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Health assessment of gasoline and fuel oxygenate vapors: Developmental toxicity in mice

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ABSTRACT

CD-1 mice were exposed to baseline gasoline vapor condensate (BGVC) alone or to vapors of gasoline blended with methyl tertiary butyl ether (G/MTBE). Inhalation exposures were 6 h/d on GD 5–17 at levels of 0, 2000, 10,000, and 20,000 mg/m^3. Dams were evaluated for evidence of maternal toxicity, and fetuses were weighed, sexed, and evaluated for external, visceral, and skeletal anomalies. Exposure to 20,000 mg/m^3 of BGVC produced slight reductions in maternal body weight/gain and decreased fetal body weight. G/MTBE exposure did not produce statistically significant maternal or developmental effects; however, two uncommon ventral wall closure defects occurred: gastroschisis (1 fetus at 10,000 mg/m^3) and ectopia cordis (1 fetus at 2000 mg/m^3; 2 fetuses/1 litter at 10,000 mg/m^3). A second study (G/MTBE-2) evaluated similar exposure levels on GD 5–16 and an additional group exposed to 30,000 mg/m^3 from GD 5–10. An increased incidence of cleft palate was observed at 30,000 mg/m^3 G/MTBE. No ectopia cordis occurred in the replicate study, but a single observation of gastroschisis was observed at 30,000 mg/m^3. The no observed adverse effect levels for maternal/developmental toxicity in the BGVC study were 10,000/2000 mg/m^3, 20,000/20,000 for the G/MTBE study, and 10,000/20,000 for the G/MTBE-2 study.

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 gasoline blended with the 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 subchronic toxicity testing (Clark et al., 2014), genotoxicity (Schreiner et al., 2014), neurotoxicity (O'Callaghan et al., 2014) and immunotoxicity (White et al., 2014). This paper describes the results of developmental toxicity testing in mice which have been submitted to EPA. Other papers in this issue report on the results of developmental and reproductive toxicity testing in rats (Roberts et al., 2014; Gray et al., 2014).

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2. Materials and methods

Two studies to meet the CAA requirements were conducted at ExxonMobil Biomedical Sciences, Inc. (EMBSI) Laboratory Operations, Mammalian Toxicology Laboratory, 1545 Route 22 East, P.O. Box 971, Annandale, New Jersey 08801. The test materials were a baseline gasoline vapor condensate (BGVC) and a vapor condensate of gasoline mixed with methyl-t-butyl ether (G/MTBE). Due to an unusual finding in the G/MTBE study, a repeat of the G/MTBE study was conducted at Huntingdon Life Sciences (HLS), Princeton Research Center, 100 Mettlers Road, East Millstone, NJ. The postmortem maternal and fetal evaluations and analyses were conducted by staff of RTI International (RTI), POB 12194, 3040 Cornwallis Road, Research Triangle Park, NC, 27709, at the HLS testing facility. All of the laboratories are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).

2.1. CAA Studies

2.1.1. Test material preparation and characterization

Test articles included two different vapor condensates: one prepared from an EPA-described "baseline gasoline" (BGVC) (US EPA, 1994), and the other from BGVC blended with methyl-t-butyl ether (G/MTBE) prepared and supplied in 100 gal gas cylinders by Chevron Research and Technology Center (Richmond, CA). The methodology for preparation and analytical characterization of the samples is described in a companion paper (Henley, et al., 2014). The test substances, as received, were considered the "pure" substance for the purpose of dosing.

2.1.2. Animal selection and care

The test animals were Caesarean-originated Virus Antibody Free (VAF) Crl:CD-1® (ICR) BR outbred albino mice supplied by Charles River Laboratories, Inc.(Portage, MI). Sexually mature males were used for mating purposes only. Females were 12–13 weeks of age and weighed 26–35 g at the start of mating.

Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, Missouri) was available ad libitum. Analysis of each feed lot used during this study was performed by the manufacturer. Water was available without restriction via an automated watering system. There were no known contaminants in the feed or water to interfere with test results of these studies. Animals were without food and water while in the exposure chambers.

2.1.3. Housing and environmental conditions

Animals were housed individually in suspended stainless steel wire mesh cages. A twelve hour light/dark cycle was controlled by an automatic timer. Temperature and relative humidity were maintained within the specified range (64–72 degrees F, and 30–70%, respectively).

2.1.4. Experimental design

The experimental design is shown in Table 1. Untreated animals were mated (1 nulliparous female; 1 male) until sufficient plug positive presumed-pregnant females were identified by the presence of a copulatory plug in the vagina. Plug-positive female mice were distributed by body weight into four different exposure groups (25/group) on gestational day (GD) 0. Presumed pregnant females were exposed to 0 mg/m^3 (air control), 2000 mg/m^3, 10,000 mg/m^3 and 20,000 mg/m^3, 6 h/day from GD 5 through GD 17. The highest exposure level represented approximately 50% of the Lower Explosive Limit of each test material. On GD 18, animals were sacrificed and evaluated as described in Section 2.1.6.

2.1.5. Administration of test substance and exposure schedule

The experimental and control animals were placed into whole-body inhalation chambers operated under dynamic conditions for at least 6 h per day for GD 5–16 at 2000, 10,000, and 20,000 mg/m^3, or for GD 5–10 at 30,000 mg/m^3 after target exposure levels were reached. The animals remained in the chambers for at least an additional 23 min (theoretical equilibration time) while the test atmosphere cleared. Females were exposed in 1.0 m^3 stainless steel and glass chambers operated at a flow rate of approximately 12–15 air changes/hour. During exposure periods, animals were individually housed in stainless steel, wire mesh cages. Flow rate and slightly negative pressure was monitored continuously and recorded approximately every 30 min. Light (ca. 30–40 foot-candles 1.0 m above the floor) and noise levels (<85 db) in the exposure room were measured pretest and at the beginning, middle and end of the study. Oxygen levels in the exposure chambers were maintained between 20.6 and 20.7%.

The control group was exposed to clean filtered air under conditions identical to those used for groups exposed to the test substance. The test substance was administered fully vaporized in the breathing air of the animals. The chamber concentrations were measured in the breathing zone of the mice by on-line gas chromatography (GC). The chromatographic analyses were used to assess the stability of the test substance over the duration of the study. Additionally, sorbent tube samples were collected once weekly and stored in a freezer for analysis by a detailed capillary GC method to compare component proportions of the test material atmosphere with the liquid test material. Homogeneity of the exposure system was validated prior to the start of each study. Particle size determination confirmed that exposures were to vapor only (see Section 3.1).

2.1.6. Experimental evaluation

Animals were examined for viability at least twice daily during the study. Body weights were recorded prior to selection and on GD 0, 5, 8, 11, 14, 17, and 18. Food consumption was measured for mated females on GD 5, 8, 11, 14, 17, and 18. A clinical examination of each female occurred prior to selection and daily during gestation. Additionally, group observations of the animals for mortality and obvious toxic signs while in the chambers were recorded at 15, 30, 45, and 60 min after initiation of the exposure and then hourly during each exposure.

Body weights were recorded on GD 18, the day of necropsy. Dams were sacrificed by CO_2 asphyxiation followed by exsanguination. A gross necropsy was performed on all confirmed-mated females. Uterine weights with ovaries attached were recorded at the time of necropsy. Uterine contents were examined and the numbers and locations of implantation sites, early and late resorptions, and live and dead (live or dead *in utero*) fetuses were counted. Ovarian corpora lutea also were counted. The uteri of all apparently non-pregnant females were stained with 10% ammonium sulfide to confirm non-pregnancy status. Evaluations of dams during cesarean section and subsequent fetal evaluations were conducted without knowledge of treatment group in order to minimize bias. Each fetus was weighed and examined externally for gross malformations and variations. Fetal sex was determined by external examination and confirmed internally only on those fetuses receiving visceral examinations. Fetuses were euthanized by hypothermia after the external examination and weighing.

The viscera of approximately one-half of the fetuses of each litter were examined by fresh dissection (Staples, 1974; Stuckhardt and Poppe, 1984) prior to decapitation of the fetus. The heads were preserved in Bouin's solution for at least two weeks, then rinsed and subsequently stored in 70% ethanol. Free-hand razor blade sections of the Bouin's-fixed fetal heads were examined for the presence of abnormalities. The remaining fetuses were eviscerated,