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Health assessment of gasoline and fuel oxygenate vapors: Subchronic inhalation toxicity

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ABSTRACT

Sprague Dawley rats were exposed via inhalation to vapor condensates of either gasoline or gasoline combined with various fuel oxygenates to assess whether their use in gasoline influences the hazard of evaporative emissions. Test substances included vapor condensates prepared from an EPA described "baseline gasoline" (BGVC), or gasoline combined with methyl tertiary butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether (G/DIPE), ethanol (G/EtOH), or t-butyl alcohol (G/TBA). Target concentrations were 0, 2000, 10,000 or 20,000 mg/m^3 and exposures were for 6 h/day, 5 days/week for 13 weeks. A portion of the animals were maintained for a four week recovery period to determine the reversibility of potential adverse effects. Increased kidney weight and light hydrocarbon nephropathy (LHN) were observed in treated male rats in all studies which were reversible or nearly reversible after 4 weeks recovery. LHN is unique to male rats and is not relevant to human toxicity. The no observed effect level (NOAEL) in all studies was 10,000 mg/m^3, except for G/MTBE (<2000) and G/TBA (2000). The results provide evidence that use of the studied oxygenates are unlikely to increase the hazard of evaporative emissions during refueling, compared to those from gasoline alone.

1. Introduction

The 1990 amendments to the Clean Air Act (CAA) mandated the use of oxygenates in motor gasoline. In 1994, the U.S. Environmental Protection Agency (EPA) issued a final rule under the Act which added new health effects information and testing requirements to the Agency's existing registration requirements. As described in more detail in a companion paper (Henley et al., 2014), requirements include inhalation exposures to evaporative emissions of the gasoline or additive in question. The health endpoints include assessments for standard subchronic toxicity, neurotoxicity, genotoxicity, immunotoxicity, developmental and reproductive toxicity, and chronic toxicity/carcinogenicity. The results of chronic toxicity testing of gasoline and gasoline combined with MTBE have already been reported (Benson et al., 2011) and reported elsewhere in this issue are the findings for genotoxicity (Schreiner et al., 2014), neurotoxicity (O'Callaghan et al., 2014), immunotoxicity (White et al., 2014), reproductive toxicity (Gray et al., 2014), and developmental toxicity testing in mice and rats (Roberts et al., 2014a,b). This paper describes the results of subchronic toxicity testing submitted to EPA.

Test substances evaluated in the 13 week toxicity studies included vapor condensates prepared from an EPA defined "baseline gasoline" (BGVC), as well as gasoline combined with methyl tertiary butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether (G/DIPE), ethanol (G/EtOH), or t-butyl alcohol (G/TBA). The goal of the studies was to provide information on the extent to which the use of oxygenates in gasoline might alter the hazard of evaporative emissions that are encountered during refueling of vehicles, compared to those from gasoline alone.

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2. Materials and methods

2.1. Test substance preparation and characterization

Gasoline and gasoline/oxygenate vapor condensates were prepared and supplied in 100 gallon gas cylinders by Chevron Research and Technology Center (Richmond, CA). Since only 5-gallon cylinders were practical for use in exposure operations, the test substance was dispensed as needed at the testing facility from the 100 gallon cylinders into 5-gallon cylinders using nitrogen pressurization. The methodology for preparation and analytical characterization of the samples is described in a companion paper (Henley et al., 2014). Test substances included vapor condensates prepared from an EPA described "baseline gasoline" (BGVC), identified as API Lot 99-01, and gasoline combined with methyl tertiary butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether (G/DIPE), ethanol (G/EtOH), or t-butyl alcohol (G/TBA).

2.2. Experimental design

Seven separate 13-week inhalation toxicity studies were conducted of gasoline and gasoline/oxygenate vapor condensates at Huntingdon Life Sciences (East Millstone, NJ). In each of these studies, a total of 120 animals were used in the main and recovery phases (Table 1) and an additional 160 animals were used in satellite studies to more closely evaluate neurotoxicity, immunotoxicity and genotoxicity (Table 2). The exposure initiation dates for the studies were: BGVC – September 13, 2000; G/MTBE – February 6, 2001; G/EtOH – April 17, 2001; G/TAME – June 26, 2001; G/ETBE – October 23, 2001; G/DIPE – February 12, 2002; and G/TBA – June 25, 2002.

After 13 weeks of exposure, 20 control and 20 high dose animals were kept unexposed for an additional 4 weeks to serve as a recovery group. Clinical chemistry, coagulation and hematology evaluations were conducted on ten males and females per treatment group after 4 and 13 weeks of exposure as well as on the recovery group at the end of the recovery period. Necropsies were conducted on 10 male and 10 female animals per exposure group at the end of 13 weeks, and on all of the recovery group animals consisting of 10 male and 10 female animals in the control and high concentration groups.

2.3. Animal selection, assignment and care

CD (Sprague–Dawley derived) [Crl: CD@ IGS BR] albino rats (approximately 6 weeks old) were received from Charles River Laboratories (Kingston, NY) for each study. Animals were acclimated for at least 16 days after receipt and examined to confirm suitability for study.

Each rat was assigned a temporary number upon receipt and then identified with a metal ear tag bearing its assigned animal number. The assigned animal number plus the study number comprised the unique animal number for each animal. In addition, each cage was provided with a cage card, which was color-coded for exposure level identification and contained study number and animal number information.

Animals considered suitable for study on the basis of pretest physical examinations, body weight data and pretest ophthalmology evaluations were randomly assigned, by sex, to control or treated groups in an attempt to equalize mean group body weights. Individual weights of animals placed on test were within ±20% of the mean weight for each sex for each study. Currently acceptable practices of good animal husbandry were followed (National Academy of Sciences, 1996). Huntingdon Life Sciences, East Millstone, NJ is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

2.4. Diet and drinking water

Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, MO) was available without restriction, except during exposures. Fresh feed was presented weekly. Analysis of each feed lot used during this study was performed by the manufacturer. There were no known contaminants in the feed which were expected to interfere with the results of this study. Water was available without restriction via an automated watering system, except during exposures. Monthly water analyses are conducted by Elizabethtown Water Company, Westfield, NJ (Raritan-Millstone Plant). In addition, water samples were collected biannually from representative rooms in the testing facility for microbiological analyses by a subcontract laboratory. There were no known contaminants in the water which were expected to interfere with the results of this study.

2.5. Housing and environmental conditions

Animals were housed individually in suspended stainless steel wire mesh cages. During exposure periods, animals were individually housed in stainless steel, wire mesh cages within a 1000 L stainless steel and glass whole-body exposure chamber. A twelve hour light/dark cycle controlled via an automatic timer was provided. Temperature and relative humidity were monitored in accordance with Testing Facility SOPs and maintained within the specified range (18–26 degrees C, and 30–70%, respectively) to the maximum extent possible. Excursions outside the specified range were not considered to have affected the integrity of the study.

During exposure periods chamber static pressure was recorded every half-hour. Chamber temperature and relative humidity were

Table 1
Main and recovery study design.

Main study		Main and recovery study animals					
		Number of animals at initiation of exposure[a]		Necropsy and microscopic pathology[b]			
				Terminal (13 weeks)		After 4 weeks recovery	
Exposure group	Target concentration (mg/m^3)	M	F	M	F	M	F
Control	0 (air only)	20	20	10	10	10	10
Low	2000	10	10	10	10	–	–
Middle	10,000	10	10	10	10	–	–
High	20,000	20	20	10	10	10	10

[a] Exposures were 6 h/day, generally 5 days/week for 13 weeks, for at least 65 exposures.
[b] Complete postmortem evaluations were performed on animals.