on the basis of the established carcinogenicity of some constituents such as benzene.

Skin lesions and mucous membranes. In humans, gasoline causes irritation to eyes at exposures up to 200 ppm in the air over a period of 30 min (3). After gasoline application to skin, a decrease in glutathione concentration, glutathione S-transferase activity, and lipid peroxidation was observed in liver and brain (18).

Some studies in refinery workers and gasoline-exposed workers indicated an increased mortality risk from malignant melanoma; whether this was the result of exposure to gasoline, benzene, or sunlight, or a combination of these factors has been difficult to establish (10).

Heart disease. No clear association has been found between gasoline exposure and refinery workers in regard to heart disease (6,7). Evaluation of risk is complicated by important related factors, e.g., cigarette smoking, a point that is particularly relevant when studying gasoline-exposed workers who, due to danger of explosion in their work environment, would naturally have a lower smoking factor than the general population (6).

Neurotoxicity. Occupational exposure to gasoline has been associated with numerous neurotoxicity signs including significant effects on intellectual capacity, modifications of psychomotor and visualmotor functions, and immediate and delayed memory (19). Other gasoline-induced neurotoxic effects (ataxia, tremor, acute or subacute encephalopathic syndrome) are ascribable to intentional use (gasoline sniffing) and not to occupational exposure (19).

Current epidemiological data are generally considered inadequate for a global assessment of health risk, but it is to be hoped that ongoing studies will remedy this situation.

Toxicity Studies on Unleaded Gasoline

Most of the animal toxicity studies that we reviewed were performed with an unleaded gasoline (UG) known as PS-6 [American Petroleum Institute (API), Washington, DC], a blend with the benzene content adjusted to 2% and regarded as a representative UG (20). The formulation for PS-6 gasoline was described in a 1995 API publication (21).

As with leaded gasoline, major toxicological problems concern carcinogenic potential, namely, liver tumors in female mice and renal tumors in male rats. In female rats, only a mild proximal tubular dysfunction was found (22).

Renal Tumors

In explaining renal tumors in F344 male rats exposed to UG, Raabe (23) suggested that the presence of hyaline droplets, or the increase in α2u-globulin in the renal tubules of UG-treated male rats, could be involved in the mechanism for this pathology. The increase was attributed to the higher composition of branched hydrocarbons in UG (24) to which α2u-globulin could bind, thus inducing renal tubule cell death followed by a proliferative sequence that increases renal tubule tumors (23,25). In any case, no clear conclusion can yet be drawn concerning the role of α2u-globulin in the development of renal disease and the association between α2u-globulin and renal carcinogenesis (25,26).

Extrapolation of male rat renal tubule tumors to humans is questionable (27): if α2u-globulin is involved in male rat kidney tumors, because it is a rat-specific protein and unique to the male rat (28–30), it would not relate to humans (30,31).

Liver Tumors

Standeven et al. (32,33) attempted to determine the mechanism of induction of liver tumors with concomitant uterine changes in B6C3F1 female mice chronically exposed to UG (PS-6) vapors and concluded that high-level exposures could produce an increase in liver tumors in female mice due to interference with estrogen hormone function. Standeven et al. (34) demonstrated an induction of estrogen metabolism in isolated hepatocytes from UG-treated mice, which suggested a potential mechanism for apparent antiestrogenic activity of UG. Tilbury et al. (35) surmised that some biological effects, such as the induction of cell turnover or altered growth control, could play a role in the carcinogenic process in PS-6 gasoline-treated mice. Furthermore, Standeven and Goldsworthy (36,37) demonstrated an induction of CYP2B, a subfamily of cytochrome P450, in female mouse liver.

Toxicity Studies on Gasoline Constituents

Individual constituents of gasoline mixtures, regardless of their presence or quantity in any one formulation, have been examined because they have been the subjects of scientific publications that provide a contribution to knowledge already acquired on the relevant toxicological profile. Particular attention has been given to the new constituents recently adopted as gasoline additives.

Organic Compounds of Lead

To improve yield and performance, fuel combustion must be rapid, and the search for suitable antiknock agents has resulted in the development of alkyl leads (tetramethyl and tetraethyl lead) as cost-effective octane enhancers. Increase in the compression ratio began in the 1950s, leading to the need for higher octave fuels; therefore, lead concentrations were increased up to the limit of 1.14 g Pb/l.

A 1990 study carried out in employees, with an average of 14 years exposure to
organic compounds of lead, demonstrated that neurotoxic damage can result from exposure to such antiknock additives (38).

As a matter of fact, it has been well known for decades that organolead compounds are potent neurotoxins on the CNS and its development. In neonatal rats, these compounds produced persistent and dose-dependent behavioral hyperreactivity and hippocampal damage; a relationship between these effects via cholinergic, but not dopaminergic, pathways was hypothesized by Booze and Mactutus (39). In a recent study carried out on cultured E18 rat hippocampal neurons (40), triethyl lead, the major metabolite of tetraethyl lead, was shown to disrupt cytoskeletal elements, particularly neurofilaments, at very low levels (nanomolar concentrations).

In humans, the organolead compounds, especially tetraethyl lead, are known to have a toxic impact on the CNS, as suggested by pathological changes in brain stem neurons and subtle cognitive and neurological deficits. Infants are the most susceptible population (41-43). Other effects are nephropathy, hematologic alteration, hypertension, congenital malformations, growth and development deficiencies, and impairment of immune system responses. Due to the probable involvement of multiple etiologic factors in the above pathological situations, the actual scientific challenge is to develop sensitive methodologies capable of detecting the rate of organolead compound involvement.

Improvements brought about by the introduction of unleaded fuels determined a gradual decrease in lead content in gasoline, which in many countries has been associated with a lowering of blood lead levels in the general population (44-48). However, over the past two decades, research has demonstrated that adverse health effects occur at levels previously considered safe (49-51). Organometals, like organolead, are difficult to eliminate from the CNS, and the injuries induced usually result in permanent neurological deficits that cause medical as well as social-economic problems (52).

**Oxygenates**

Oxygenates are used as antiknock agents in place of lead derivatives and as substitutes for high octane aromatics in fuel (53,54); by permitting a more efficient fuel combustion, they diminish exhaust emissions of carbon monoxide and hydrocarbons. Oxygenate concentrations in gasoline formulations vary from 0.7% by volume in leaded gasoline [mostly methyl tertiary butyl ether (MTBE)] currently used in some European countries to 15% in some types of U.S. oxygenated gasolines.

Oxygenates include substances such as ethanol, methanol, MTBE, ethyl tertiary butyl ether (ETBE), tertiary butyl alcohol (TBA), and tertiary amyl methyl ether (TAME).

**Ethanol**. Ethanol is a chemical to which man is particularly exposed. It is widely used as an industrial solvent and is also consumed in alcoholic beverages. Abundant literature exists on the effect of ethanol ingestion (CNS, endocrine, renal, gastrointestinal, hepatic, and cardiovascular systems), which is outside the purpose of this paper.

In reference to ethanol use as a gasoline additive (approximately 10% volume), it is worth noting that exposure to ethanol in gasoline should not increase toxicity risk for human health. In fact, the potential levels of exposure are much lower than the levels associated with the toxic effects observed in experimental animals or in humans, as described by Burbacher (19) and Reese and Kimbrough (55).

**Methanol**. Methanol can be used as a fuel either alone or as a gasoline additive. Its toxicological profile has been widely studied, and only recent references are reported here.

Methanol is well absorbed in humans following inhalation, ingestion, or cutaneous exposure; it produces a transient mild depression of the CNS with headache, vertigo, and vomiting. It is oxidized to formaldehyde in the liver and then to formic acid, which contributes to the metabolic acidosis that occurs in acute methanol poisoning. Intoxication has delayed onset characterized by acidosis, mental confusion, ocular toxicity with visual disturbance [recently suggested (56,57)] as due to intraretinal metabolism of methanol, rather than to elevated blood formate levels, reversible or permanent blindness, and, in severe cases, death. These effects represent a classic example of lethal synthesis in which toxic metabolites can cause fatality after a characteristic latency period (58).

A significant increase in incidence of exencephalia and cleft palate has been observed in mice, as well as an increase in embryo/fetal death, after inhalation of high concentrations of methanol (59). Cummings (60) obtained similar results together with reduced uterine pregnancies and implantations in rats and mice. An in vitro study performed by Andrews et al. (61) reported a dysmorphic effect of methanol on rat and mouse embryos and suggested a higher sensitivity in the mouse to the developmental toxicity of methanol.

In a cellular culture of a specific and sensitive target, craniofacial tissue, methanol selectively affected sensitive cell populations and modified proliferation and cell fate (62). Lee et al. (63), studying effects of methanol vapors on testicular morphology and on testosterone production in rats, hypothesized that methanol could potentially accelerate age-related degeneration of the testes.

Two papers on methanol inhalation toxicity that are especially pertinent to the subject include a 1995 study in which no interactive effects between methanol and gasoline were found in a short-term inhalation study in rats (64), and a study on perinatal exposure to low concentrations of methanol, which resulted in no significant abnormalities in the brains of treated rats (65).

There is little evidence from available information of human health effects from low-level exposure which demonstrates that methanol vapors from motor vehicle fuel can cause acute adverse effects to health (55,66). Lee et al. (67) demonstrated that after a 6-hr exposure to the current methanol threshold limit value (TLV) of 200 ppm, the formate does not accumulate in blood in human subjects at rest or during exercise. This result was confirmed by other authors (68,69).

The neurotoxic effects recorded at current TLVs (70) are not significant in assessing human risk from methanol as a fuel or additive; in fact, Costantini (66) found levels lower than 50 ppm in most exposure scenarios, but reported methanol exposure levels from methanol-fueled car emissions at approximately 150 ppm in public garages. For the most part, the general public's exposure to methanol would be brief but repetitive, and further studies on the relationship between chronic low-level exposure and subtle changes in CNS function need to be performed (19,66,71).

**Methyl tertiary butyl ether**. MTBE is an aliphatic ether, a volatile, colorless, and inflammable liquid, currently the most widely used oxygenate. It has been employed as an octane enhancer in gasoline blends at concentrations up to 7% since 1979. In 1992, in those areas in the United States where carbon monoxide exceeded national standards, MTBE was raised to 15% by volume to reduce atmospheric pollution. Raising the oxygen content in gasoline reduces emissions of carbon monoxide, especially in older cars.

**Experimental animal data**. Acute toxicity data show a low order of MTBE toxicity in rodents following inhalation, oral administration, or cutaneous exposure (72-75). In subchronic and chronic toxicity studies (75-79), MTBE appears to have low systemic toxicity; the main findings observed in rats were depressant effects on the CNS, typical of similar ethers. Such effects were transient and completely reversible (75,77,79); no histopathological changes in tissues of the peripheral or central nervous system occurred (75,79). Other target organs following repeated oral or inhalatory
exposure at high doses have been identified as liver, kidneys, and adrenal glands (77–79). No significant immunological, cardiovascular, hematological, or pulmonary effects in animals have been observed (73,76,77,79). Finally, MTBE appears to be slightly irritating to eyes and mucous membranes (72–74).

In rats and mice, MTBE is rapidly and completely absorbed by inhalation and ingestion, whereas absorption following dermal exposure is limited (80). The main metabolites deriving in equimolar amounts from the oxidative demethylation of MTBE are TBA and formaldehyde (55,81–83). TBA is oxidized to 2-methyl-1,2-propanediol and α-hydroxysisobutyric acid (55,74,80,82). Brady et al. (81) indicated a selective increase in hepatic microsomal oxidase activity (P450) in rats treated with MTBE, showing that MTBE may stimulate its own metabolism. After inhalation exposure in rats, MTBE is rapidly exhaled (20–70% depending on dose); the remainder is eliminated through urine (55,80).

MTBE has not been found to induce adverse effects on fetal development in rabbits, rats, or mice (84–87). Furthermore, adverse effects on reproduction have not been observed in one- and two-generation studies in rats (88,89).

After high MTBE exposure, female mice showed increased incidence of hepatocellular adenoma and male rats showed increased incidence of renal tubular cell tumors and interstitial-cell testicular tumors (78). A nongenotoxic mechanism is supported by the substantial lack of in vitro and in vivo evidence of mutagenicity and other genotoxic endpoints in both mammalian and bacterial systems (90–95). It could be maintained that an MTBE carcinogenic effect can result from cellular injury, which is induced only by high doses (78).

In particular, the species (mice) and sex (female) specific MTBE hepatocellular adenoma at high doses suggests an involvement of antiestrogenic-like effects ascribable to MTBE. The antiestrogenicity is often linked to hepatic tumor promoting activity as described for UG and for several other chemicals (33). Evidence of an antiestrogenic effect was recently confirmed by Moser et al. (96,97). The question of how MTBE elicits these antiestrogenic effects remains unanswered. Experiments under way at the Chemical Industry Institute of Toxicology (CIIT) are showing that MTBE does not produce its antiestrogenic effects through interaction with the estrogen receptor (S. Borghoff, unpublished data).

In contrast with what has been previously suggested (98), a lack of involvement of formaldehyde (metabolic product of MTBE) in the onset of mouse liver tumors has recently been reported (99). In conclusion, nongenotoxic hormonally related mechanisms appear to be the most plausible explanation for the development of these tumors, as suggested by the National Research Council (100). In any case, such a manifestation in a single animal species (mouse) relating exclusively to the female sex, renders any extrapolation to humans questionable to say the least.

Kidney tumors in male rats are associated with concurrent nephrotoxicity characterized by increased concentrations of rat-specific α2u-globulin in renal tubules (77,78,101–103). An 1997 in vitro study (104) demonstrated that the binding of MTBE to this protein contributes to the uptake and accumulation of MTBE in male rat kidney in vivo (105).

Knowing the role of α2u-globulin in the renal toxicity of MTBE is useful in assessing human risk because the protein involved is present only in male rats and not in humans (28–31,106). Due to the above considerations, these kidney tumors cannot be used as weight of evidence in the assessment of human cancer risk. Finally, the increase in interstitial-cell testicular tumors in rats does not appear to be significant because an increase in testicular tumors is a common occurrence in aging rats of this strain (78,107,108).

A 1995 study (109) reported an increase in lympho–hematopoietic cancers in rats treated with high doses of MTBE dissolved in olive oil by oral gavage. This study is fraught with uncertainty because it employs only two doses, both high, of MTBE and was carried out in highly inbred rats having a significant incidence of spontaneous tumors of hemolymphoreticular tissue; furthermore, oral administration is subject to a hepatic first pass, and forced-feeding itself can induce a toxicological response. These considerations are shared by Mennear (110) who, furthermore, in accordance with an opinion issued by the National Toxicology Program working committee in 1986, contested the scientific validity of combining lymphomas and leukemias for statistical purposes.

Also, a study on oral toxicity of MTBE has less value for estimating cancer risk to humans than an inhalation study when normal human exposure is by inhalation; furthermore, a single high concentrated oral dose is vastly different to any possible human exposure. It is therefore impossible to evaluate this study or accept its statement that "... MTBE must be considered an animal carcinogen" (109).

Finally, tumor findings in rats and mice are not relevant to humans and support the conclusion that exposure to MTBE does not pose an added cancer risk for the population in respect to whole gasoline, considering the current levels maintained in MTBE-oxygenated gasoline and the MTBE exposure levels in marketing and distribution workers, as well as in the general population (111–115).

The EPA, on the basis of limited animal evidence, has not provided a final carcinogenicity classification for MTBE, but tentatively suggested that MTBE be classified as a possible human carcinogen (Group C) (116).

**Human and epidemiological studies.** To date, information regarding MTBE effects on human health is limited. Some data (117–119) ensuing from clinical use (MTBE infusion for gallstone treatment) are irrelevant to the evaluation of MTBE toxicity when it is used as an additive to gasoline (55). In humans, the principal route of absorption remains inhalation (55,120); skin application shows little absorption (121). MTBE uptake and distribution in male volunteers has been reported (122). In vitro metabolism of MTBE in human liver has recently been studied by Hong et al. (123).

When MTBE-oxygenated fuels were introduced in the winter of 1992 in Fairbanks and Anchorage, Alaska (4 years later than in other states in the United States), acute health complaints were reported. These complaints were characterized by three types of nonspecific symptoms at varying levels in the respiratory tract and eyes (i.e., burning of the nose, throat, and eyes, cough), the gastrointestinal tract (nausea), and the CNS (headache, dizziness, feelings of disorientation).

While preliminary studies by public health officials seemed to indicate a positive association between these symptoms and acute exposure to MTBE-oxygenated gasoline (124,125), further data, either from studies in exposed workers (126–132) or from human exposure under controlled experimental conditions (133–136), did not substantiate these results. In Alaska, research using a wide-range questionnaire showed no exposure–response relationship (126), emergency room visits for headache did not increase (129), and insurance claim statistics for respiratory complaints revealed no significant difference to pre-MTBE-oxygenated fuel periods (131). Other studies in Stamford, Connecticut, and Albany, New York, did not show any association between symptoms and exposure (127,128). Results in favor of MTBE were also obtained in a study performed in 1994 in garage workers in New Jersey (130). A recent Finnish study (132) showed no statistically significant differences in neuropsychological symptoms.
that occurred between chronically exposed tanker drivers and milk delivery drivers.

Finally, the results of experimental studies in humans in controlled MTBE exposure chambers showed no effects on health (133–136).

Several hypotheses have been formulated to explain these findings, which are in contrast with previously mentioned health complaints. The first is that certain individuals, abnormally sensitive to chemicals, may be particularly sensitive to low concentrations of MTBE. This was studied but not confirmed by Fiedler et al. (137). Another hypothesis infers an effect brought about by media exposure regarding antipathy toward the oxyfuels program, a form of suggestion that causes people to attribute symptoms to MTBE exposure. A third hypothesis blames the distinctive and unpleasant odor of MTBE for being a trigger for stress-related symptoms and for increasing existing symptom awareness.

MTBE determination methods in gasoline vapors and on MTBE degradation in soil systems have also been reported (138–140).

Tertiary butyl alcohol. Animal toxicity studies with TBA indicated the urinary tract is the target organ in rodents, with males being more sensitive than females (141). Takahashi et al. (142) confirmed that TBA, similar to MTBE, induces nephropathy in Fischer 344 male rats by increasing renal accumulation of hyaline protein, which is consistent with α2u-globulin. Increased incidence of renal tubular adenomas or carcinomas in male F344 rats with lifetime exposure to TBA via drinking water has been reported (143,144). In female mice, oral administration of TBA induced a statistically significant increase in thyroid adenomas (143,144). TBA does not show mutagenic activity (90,91,116,145).

In humans, irritation of eyes, nose, and skin have been reported after short-term exposure; defatting of the skin and dermatitis have been recorded after long-term exposure (146).

Tertiary amyl methyl ether. TAME received serious consideration as an oxygenate as early as 1991 (147). Although the octane content is slightly lower than in other ethers, it compares favorably for vapor pressure, boiling point, energy density, and water mixability.

TAME causes a significant but transient CNS depression akin to, but slightly more severe than, that resulting from MTBE exposure at the same levels (147,148).

In a short-term study in rats, oral treatment with TAME induced minimal changes in clinical chemistry and hematology findings and a reduction of food consumption and animal body weights in high dose groups (149).

Studies with TAME did not demonstrate any evidence of genotoxicity in an Ames assay or in mouse micronucleus assay (147,149); a preliminary report submitted by Chevron (R.D. Cavalli, unpublished data) to the Toxic Substances Control Act section 8(e) coordinator refers to an increased incidence of cleft palate in some of the pups after exposure of pregnant mice to high concentrations, while no effects were noted in rats. The same report refers to a positive TAME concentration-related response in an in vitro study of chromosome effects in Chinese hamster ovary (CHO) cells after metabolic activation.

The kinetics and mechanisms of atmospheric removal of TAME have been well described in a 1994 report (150).

Ethyl tertiary butyl ether. ETBE can be used as a gasoline antiknock additive, but its use is limited due to the high cost of ethyl alcohol. In a CASE (Computer-Automated Structure Evaluation) study where the ETBE structure was compared with the structures of recognized determinants of carcinogenicity in rodents, ETBE was predicted to be neither a genotoxicant nor a carcinogen (93).

Other Additives

Ethylene dibromide (EDB) and ethylene dichloride (EDC). Leaded gasoline scavengers (EDB and EDC) are required to remove lead from engines. These compounds provide halogen atoms that react with lead to form the volatile products which escape through the exhaust. EDB and EDC are irritating to skin and eyes and are metabolized by an oxidative pathway (cytochrome P450) followed by conjugation (glutathione S-transferase) (151,152). Their metabolites play an important role in exerting toxicity (151–154).

In 1987, IARC classified EDB as probably carcinogenic to humans (Group 2A) (155) and EDC as possibly carcinogenic to humans (Group 2B) (156).

Methylocyclopentadienyl manganese tri-carbonyl (MMT) is an effective octane enhancer used in Canada since 1976 (157); it was recently approved for use in the United States as a gasoline additive (158), but is not currently in use in Europe. According to Lynam et al. (159), due to MMT’s low vapor pressure and short half-life in sunlight, it is unlikely that significant concentrations of MMT occur in the environment from its use as a gasoline additive.

The combustion of MMT in gasoline may be a source of environmental contamination and manganese exposure [it has been reported that over 99.9% of Mn from MMT is converted into inorganic oxides of Mn during fuel combustion (159)]. The effects of Mn on lungs and on the nervous system are common knowledge. In some occupational studies, a relationship has been indicated between high inhalation exposure to Mn, neurological signs, and neurodegenerative disorders (160–165).

Information is scarce on potential health risk derived from MMT as a gasoline additive (157,166). In a Canadian study (166), it was reported that MMT use in gasoline is a potentially important source of Mn for the occupationally exposed population, even if recorded Mn values remain well below established limits for occupational and environmental airborne exposure. This was also reported by Frumkin and Solomon (158). Sierra et al. (167) obtained data on Mn environmental exposure in 35 garage mechanics and suggested that less than 10% of Mn exposure was due to MMT.

According to Frumkin and Solomon (158), the critical question is whether the additional population exposure resulting from widespread use of MMT converted to Mn could lead to toxic effects.

The disposition and toxicity of MMT in the brains of 12-month-old MMT-treated mice was reported in 1994 (168).

Benzene

Benzene is an aromatic organic hydrocarbon present in leaded gasoline, and it is sometimes added as a blending agent to UG to improve antiknock characteristics. European Union requirements state that the benzene added to leaded and unleaded gasolines must not exceed 5%; however, in some European countries, as in the United States, fuels are currently available with an even lower benzene content (1%).

The toxic effects of benzene, some of which also pertain to other aromatic hydrocarbons, are well known (169,170).

Hematopoietic toxicity is the major effect and is unique to benzene.

Chronic occupational exposure to benzene (with inhalation as the main route of absorption) causes bone marrow injury and hematopoietic toxicity, including leukopenia, lymphocytopenia, aplastic anaemia and leukemia (acute myelogenous leukemia). Further epidemiological reports on leukemia have appeared in recent literature (171–173).

Unlike leukopenia and lymphocytopenia (174,175), benzene-induced leukemia has only been observed in humans: no satisfactory animal model exists that can consistently reproduce the human disease (176).

The mechanism of the leukemogenic effect of benzene is not fully understood. The modified base 8-hydroxy-deoxyguanosine has been suggested as a sensitive marker
of DNA damage due to hydroxyl radical attack at the C8 position of guanine (177). Biochemical mechanisms of leukemia have been reviewed and discussed by Snyder and Kalf (178). In 1987, IARC classified benzene as a human carcinogen (179).

Biotransformation in liver is thought to be necessary for its hepatotoxicity and carcinogenicity, and differences in species sensitivity have been ascribed to hepatic metabolism (180,181). Benzene biotransformation is complex, yielding glucuronide and sulfate conjugates of phenol, quinol, and catechol; 1-Phenylmercapturic acid; muconaldehyde; and trans,trans-muconic acid by ring scission. Furthermore, inhalation of benzene may stimulate microsomal mixed-function oxidase, cytochrome P450, responsible for the oxidation of benzene and for the generation of oxygen radicals (182). Formation of oxygen radicals could be a major cause of benzene toxicity, with involvement of multiple mechanisms including synergism between arylating and glutathione-depleting reactive metabolites and oxygen radicals (183). Because benzene and its hydroxylated metabolites are substrates for the same cytochrome P450 enzymes, competitive interactions among metabolites are possible (184).

Metabolic interactions between benzene and ethanol (185) and between benzene and gasoline (186) vapors have been reported. A critical question is whether the low concentrations of benzene that occur in the environment derived from its use as a gasoline component could pose a significant human health risk (187). In this regard, Weisel et al. (188) state that the amount of metabolism by ring-opening pathway is greater at low exposure in humans than at high exposure, and maintain that care is needed when extrapolating potential health risk from high to low dose.

In a Swedish study, the increased risk of acute myeloid leukemia in gasoline station attendants was associated with the benzene content in gasoline (15). In their 1996 study, Raabe and Wong (189) combined studies in cohorts of petroleum workers in the United States and the United Kingdom in a single database for cell-type-specific leukemia analysis; they did not find an increase in acute and chronic myeloid leukemia or in acute lymphocytic or chronic lymphocytic leukemia. There was also no excess risk of leukemia or acute myeloid leukemia reported in an Italian study (190) or in a Nordic study (9), both carried out in service station attendants.

Furthermore, an analysis of the published case-control studies conducted in 1996 by Bezabeh et al. (191) indicated that exposure to petroleum products and employment in petroleum-related occupations does not represent a risk factor for multiple myeloma. Wong and Raabe (192) recently published a study in a multinational cohort of more than 250,000 petroleum workers; the pooled analysis indicates no increased risk of multiple myeloma as a result of exposure to benzene.

A genotoxic effect at relatively low levels of benzene exposure (0.1 ppm) has been reported in a recent study in humans (193). Various papers reporting benzene blood levels in groups occupationally exposed to gasoline indicate that occupational activities are significant determinants of blood benzene concentrations (194–197). A global evaluation of risks from benzene exposure is reported by Paustenbach et al. (198) and Hughes et al. (199).

**Alkylbenzenes: Toluene and Xylene**

Alkylbenzenes are single ring aromatic compounds containing one (toluene) or more (xylene) saturated aliphatic side chains.

Toluene and xylene, occurring in small amounts in gasoline blends and standard gasoline formulations as a result of the octane process, are mainly absorbed by inhalation (acute irritation of eyes and respiratory tract) and through the skin. Direct skin contact promotes defatting of the keratin layer, with vasodilation, erythema, and dry, scaly dermatitis. Xylenes are more potent skin irritants than benzene or toluene (55).

Other than liver, kidney, and heart damage, dysfunction in the CNS is the principal health consequence of exposure to alkylbenzenes. The effects vary from severe neurological disorder (acute inhalation due to sniffing abuse) to deficits in neurobehavioural function in occupationally exposed groups.

Neurobehavioural effects deriving from subacute exposure to toluene have been investigated in rats (200) and mice (201). In a rat model for Parkinson's disease, Cintra et al. (202) observed that toluene challenged the dopaminergic nigrostriatal system. Persistent damage to CNS functions resulting from rat subchronic exposure to m-xylene has also been reported (203).

Pryor et al. (204) observed that chronic exposure to toluene produced an irreversible progressive time- and dose-dependent hearing loss. While this auditory impairment was recorded in laboratory animals at high toluene exposures as compared to permissible exposure levels (205), Liu and Fechter (206) demonstrated that the toxic effect in cochlear cells can be observed in vitro at toluene concentrations that are more realistic for human occupational exposure. Finally, effects on the thyroid gland (mild reduction of follicle size) have been reported after toluene inhalation in female rats (207).

Recent papers on embryotoxic potential indicate that toluene (at much higher levels than those in the natural environment) induce some fetotoxic, but not teratogenic, effects (208–210), while xylenes appeared to be embryotoxic/fetotoxic and teratogenic, causing an increase in preimplantation losses, skeletal anomalies, reduced fetal body weight, and delayed development (211,212).

Wilkins (213) presented a 1997 update on toluene teratogenicity.

Because of the wide industrial use of these alkylbenzenes, studies continue to describe the effects of these compounds in occupationally exposed groups (214–217). Due to the scarce information available, the long-term effects on human health from the use of alkylbenzenes as additives in gasoline remain uncertain (19,218).

Greenberg (219) pointed out that because subgroups that are sensitive to neurobehavioural effects of toluene exist, adequate measures of protection for the general population are required (even if atmospheric levels of toluene in urban environments are about 10 times lower that the inhalation reference concentration (RfC) fixed at 0.1 ppm for general populations chronically exposed to toluene). Lagorio et al. (194), Hakkola and Saarinen (196), and Lawryk et al. (220) presented evaluations of levels in various exposure scenarios.

The principal metabolites of alkylbenzenes appear to have a low order of toxicity and are readily excreted. Thus, toluene is oxidized at the level of the methyl group to benzoic acid, which is conjugated with glycine to form hippuric acid and then excreted. Hippuric acids are also metabolites of xylene. Tardif et al. (221) suggested that there is a metabolic interaction between toluene and xylene that affects the metabolic disposition of both chemicals. An exhaustive picture of toluene and xylene effects in plants and aquatic animals has been reported by Nielsen and Howe (208) and Crookes et al. (211).

In 1989, IARC reviewed the database for toluene (222) and xylene (223) and concluded that there was inadequate evidence for carcinogenicity of both compounds in humans and in experimental animals; thus, toluene and xylene were not classifiable as to their carcinogenicity in humans (Group 3).

In a 1990 study, no evidence of carcinogenic activity was found in male or female F344 rats and B6C3F1 mice exposed to toluene by inhalation (224).

**Final Considerations**

Toxicological data obtained in laboratory animals (including carcinogenic, teratogenic, and mutagenic activities) and epidemiological results from human studies in relation to
gasoline exposure (published during the last 7 years) provide a broad picture of the current aspects of the essential toxicological properties of gasoline.

Knowledge of the intrinsic toxic properties of gasoline and its constituents is undoubtedly important, but to identify the potential toxicity, i.e., the damage (including carcinogenic effects) that can occur in man and his environment under normal conditions of use, is even more important.

Concerning gasoline carcinogenicity, any potential cancer risk in humans from gasoline cannot yet be identified with precision for several reasons:

- The diverse and different constituents of gasolines (leaded, unleaded, oxygenated, reformulated gasolines)
- The inconclusive evidence of carcinogenic risk in animals from some constituents
- The carcinogenic effect ascribed to some constituents detected in experimental animals but ascribable to mechanisms (e.g., α2u-globulin in gasoline) that are not present in humans
- The insufficient data for determination of long-term effects in occupationally exposed cohorts
- The inadequate information on general population exposure.

Despite this, it should be noted that many individual investigators (109, 225–229) who report results of carcinogenicity studies in experimental animals (often with insignificant or only borderline incidence of carcinogenic events) often suggest direct extrapolation of such results to humans. This abnormal situation is further aggravated by interpretative manipulation of valid data by nonexperts or by scientific dabbler, and by diffusion through newspapers and other mass-media messages of real and presumed hazards deriving from gasolines. Lotti (230), referring to any toxicological study, indicated that the simple modification of a form of expression by using “a variety of figures of speech, lexical forms and rhetorical emphasis” can send completely different messages.

In reference to the carcinogenic potential of a chemical, the assumption that what is carcinogenic in animals is also carcinogenic in humans is “biologically plausible and prudent,” according to IARC (231), and there are many scientists who agree. No single investigator or expert of mass-media communication can claim carcinogenicity and/or toxicity for a chemical. Only a responsible health regulatory agency (such as IARC, the EPA, etc.) can adequately express judgment on the toxicity and carcinogenicity of any chemical (including gasoline) in laboratory animals after reviewing and assessing all available data, discarding irrelvant and confounding data, and utilizing up-to-date studies in toxicokinetics, metabolism, and receptor binding. Indeed, every chemical entity represents a unique case due to its structure, toxicological properties, and circumstances of use (232).

This overview provides a reference source, covering the period 1990–1997, for the toxicological aspects of gasoline and its constituents. By reviewing and summarizing the concordant and conflicting results of such studies, we intended to highlight the considerable difficulties that exist for any precise identification of potential toxic effects on health, meaning the risk for humans, especially in relation to the need for a correct and adequate handling of other variables such as preexisting pathological conditions, cigarette smoking, drug use, etc. Furthermore, wide margins of uncertainty in risk assessment from gasoline exposure also derive from the variable characteristics of the different gasoline mixtures, as well as from incomplete results or incorrect scientific approach and/or performance of some toxicological studies.

Thus, the problem regarding the potential toxicity of gasoline is still open, even though it is clear that modern unleaded gasolines present less risk to human health due to the lower quantities of benzene and lead. With further changes in the formulation of gasoline, manufacturing processes, and methods of distribution, the future promises even lower levels of risk to health and the environment.

The evaluation of potential health effects of gasoline represents a very complex process, with wide margins of uncertainty. On the other hand, uncertainty is a characteristic of any scientific conclusion, while certainties are frequently, perhaps justifiably, requested by regulatory authorities in order to make better decisions and correct political choices. Moreover: “The demand for [an absolute] certainty is a sign of weakness, and by persisting, induces paralysis” [Mark Rutherford (233)].

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