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Toxicological assessments of rats exposed prenatally to inhaled vapors of gasoline and gasoline–ethanol blends ☆,☆☆

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ABSTRACT

The primary alternative to petroleum-based fuels is ethanol, which may be blended with gasoline in the United States at concentrations up to 15% for most automobiles. Efforts to increase the amount of ethanol in gasoline have prompted concerns about the potential toxicity of inhaled ethanol vapors from these fuels. The well-known sensitivity of the developing nervous and immune systems to ingested ethanol and the lack of information about the neurodevelopmental toxicity of ethanol-blended fuels prompted the present work. Pregnant Long–Evans rats were exposed for 6.5 h/day on days 9–20 of gestation to clean air or vapors of gasoline containing no ethanol (E0) or gasoline blended with 15% ethanol (E15) or 85% ethanol (E85) at nominal concentrations of 3000, 6000, or 9000 ppm. Estimated maternal peak blood ethanol concentrations were less than 5 mg/dL for all exposures. No overt toxicity in the dams was observed, although pregnant dams exposed to 9000 ppm of E0 or E85 gained more weight per gram of food consumed during the 12 days of exposure than did controls. Fuel vapors did not affect litter size or weight, or postnatal weight gain in the offspring. Tests of motor activity and a functional observational battery (FOB) administered to the offspring between post-natal day (PND) 27–29 and PND 56–63 revealed an increase in vertical activity counts in the 3000- and 9000-ppm groups in the E85 experiment on PND 63 and a few small changes in sensorimotor responses in the FOB that were not monotonically related to exposure concentration in any experiment. Neither cell-mediated nor humoral immunity were affected in a concentration-related manner by exposure to any of the vapors in 6-week-old male or female offspring. Systematic concentration-related differences in systolic blood pressure were not observed in rats tested at 3 and 6 months of age in any experiment. No systematic differences were observed in serum glucose or glycated hemoglobin A1c (a marker of long-term glucose homeostasis). These observations suggest a LOEL of 3000 ppm of E85 for vertical activity, LOELs of 9000 ppm of E0 and E85 for maternal food consumption, and NOELs of 9000 ppm for the other endpoints reported here. The ethanol content of the vapors did not consistently alter the pattern of behavioral, immunological, or physiological responses to the fuel vapors. The concentrations of the vapors used here exceed by 4–6 orders of magnitude typical exposure levels encountered by the public.

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1. Introduction

Sources of renewable fuels and alternative energy have been topics of legislation during the past decade. The Energy Policy Act of 2005 (P.L. 109–58) established a Renewable Fuel Standard (RFS) program to incorporate renewable fuels in the American automotive fuel supply. Two years later, the Energy Independence and Security Act of 2007 (EISA, P.L. 110–140) was enacted to encourage energy independence and to limit climate change by reducing greenhouse gas emissions from automobiles through increased use of renewable fuels. EISA required the U.S. Environmental Protection Agency (EPA) to revise the

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RFS to increase the volume of renewable components of transportation fuels from 9 billion gal in 2008 to 36 billion gal by 2022 (EPA-HQ OAR-2010-0133; FRL-9234-6, Federal Register, Vol: 75, No. 236, Dec. 2010).

Increased use of alternative fuels and the large population likely to inhale their vapors argue for information about the public health impacts of evaporative emissions of these fuels. Ethanol is the only current commercially-viable alternative fuel for spark-ignition engines, and it constituted 95% of the biofuel produced in the U.S. in 2009 (EPA, 2011). Health consequences of ingested ethanol are well known. In addition to its acute effects in adults, consumption during pregnancy has been linked to a range of teratogenic effects known as fetal alcohol spectrum disorder (FASD), which includes craniofacial malformations, persistent neurodevelopmental and neurocognitive deficits and immune system dysfunction (Abel, 2006; Kane et al., 2012; Mukherjee et al., 2005). The magnitude and severity of the deficits have been linked to the peak blood ethanol concentration (BEC) in the mother, but are also influenced by the number of exposures and the developmental stage at which exposure occurs (Driscoll et al., 1990; Riley and McGee, 2005; Zajac and Abel, 1992). Significant changes in cognitive function have been reported in rats whose mothers were exposed to ethanol by the oral route during pregnancy at maternal BECs of about 80 mg/dL (Savage et al., 2002), indicating that serious effects of prenatal ethanol exposure can occur in the absence of morphological abnormalities. These well-documented effects of ethanol were observed after dosing by the oral route, rather than by inhalation, the relevant route for exposures to fuel vapors.

For these reasons, previous studies from this laboratory assessed the neurodevelopmental, immunological and physiological impacts of maternal inhalation of ethanol in a rat model. These studies found no systematic, dose-related effects of prenatal ethanol in adult offspring on sensory systems (Boyes et al., 2014), unconditioned behaviors, immune function, blood pressure or clinical markers of liver and kidney functions (Beasley et al., 2014), even at maternal BECs approaching 200 mg/dL. Clear effects on cognitive function were limited to increased anticipatory responses in a choice reaction time test in rats whose mothers had been exposed during pregnancy to 21,000 ppm ethanol and an estimated BEC of 192 mg/dL although other changes that were not concentration-dependent were observed on cue learning and reference memory (Oshiro et al., 2014).

Compared to ethanol, the effects of gasoline vapors are less thoroughly studied. Low reproductive and developmental toxicity of gasoline vapors has been reported, albeit at concentrations several orders of magnitude above those expected in garages or from fueling operations, which rarely exceed 100 ppb (Zielinska et al., 2007). For example, McKee et al. (2000) used a vapor recovery unit to collect a relatively volatile fraction of gasoline, similar to the vapors likely to be airborne during fueling operations. They reported that rats exposed to 5000, 10,000, or 20,000 μg/m^3 (approximately 1900, 3700, or 7500 ppm) of this vapor during gestation showed no treatment-related effects other than hyaline droplet nephropathy in the kidneys of the male rats. Because this effect is specific to the male rat kidney (Hard et al., 1993), it did not raise concern for human health. Reproductive performance was not affected: maternal growth and litter sizes in the F1 generation were normal and the offspring survived and grew normally; no effects were seen in the F2 generation. Generally null findings consistent with this study have been reported from rats exposed to vapors of unleaded gasoline (Roberts et al., 2001) and gasoline containing 10% ethanol (Gray et al., 2014; Roberts et al., 2014) at concentrations up to 9000 ppm. Similarly low toxicity has been reported from rodent studies with high flash aromatic naphtha, a product of petroleum refining that contains 70–80% 9-carbon aromatics (McKee et al., 1990; Schreiner et al., 2000). A subchronic study of the effects of vapors from a blend of gasoline and 10% ethanol (E10) in adult rats identified increases in glial fibrillary acidic protein in the brains of males (Clark et al., 2014), suggesting the possibility of neurotoxicity from ethanol-blended gasolines.

None of these prior studies evaluated the potential developmental neurotoxicity of gasoline vapors, or the effects of vapors from fuels containing more than 10% ethanol. The present experiments were conducted to address these issues and the widely-recognized concerns regarding the effects of gestational exposure to ethanol on fetal development. In these experiments, we exposed pregnant rats to vapors of ethanol, gasoline, and two gasoline–ethanol mixtures. We targeted the developing fetus by exposing pregnant rats during the second and third weeks of gestation, a critical period of development during which orally-administered ethanol impairs development of many physiological systems including the CNS (Becker, 1996; Savage et al., 2002), the immune system (Jerrells and Weinberg, 1998), neuroendocrine functions (Zhang et al., 2005) including glucose homeostasis (Chen and Nyomba, 2003) and insulin regulation (Chen and Nyomba, 2004), and cardiovascular functions (Ren et al., 2002).

Our studies were designed to compare a wide range of concentrations of ethanol in gasoline, including neat ethanol (E100), gasoline blended with either 85% or 15% ethanol (E85 or E15), or gasoline without ethanol (E0). This strategy enables quantitative comparisons of dose–effect relationships as a function of ethanol concentration, so that the toxicity of any blend could be estimated. Given that public exposure to these mixtures would occur primarily by breathing vapors during fueling and parking in enclosed garages, the exposures were conducted via the inhalation route.

Because gasoline is a complex mixture of hydrocarbons with a wide range of volatilities, and because repeated daily exposures were planned during the animals' pregnancies, it was important to generate an airborne mixture that remained consistent across hours within each exposure day and across days of gestation. Because vapors generated by heating the entire mixture would change in composition as the more volatile components evaporated first and the less volatile components followed later, we adopted the strategy of exposing the rats to the vapors of condensed evaporative emissions of these fuels (Henley et al., 2014). These vapor condensates (VCs) were generated prior to the experiments by collecting and compressing the most volatile 10% of each fuel mixture; exposures were then conducted simply by warming and releasing the vapor into chambers in which the rats were housed.

The studies of inhaled E100 (ethanol) have been reported previously (Beasley et al., 2014; Boyes et al., 2014; Oshiro et al., 2014). The results of tests of the toxicity of the fuel blends are being reported similarly in three parts. This report documents effects of exposure to the fuels in pregnant rats and their offspring on tests not involving cognitive or sensory functions, which will be reported separately. Tests in the offspring reported here include screening-level assessments of behavioral development using a well-validated battery of tests of unconditioned behaviors (functional observational battery, FOB) and motor activity. Because published evidence (see above) suggested that heart function and glucose homeostasis might be targets of inhaled ethanol and gasoline vapors, systolic blood pressure and serum concentrations of glucose and glycated hemoglobin A1c (GHbA1c, a marker of long-term glucose status) were measured to test these hypotheses. Tests of immune function include humoral and cell mediated immunity.

2. Materials and methods

2.1. Overall design

Three experiments were conducted with the same basic design that was used for the ethanol study (Beasley et al., 2014). In each experiment, pregnant rats were exposed to air or one of three concentrations of vapor of a single fuel blend (E0, E15, or E85) daily for 6.5 h from gestational day (GD) 9 to 20. To evaluate the effects of housing in the exposure chamber during gestation, an additional control group was added to the E0 experiment. This 'cage-control' group was kept in the vivarium during pregnancy and allowed to deliver their offspring