

# PREDICTING THE TOXICITY OF GASOLINE VAPORS BASED ON KNOWLEDGE OF FUEL COMPONENTS

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## ABSTRACT

The toxicity of chemicals in mixtures such as gasoline may differ greatly from that observed when the chemicals are tested as pure compounds. For example, metabolic activation is the critical first step in the development of toxicity after exposure to benzene. Gasoline components inhibit benzene metabolism and thus reduce benzene's toxicity. The extent to which inhibition occurs depends on the gasoline vapor composition and inhaled concentration. Gasoline vapors vary in inhibitory effects based on the aromatic content of the mixture. Additionally, metabolic inhibition is dependent on concentration, with inhibition increasing with increasing concentration. The challenge in estimating the effect of gasoline components on the inhibition of benzene metabolism is to determine the shape of the concentration-inhibition function.

## INTRODUCTION

Benzene is a ubiquitous industrial and environmental pollutant (Runion and Scott, 1985). It is present in automobile emissions, both evaporative and combusive, and has been detected in cigarette smoke (Wallace, 1990; Wallace and Pellizzari, 1986). Exposure to benzene is most likely associated with coexposure to other volatile organic chemicals normally present in the environment.

Benzene is myelotoxic and carcinogenic at high concentrations. Epidemiology studies have shown that people develop blood dyscrasias, such as pancytopenia, aplastic anemia, and acute myelogenous leukemia following repeated exposure to high concentrations of benzene (Goldstein, 1977; Rinsky et al., 1987). Cytogenetic damage has been observed in humans who have developed benzene-associated hemopathies, especially leukemia (Huff et al., 1989). This correlation between cytogenetic damage in leukemia suggests that cytogenetic alterations in bone marrow cells may be a good marker for genetic alterations in bone marrow stem cells that precede the development of leukemia.

Benzene is not thought to be a direct-acting agent in the bone marrow, but rather is converted to bioactive metabolites (in the liver) which cause myelotoxicity (Irons, 1985; Eastman et al., 1987; Barale et al., 1990). The metabolism of benzene involves a series of oxidations of the benzene ring by cytochrome P450 monooxygenases (Figure 1). After absorption into the blood and translocation to the liver, benzene is metabolized by cytochrome P450 2E1 to its major metabolite, phenol (Figure 1; Smith et al., 1989). Phenol is further oxidized by the same cytochrome P450 to the polyhydroxylated metabolite, hydroquinone (Koop et al., 1989; Schlosser et al., 1993). Both phenol and hydroquinone can translocate in the blood to the bone marrow where they interact with critical blood cell components. Alternatively phenol and hydroquinone are detoxified by Phase II conjugating enzymes such as sulfotransferases and glucuronyl transferases. Muconaldehyde, a reactive metabolite of benzene, is also thought to be formed by a two step oxidation of benzene although the mechanism and isozyme involved are unknown.

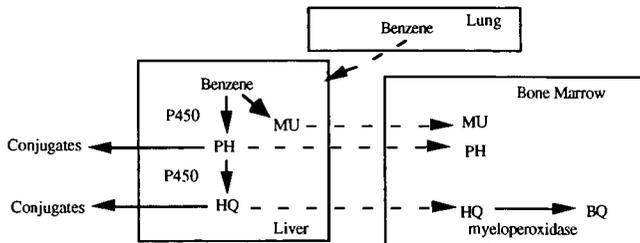


Figure 1. Metabolic scheme for benzene and its major metabolites. BQ = Benzoquinone, HQ = Hydroquinone, PH = Phenol, MU = Muconaldehyde

## INTERACTIONS WITH OTHER VOLATILE ORGANICS

The multiplicity of benzene's metabolic pathways provides opportunities for modulation of benzene metabolism, either by competition with other organic chemicals for available

enzyme sites, by induction or inhibition of the oxidation or conjugation enzymes, or by direct competition among benzene and its metabolites for enzyme sites. Other volatile organics can modulate the toxicity and metabolism of benzene. Gad-El-Karim et al. (1984) investigated the genotoxicity of benzene in mice treated orally with benzene or combinations of benzene and toluene. Benzene alone was clastogenic to bone marrow cells and elevated numbers of micronucleated polychromatic erythrocytes (micronuclei, MN) were detected in mice receiving benzene compared with controls. When both benzene and the aromatic hydrocarbon, toluene, were coadministered, the clastogenic effect of benzene was reduced considerably. Similar results were noted when chromosomal aberrations were analyzed. These investigators hypothesized that toluene inhibited the metabolism of benzene and that one or more metabolites of benzene were responsible for the myeloclastogenic effects.

Andrews et al. (1977) used incorporation of  $^{59}\text{Fe}$  into maturing red blood cells to evaluate the effects of benzene on erythropoiesis. Mice were given injections of benzene alone or combinations of benzene plus toluene. In parallel studies, the effect of toluene on the pharmacokinetics of benzene and its metabolites was also investigated. Coadministration of toluene and benzene resulted in reduction in the quantity of benzene metabolites measured in urine compared with the benzene-only exposed group. Unmetabolized benzene was exhaled. Coexposure to toluene also counteracted the benzene-induced reduction in  $^{59}\text{Fe}$  uptake. Thus, toluene both reduced benzene metabolism and protected against benzene-induced suppression of iron utilization by red cells. The concentration of benzene in bone marrow was similar in mice given only benzene compared with mice given benzene and toluene. In contrast, concentrations of benzene metabolites in bone marrow of mice given benzene alone were much higher than those found when benzene was coadministered with toluene. In summary, the observation that toluene reduced both the level of benzene metabolites and the inhibition of iron uptake suggests that metabolism of benzene is closely related to its hematotoxicity. Toluene protects against benzene-induced hematotoxicity by reducing the level of benzene metabolites in bone marrow through suppression of benzene metabolism.

Mutual metabolic suppression between benzene and toluene also occurs in people. For example, Inoue et al. (1988) examined both the exposure concentration during a workshift and the benzene metabolite concentrations in urine of male Chinese workers exposed to either benzene, toluene, or a mixture of both chemicals. Urinary levels of the benzene metabolites phenol and hydroquinone were lower in the workers exposed to both toluene and benzene compared with those exposed to benzene alone. The investigators hypothesized that biotransformation of benzene to its hydroxylated metabolites in people is suppressed by coexposure to toluene.

Gasoline, one important source of environmental exposure to benzene, also contains toluene and other aromatic and aliphatic hydrocarbons such as xylene and hexane. These hydrocarbons could inhibit benzene metabolism. Travis et al. (1992) examined the effect of coexposure to gasoline vapor on the metabolism of benzene. Coexposure to gasoline vapors increased the maximum rate for benzene metabolism and decreased the apparent affinity of the enzyme for benzene. Generally, enzymatic inhibition is associated with a decrease in the maximum rate and an increase in the apparent affinity. Thus, the results of this study are in contrast with the demonstration of the inhibition of benzene metabolism by toluene previously reported by these investigators using a similar experimental system (Purcell et al., 1990). Clearly more research on the interaction of benzene with gasoline components is necessary to adequately assess the potential human health risks for this environmentally important mixture.

#### MODEL SIMULATIONS OF BENZENE-GASOLINE INTERACTIONS

Figures 2 and 3 show the results of physiologically-based pharmacokinetic model simulations of four different benzene-gasoline exposure scenarios. Figure 2 assumes that the major gasoline components are aromatic chemicals. Figure 3 assumes that the major gasoline components are aliphatic chemicals. Simulations in the A panels are for an exposure to gasoline at the threshold limit value (300 ppm) and simulations in the B panels are for an exposure at 2000 ppm, the concentration used in the chronic toxicity studies with unleaded gasoline. Comparison of simulations for the aromatic and aliphatic volatiles at the high doses (Panel B) compared with the lower dose (Panel A) demonstrates the dose dependence of inhibition by gasoline components on benzene metabolism. Less benzene is metabolized (more inhibition) at the higher (B) compared with the lower (A) dose. This can be determined by comparing the differences for the simulations for benzene alone to benzene plus other volatiles for each figure. At the low dose, the difference is small; at the high dose, the difference is large.

A comparison of the relative effect of gasoline vapor composed primarily of aromatics can be seen by comparing results of model simulations at the low dose (Figures 2A and 3A). The aromatic chemicals appear to be better inhibitors of benzene at this lower concentration than do aliphatics. Again, this can be seen by the difference in the predicted benzene metabolized when benzene is given alone compared with the predictions when benzene plus other volatiles are given. The results of these simulations suggest that the scientific answer to the question, "Does gasoline inhibit benzene metabolism?", is that inhibition depends both on dose and on chemical composition.

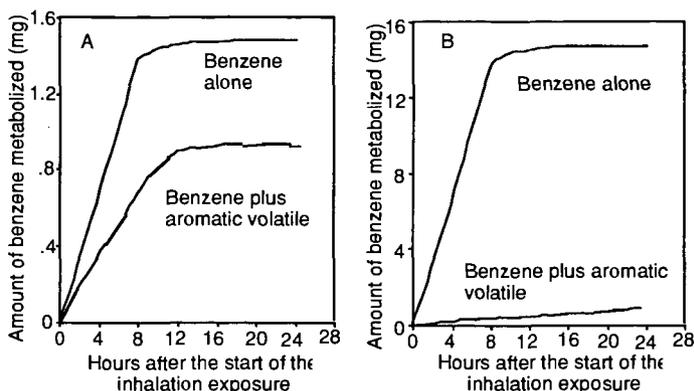


Figure 2. Model simulations for the effect of aromatic volatiles on the metabolism of benzene by one mouse after an 8-hr exposure to (A) 300 ppm of aromatics together with 5 ppm benzene or to a 5 ppm benzene exposure only or to (B) 2000 ppm of aromatics together with 40 ppm benzene or to a 40 ppm benzene exposure only.

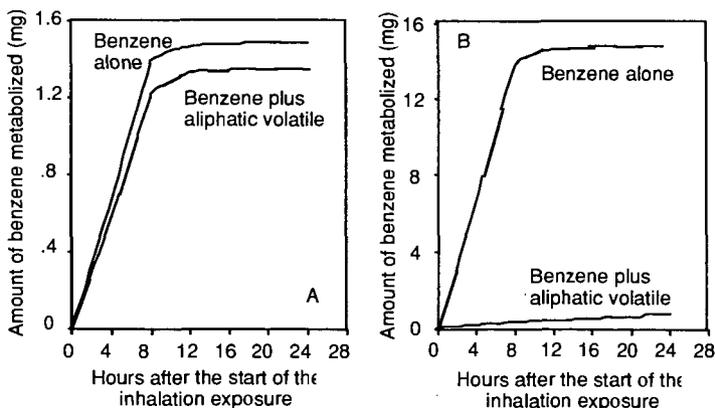


Figure 3. Model simulations for the effect of aliphatic volatiles on the metabolism of benzene by one mouse after an 8-hr exposure to (A) 300 ppm of aliphatics together with 5 ppm benzene or to a 5 ppm benzene exposure only, or to (B) 2000 ppm of aliphatics together with 40 ppm benzene or to a 40 ppm benzene exposure only.

## CONCLUSIONS

There are two major factors that influence the potential interaction of benzene and gasoline components. These issues relate to the composition of the gasoline vapor and the inhaled concentration (or dose) of the vapor. Some components of gasoline may be better inhibitors of benzene metabolism than are others. The aromatic components, such as xylene and toluene, may be more effective competitors against benzene for active enzyme sites by nature of their greater solubility in tissues compared with benzene. Concentrations of these chemicals might be higher in the metabolizing organs such as liver and therefore these aromatics might be better able to compete with the benzene for active metabolic sites.

The fractional composition of volatile gasoline components changes with increasing vaporization temperature. Of most importance is the composition of the vaporized fuel relative to the whole gasoline. With increasing temperature, the aliphatic volatile organics make up an increasing percentage of the total percent of the gasoline organics and the aromatics become a decreasing percent. The scientific question relevant for risk assessment relates to the range of composition of vapors that people are likely to be exposed to when using gasoline.

Inhibition of one chemical on the metabolism of another is typically a dose-dependent phenomenon. At very high doses, inhibition can approach 100%. At very low doses, inhibition may be insignificant. The challenge in estimating the effect of gasoline components on the inhibition of benzene metabolism is to determine the shape of the concentration-inhibition function. The added complexity is that the shape of the benzene concentration-target tissue dosimetry function is also highly nonlinear. In actuality, it is not just the effect of gasoline components on the total benzene metabolized that must be addressed, but also the effect of possible inhibition of the formation of oxidized metabolic products such as hydroquinone.

#### ACKNOWLEDGMENTS

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