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Selective cognitive deficits in adult rats after prenatal exposure to inhaled ethanol☆

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ABSTRACT

Increased use of ethanol blends in gasoline suggests a need to assess the potential public health risks of exposure to these fuels. Ethanol consumed during pregnancy is a teratogen. However, little is known about the potential developmental neurotoxicity of ethanol delivered by inhalation, the most likely route of exposure from gasoline–ethanol fuel blends. We evaluated the potential cognitive consequences of ethanol inhalation by exposing pregnant Long Evans rats to clean air or ethanol vapor from gestational days 9–20, a critical period of neuronal development. Concentrations of inhaled ethanol (5000, 10,000, or 21,000 ppm for 6.5 h/day) produced modeled peak blood ethanol concentrations (BECs) in exposed dams of 2.3, 6.8, and 192 mg/dL, respectively. In offspring, no dose-related impairments were observed on spatial learning or working memory in the Morris water maze or in operant delayed match-to-position tests. Two measures showed significant effects in female offspring at all ethanol doses: 1) impaired cue learning after trace fear conditioning, and 2) an absence of bias for the correct quadrant after place training during a reference memory probe in the Morris water maze. In choice reaction time tests, male offspring (females were not tested) from the 5000 and 10,000 ppm groups showed a transient increase in decision times. Also, male offspring from the 21,000 ppm group made more anticipatory responses during a preparatory hold period, suggesting a deficit in response inhibition. The increase in anticipatory responding during the choice reaction time test shows that inhaled ethanol yielding a peak BEC of ~200 mg/dL can produce lasting effects in the offspring. The lack of a dose-related decrement in the effects observed in females on cue learning and a reference memory probe may reflect confounding influences in the exposed offspring possibly related to maternal care or altered anxiety levels in females. The surprising lack of more pervasive cognitive deficits, as reported by others at BECs in the 200 mg/dL range, may reflect route-dependent differences in the kinetics of ethanol. These data show that response inhibition was impaired in the offspring of pregnant rats that inhaled ethanol at concentrations at least 5 orders of magnitude higher than concentrations observed during normal automotive transport and fueling operations, which rarely exceed 100 ppb.

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1. Introduction

The Energy Independence and Security Act (EISA), passed by Congress in 2007, mandated the United States to decrease dependence on foreign oil supplies, reduce energy consumption, and address climate change. The EISA required the Environmental Protection Agency (EPA) to revise the Renewable Fuel Standards (RFS) by requiring the volume of renewable fuel[2] blended into transportation fuel to be increased

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[2] As defined by EISA, the generation and use of a "renewable fuel" must reduce greenhouse gas emissions by at least 20% compared to petroleum fuels. RFS2 goals are reviewed in EPA-HQ OAR-2010-0133; FRL-9234-6, Federal Register, Vol: 75, No. 236, Dec. 2010.

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from 9 billion gallons in 2008 to 36 billion gallons by 2022. In response to these new goals (RFS2), the demand for ethanol blended with gasoline has increased. In 2009, 95% of renewable fuels produced in the U.S. were made from corn ethanol, and this trend is likely to continue as long as ethanol remains the dominant renewable fuel (EPA/600/R-10/183F, 2011). Indeed, the percentage of ethanol used in gasoline was recently approved for increase in the United States from 10% (E10) to 15% (E15) with some restrictions (www.epa.gov/otaq/regs/fuels/additives/e15).

In light of the potential benefits of these new fuels to energy independence and to climate change, it is important to assess the possible human health hazards associated with ethanol fuel blends, especially in sensitive populations. Ethanol is a well-known teratogen and neurotoxicant. High levels of ethanol consumption have long been associated with fetal alcohol syndrome (FAS), a classification of effects that includes craniofacial malformations, slowed growth, and mental retardation (Jones and Smith, 1973). More recent research has indicated that deficits in cognitive functions (i.e. learning, memory and attentional processes) can also occur in children exposed gestationally to more moderate levels of alcohol (Berman and Hannigan, 2000; Coles et al., 1997, 2002; Driscoll et al., 1990; Eckardt et al., 1998; Streissguth et al., 1999). Some deficits have also been reported in children exposed to as little as one drink per week (Jacobson et al., 1994; Sood et al., 2001). Although these studies rely on the self-reporting of alcohol consumption in their subjects, and dosages are averaged over time and largely speculative (Abel, 2006), some alcohol clinicians and researchers advise that no amount of alcohol is safe for consumption during pregnancy (Guerri et al., 1999; Jacobson et al., 1994; Streissguth et al., 1999; Vaglenova and Petkov, 1998).

Animal research with ethanol has revealed a qualitatively similar continuum of effects in rats exposed prenatally to ethanol as is observed in humans (Driscoll et al., 1990; Riley et al., 2011; Zajac and Abel, 1992). These studies show complex neuroanatomical and neurophysiological changes in response to prenatal ethanol exposure (Berman and Hannigan, 2000; Guerri, 1998; Guerri et al., 2009). The hippocampus, neocortex and cerebellum are particularly susceptible to ethanol and are believed to underlie effects observed on spatial learning and memory, attentional processes and motor coordination (Berman and Hannigan, 2000; Carneiro et al., 2005; Guerri, 1998, Savage et al., 2010; Valenzuela et al., 2012). The severity of developmental effects from ethanol or alcohol in humans and animals has been linked with the peak blood ethanol concentration (BEC) achieved in the mothers during gestation but is also influenced by the timing and frequency of exposure (Bonthius and West, 1990; Driscoll et al., 1990; Guerri et al., 2009; Streissguth and Martin, 1983).

Although the vast ethanol literature raises potential human health concerns for its use in gasoline, all of the human data and a majority of the animal data are based on studies in which mothers consumed ethanol orally during pregnancy (Zajac and Abel, 1992). Given the large population likely to be exposed to fuels containing ethanol, the hazard of exposure to ethanol vapors alone and in combination with gasoline needs to be evaluated.

Little is known about the developmental effects of inhaled ethanol, the main route of concern for exposure to ethanol–biofuel blends at the gas pump, and even less is known about the developmental toxicity of inhaled ethanol vapors in combination with gasoline vapors. A few studies have measured the developmental effects of inhaled ethanol and gasoline vapors. Nelson et al. (1985b) concluded that inhaled ethanol (20,000 ppm for 7 h daily throughout gestation) was not teratogenic in rats even when the inhaled concentration yielded a BEC of ~200 mg/dL and produced narcosis in the mothers. Tests of neuromuscular ability, activity, avoidance conditioning, and performance of a progressive fixed ratio schedule of reinforcement were negative in animals exposed to 10,000 or 16,000 ppm ethanol throughout gestation, which yielded BECs of 3 and 50 mg/dL, respectively (Nelson et al., 1985a, 1988). Additionally, a significant decrease in serotonin levels in the cerebellum and increased met-enkephalin levels in many brain areas of exposed offspring were reported in the low-dose group; however, the magnitude of these effects was not linearly related to the concentrations nor was it consistent across brain regions (Nelson et al., 1988). Behavioral tests and neurochemical assays were not conducted in offspring exposed to the 20,000 ppm concentration.

Another study investigated the developmental effects of inhaled vapor condensates made from a blend of gasoline and 10% ethanol (E10), and reported slight maternal toxicity (reduced weight gain) but no fetal toxicity at concentrations of vapor condensates ranging from 2000 to 20,000 mg/m^3 (~1061 to 10,615 ppm) inhaled 6 h daily from gestation day (GD) 5 to 20; however, no tests of cognitive function or other neurobehavioral assessments were conducted in the offspring (EPA-HQ-OAR-2003-0065, 2008). No developmental studies were found that looked at blends of 15% ethanol or higher.

To assess the risk of inhaled ethanol–gasoline blends, we began a multifaceted study to evaluate the toxicity of a range of ethanol–gasoline blends including ethanol alone (E100) and vapor condensates made from gasoline alone (E0) and blends of 15% and 85% v/v ethanol/gasoline (E15 and E85, respectively). Each blend was assessed separately, beginning with E100. Here, we report findings from cognitive function tests conducted in adult offspring of rats exposed to concentrations of 0, 5000, 10,000, or 21,000 ppm of ethanol vapor for 6.5 h daily from GD 9 to 20. This developmental period includes a critical stage for neuroepithelial cell proliferation and migration (GD 12–20) in rats that is important for CNS development (Rice and Barone, 2000). Ethanol exposure via the oral route during this time period has been associated with a reduced number of neurons and glial cells in the neocortex and hippocampus (Guerri, 1998; Gressens et al., 1992; Miller, 1992, Miller, 1995; Valles et al., 1997) and deficits in spatial learning, memory, and attention in exposed offspring (Berman and Hannigan, 2000; Hannigan et al., 1993; Hausknecht et al., 2005; Nagahara and Handa, 1997; Neese et al., 2004; Wilcoxon et al., 2005). Information is needed regarding the possibility of similar effects from inhaled ethanol.

Cognitive tests were chosen to assess learning, memory and attentional processes. The spatial learning and memory tests selected have been used extensively in rats and have been shown to be sensitive to prenatal ethanol exposure. These tests include trace fear conditioning to assess cue and context learning (Gilbert, 2011); the Morris water maze to assess spatial learning, reference, and working memory (Morris, 1981; Steele and Morris, 1999); delayed spatial alternation (Widholm et al., 2004) and delayed match to position (Bushnell et al., 1991; Bushnell and Oshiro, 1996), to assess spatial and working memory, respectively. A choice reaction time task was employed to assess attentional processes (Brown and Robbins, 1991). Although not widely used in prenatal ethanol research in rodents, reaction time has been used in human ethanol research (Burden et al., 2005; Coles et al., 1997; Simmons et al., 2002) and in both species to study motor disorders (i.e. Parkinson's disease), cognitive slowing (i.e. in aging) (reviewed in Blokland, 1998) and disorders such as attention deficit/hyperactivity disorder (ADHD, Leth-Steensen et al., 2000; Zahn et al., 1998); the latter is often diagnosed in children with FAS due to impaired attentional processes and increased impulsivity in these children (Fryer et al., 2007).

The concentrations of ethanol used in the current study (5000–21,000 ppm) are several orders of magnitude higher than exposures likely achieved at the gas pump (likely in the ppb range; http://www.epa.gov/otaq/regs/fuels/additive/e15/documents/420r12010.pdf, p. 15; Zielinska et al., 2007). The high concentration was selected to achieve a BEC in the pregnant rat that is associated with developmental deficits in the offspring (~200 mg/dL) in oral dosing scenarios. Achieving this BEC required 6 h of constant exposure to 21,000 ppm, the highest achievable concentration within the limits required by safety considerations. The lower concentrations were selected as whole-number fractions of the high concentration. Given that the lowest reported