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

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ABSTRACT

Gasoline-vapor condensate (BGVC) or condensed vapors from gasoline-blended with methyl t-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) were evaluated for developmental toxicity in Sprague–Dawley rats exposed via inhalation on gestation days (GD) 5–20 for 6 h/day at levels of 0 (control filtered air), 2000, 10,000, and 20,000 mg/m^3. These exposure durations and levels substantially exceed typical consumer exposure during refueling (<1–7 mg/m^3, 5 min). Dose responsive maternal effects were reduced maternal body weight and/or weight change, and/or reduced food consumption. No significant malformations were seen in any study. Developmental effects occurred at 20,000 mg/m^3 of G/TAME (reduced fetal body weight, increased incidence of stunted fetuses), G/TBA (reduced fetal body weight, increased skeletal variants) and G/DIPE (reduced fetal weight) resulting in developmental NOAEL of 10,000 mg/m^3 for these materials. Developmental NOAELs for other materials were 20,000 mg/m^3 as no developmental toxicity was induced in those studies. Developmental NOAELs were equal to or greater than the concurrent maternal NOAELs which ranged from 2000 to 20,000 mg/m^3. There were no clear cut differences in developmental toxicity between vapors of gasoline and gasoline blended with the ether or alcohol oxygenates.

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 are the findings for subchronic toxicity testing (Clark et al., 2014), genotoxicity (Schreiner et al., 2014), neurotoxicity (O'Callaghan et al., 2014), immunotoxicity (White et al., 2014), reproductive toxicity (Gray et al., 2014), n2band developmental toxicity testing in mice (Roberts et al., 2014a). This paper describes the results of developmental toxicity testing in rats.

2. Materials and methods

Six separate studies were conducted by ExxonMobil Biomedical Sciences, Inc. (EMBSI) Mammalian Toxicology Laboratory, Annandale, New Jersey, of a gasoline vapor condensate (BGVC)

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and vapor condensates of gasoline mixed with methyl-t-butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), ethanol (G/EtOH), or t-butyl alcohol (G/TBA). The gasoline/diisopropyl ether (G/DIPE) study was conducted at Huntingdon Life Sciences Princeton Research Center, East Millstone, NJ. Both of the laboratories are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).

2.1. Test material 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). The test material 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).

2.2. Animal selection and care

The test animals were Cesarean-originated Virus Antibody Free (VAF) Crl:CD®(SD)IGSBR outbred albino rats supplied by Charles River Laboratories, Inc, Raleigh, NC. Sexually mature virgin females were allocated to the study groups after confirmation of mating. Sexually mature males were used for mating purposes only in the EMBSI studies and not involved in the actual exposures to test materials. HLS employed timed mated females shipped from Charles River Laboratories to arrive no later than GD4 for the G/DIPE study.

Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, MO) was available without restriction. 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 expected to interfere with the results of this study. Animals were without food and water while in the exposure chambers.

2.3. 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. For all studies temperature and relative humidity were maintained within the specified range (18–24 °C, and 30–70% relative humidity, respectively). Light (maintained approximately 30–40 foot-candles at 1.0 m above the floor) and noise levels (maintained below 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 19.0 and 20.7%.

2.4. Experimental design

The experimental design is described in Table 1. Untreated animals were mated (1 nulliparous female with 1 male) until sufficient presumed pregnant females were identified by the presence of a copulatory plug in the vagina. Plug positive female rats were distributed by body weight into four different exposure groups (25/group) on gestation day [GD] 0; for the G/DIPE study, timed-pregnant animals were distributed by body weight on GD 4. 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 to GD 20. The highest exposure level represented approximately 50% of the lower explosive limit (LEL) for each material.

2.5. 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 after target exposure levels were reached from GD 5 through GD 20. 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 approximately 12–15 air changes/hour. Flow rate and slightly negative pressure were monitored continuously and recorded approximately every 30 min.

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 rats by on-line gas chromatography (GC). These chromatographic analyses were used to assess the stability of the test substance over the duration of the study. Analytical concentrations of G/DIPE in the HLS study were determined by infrared spectrometry. 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.

Distribution samples were drawn from twelve different points within the exposure chambers at each exposure level during the validation of the exposure system to determine homogeneity of exposure concentrations. A particle size determination of the aerosol portion of the test atmosphere was conducted at least once during the chamber trials from the 0 mg/m^3 and 20,000 mg/m^3 concentrations.

2.6. Experimental evaluation

Animals were examined for viability at least twice daily during the study. Body weights were taken prior to selection, and on GD 0 (EMBSI studies), 5, 8, 11, 14, 17, 20 and 21. Food consumption was measured for mated females on GD 5, 8, 11, 14, 17, 20 and 21. A clinical examination was given to each female 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 regularly during each exposure.

Dams were sacrificed by CO_2 asphyxiation followed by exsanguination on GD 21. 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, corpora lutea and the numbers and locations of implantation sites, early and late resorptions, and live and dead (alive or dead *in utero*) fetuses were counted. The uteri of all apparently non-pregnant females were stained with 10% ammonium sulfide to confirm non-gravid status. Evaluations of dams during necropsy and subsequent fetal evaluations were conducted without knowledge of treatment group in order to minimize bias.

Fetuses were counted, 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 CO_2 asphyxiation in the EMBSI studies and by intraperitoneal sodium pentobarbitol in the HLS study.

The viscera of approximately one-half of the fetuses of each litter were examined by fresh dissection (Staples, 1974; Stuckhardt

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