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Neurophysiological assessment of auditory, peripheral nerve, somatosensory, and visual system function after developmental exposure to gasoline, E15, and E85 vapors☆,☆☆

David W. Herr [a,*], Danielle L. Freeborn [a], Laura Degn [a], Sheppard A. Martin [a], Jayna Ortenzio [b], Lara Pantlin [c], Charles W. Hamm [a], William K. Boyes [a]

[a] Neurotoxicology Branch, Toxicity Assessment Division, MD B105-04, NHEERL, ORD, U.S. E.P.A., Research Triangle Park, NC 27711, USA
[b] Student Contractor to the U.S. E.P.A. Research Triangle Park, NC 27711, USA
[c] Meredith College, Raleigh, NC 27607, USA

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ABSTRACT

The use of gasolines blended with a range of ethanol concentrations may result in inhalation of vapors containing a variable combination of ethanol with other volatile gasoline constituents. The possibility of exposure and potential interactions between vapor constituents suggests the need to evaluate the possible risks of this complex mixture. Previously we evaluated the effects of developmental exposure to ethanol vapors on neurophysiological measures of sensory function as a component of a larger project evaluating developmental ethanol toxicity. Here we report an evaluation using the same battery of sensory function testing in offspring of pregnant dams exposed during gestation to condensed vapors of gasoline (E0), gasoline blended with 15% ethanol (E15) or gasoline blended with 85% ethanol (E85). Pregnant Long-Evans rats were exposed to target concentrations 0, 3000, 6000, or 9000 ppm total hydrocarbon vapors for 6.5 h/day over GD9 – GD20. Sensory evaluations of male offspring began as adults. The electrophysiological testing battery included tests of: peripheral nerve (compound action potentials, nerve conduction velocity [NCV]), somatosensory (cortical and cerebellar evoked potentials), auditory (brainstem auditory evoked responses), and visual functions. Visual function assessment included pattern elicited visual evoked potentials (VEP), VEP contrast sensitivity, dark-adapted (scotopic) electroretinograms (ERGs), light-adapted (photopic) ERGs, and green flicker ERGs. The results included sporadic statistically significant effects, but the observations were not consistently concentration-related and appeared to be statistical Type 1 errors related to multiple dependent measures evaluated. The exposure concentrations were much higher than can be reasonably expected from typical exposures to the general population during refueling or other common exposure situations. Overall the results indicate that gestational exposure of male rats to ethanol/gasoline vapor combinations did not cause detectable changes in peripheral nerve, somatosensory, auditory, or visual function when the offspring were assessed as adults.

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1. Introduction

The use of biofuels has become widespread due to a growing interest in sustainable energy sources and a desire for independence from foreign oil supplies. The U.S. Energy Policy Act of 2005 (Public Law 109–58) created a renewable fuel standard (RFS) federal program that requires renewably sourced fuels (e.g. ethanol) blended into gasoline in increasing amounts over years. Currently, more than 95% of U.S. gasoline contains up to 15% ethanol (http://www.afdc.energy.gov/fuels/ethanol_blends.html) and, according to goals of the RFS, 36 billion gallons of renewable fuel is to be blended into the supply by the year 2022.

Gasoline is a complex and variable mixture, typically containing hundreds of individual hydrocarbon constituents in the C4–C12 range. It is important to consider the possibility that metabolic interactions may occur between the components of gasoline, resulting in increased

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* Corresponding author at: 109 T.W. Alexander Drive, MD B105-04, NHEERL/TAD/NB, U.S. EPA, Research Triangle Park, NC 27711, USA.

E-mail addresses: Herr.david@epa.gov (D.W. Herr), Freeborn.danielle@epa.gov (D.L. Freeborn), Degn.laura@epa.gov (L. Degn), sam506@gmail.com (S.A. Martin), Ortenzio.jayna@epa.gov (J. Ortenzio), lpantlin@rams.colostate.edu (L. Pantlin), Hamm.charlesw@epa.gov (C.W. Hamm), Boyes.william@epa.gov (W.K. Boyes).

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toxicity. Haddad et al. (2000a, 2000b) developed physiologically based pharmacokinetic models for multiple constituents of gasoline, and found that competition for metabolic enzymes could predict changes in blood levels of the compounds. Another pharmacokinetic model has been developed that considers gasoline as a complex mixture, allowing comparisons of changes in metabolism with that of single constituents (Dennison et al., 2004). Previously, Tardif et al. (1997) examined pharmacokinetic parameters for three gasoline constituents (toluene, *m*-xylene, ethylbenzene) and concluded that exposure to permissible concentrations for the mixture would be unlikely to result in biologically significant alterations in pharmacokinetics. However, none of these studies examined possible metabolic interactions if ethanol was blended with gasoline.

The U.S. EPA issued a rule in 1994 under Section 211(b) of the Clean Air Act that required fuel and fuel additive manufacturers to evaluate the potential toxicity of inhaled vapors of gasoline with and without addition of a number of compounds, which were then considered to be potential oxygenate additives. The American Petroleum Institute (API) organized an industrial consortium, referred to as the 211(b) research group, to conduct the required studies. Subsequently, multiple studies were completed of potential toxicity of baseline gasoline vapor condensates (BGVC) alone, and BGVC with the addition of several oxygenate compounds, one of which was (EtOH). Each oxygenate was added to the baseline gasoline at the maximum expected treatment rate for that oxygenate, which was 10% for EtOH. These studies included a range of potential health outcomes of concern including subchronic inhalation toxicity to adults (including neurotoxicity) and developmental and reproductive toxicity. The results of these multiple studies were provided to the EPA and are publically available in the docket for this test rule (Federal Docket, 2003). In addition, the summary results of these studies have been published recently in a series of peer-reviewed publications (Swick et al., 2014).

Briefly, baseline gasoline and gasoline blended with ethanol (up to 20,000 mg/m^3) showed no adverse reproductive findings (Gray et al., 2014; Roberts et al., 2014). Similarly, inhalation of industry average gasoline (from 1990; up to 9000 ppm) resulted in no differences between control and treated groups in malformations, total variations, resorptions, fetal body weight or viability (Roberts et al., 2001). Low toxicity was also reported from inhalation of high flash aromatic naphtha (used to create higher gas octane blending components) (McKee et al., 1990; Schreiner et al., 2000). Additionally, the volatile fraction of gasoline was collected and rats were exposed to concentrations of approximately 1900, 3700, and 7500 ppm (McKee et al., 2000). No reproductive parameters were affected in that study. The only observed effect was hyaline droplet nephropathy in kidneys of the parental male rats which is male rat specific and not relevant to human health risk assessment (Hard et al., 1993).

A suggestion of neurotoxicity produced by exposure to BGVC with ethanol (6 h/day, 5 days/week, for 13 weeks) was reported by O'Callaghan et al. (2014). A small, but statistically significant, increase in glial fibrillary acidic protein (GFAP) was found only in the cerebellum of exposed adult male rats, but not in female rats. These findings provide only modest evidence for neurotoxic effects in exposed adult rats. However, substances that cause neurotoxicity in adult animals may be potential developmental neurotoxicants. Therefore, there is a potential concern for developmental neurotoxicity from combined exposure to gasoline ethanol blends, in particular considering the large populations potentially exposed, albeit intermittently, to gasoline–ethanol vapors.

The series of studies conducted under the 211(b) program did not include an evaluation of developmental neurotoxicity. None of the studies examined functional outcomes (such as sensory function) in the offspring of exposed dams. In addition, the gasoline ethanol blends in the 211(b) studies contained 10% ethanol (E10). Currently fuels in the U.S. are routinely blended with up to 15% ethanol (E15) for general use, and may include up to 85% ethanol (E85) for "flexible fuel" vehicles. Another consideration is that the composition of baseline gasoline in the U.S. has changed since mid-1990s when the baseline fuel requirements for the 211(b) program were established. Modern gasoline, in particular that used in areas designated and being non-attainment for ground-level ozone, has been reformulated to reduce evaporative and combustion emissions including those of volatile organic compounds, toxic air pollutants and NOx emissions. Therefore, the evaporative emissions from modern gasoline have a different composition than those evaluated in the 211(b) program. It may be postulated that emissions from modern reformulated gasoline would be less toxic than those of the 211(b) baseline gasoline vapor condensates (BGVC), but this assumption has not been tested experimentally.

In a previous paper (Boyes et al., 2014), the developmental effects of ethanol inhalation at various concentrations (0, 5000, 10,000, or 21000 ppm) were investigated in pregnant Long-Evans rats. Peripheral nerve, auditory, and visual endpoints were examined and showed minimal effects that could be attributed to exposure to ethanol vapors. Due to the increasing amounts of ethanol blended with gasoline (and the potential for metabolic interactions between ethanol and gasoline constituents), it is important to analyze developmental effects resulting from inhalation of not just ethanol, but also those of gasoline vapors alone and with various concentrations of ethanol.

In the present study, the developmental effects of different ethanol gasoline blends were investigated (0, 15, 85% ethanol; E0, E15, E85). Pregnant dams in the second to third week of gestation were exposed to several concentrations (0, 3000, 6000, 9000 ppm) of each blend. Brainstem auditory evoked responses (BAERs), compound nerve action potentials (CNAPs), nerve conduction velocity (NCV), somatosensory evoked potentials recorded from the cortex (SEP_{cortex}) and cerebellum ($SEP_{cerebellum}$), steady state visual evoked potentials (VEPs) and electroretinograms (ERGs) were recorded from the adult offspring of dams who had previously experienced gestational inhalation. This allows comparisons of the results from inhalation of gasoline vapors only (E0) and two mixtures ethanol and gasoline (E15 and E85) with the lack of effects after gestational inhalation of ethanol (Boyes et al., 2014), as well as investigation of toxicological interactions that may occur after inhalation of increasing concentrations of ethanol–gasoline blends.

2. Materials and methods

2.1. Animals and exposure

In three separate studies, pregnant Long-Evans rats (Charles River Laboratories, Raleigh, NC) were obtained (E0 = 84 dams (12 dams used as cage controls, see Bushnell et al., 2015 for details), E15 and E85 = 72 dams) and exposed to control air or one of three concentrations (3000, 6000, or 9000 ppm) of three different vapor condensates made from: 1) a feedstock of 100% summer-grade gasoline (E0, 60% by weight Conventional Regular Unleaded Gasoline, Western Refining Company, El Paso, TX and 40% by weight Conventional Premium Unleaded Gasoline, Chevron Products Company, Richmond, CA), 2) a feedstock made from gasoline–ethanol blend containing 85% summer-grade gasoline and 15% fuel grade EtOH (E15; denatured ethanol, Plains Terminal, Richmond, CA), and 3) a feedstock made from gasoline–ethanol blend containing 15% summer-grade gasoline and 85% fuel grade EtOH (E85). Details regarding generation of the vapors and their composition, animal handling, and randomization of pups to dams (within treatment groups) are detailed in Bushnell et al. (2015). Vapor condensates for E0, E15, and E85 were generated by Chevron USA, Inc. (Richmond, CA) be slowly heating 1000 gal of each feedstock, drawing off the vapor, and condensing it. This process continued until a 10% volume of the feedstock was obtained (Bushnell et al., 2015). The condensates were shipped to EPA, where they were used to generate the test atmospheres. An evaporator was set to 15 °C above the boiling point of the condensate, and the vapors were directed into the exposure chambers (Bushnell et al., 2015).Animals