Evaluation of RFA/GrE Submission in Partial Fulfillment of 40 CFR 79 Tier 1 and Tier 2 E15 Registration Requirements

United States Environmental Protection Agency

Evaluation of RFA/GrE Submission in Partial Fulfillment of 40 CFR 79 Tier 1 and Tier 2 E15 Registration Requirements

U.S. Environmental Protection Agency

United States Environmental Protection Agency

EPA-420-R-12-010
February 2012

Evaluation of RFA/GrE Submission in Partial Fulfillment of 40 CFR 79 Tier 1 and Tier 2 E15 Registration Requirements[1]

Introduction

In 2009, Growth Energy and 54 ethanol manufacturers submitted an application to EPA for a waiver under section 211(f)(4) of the Clean Air Act (CAA or "the Act"). The application sought a waiver for gasoline-ethanol blends of up to 15 vol% ethanol (E15) for all motor vehicles and nonroad vehicles, engines, and equipment.[2] On April 21, 2009, EPA published notice of receipt of the application and requested public comment on all aspects of the application to assist the Administrator in determining whether the statutory basis for granting the waiver request had been met (74 FR 18228).

On October 13, 2010, EPA took two actions on the waiver request based on the information available at that time ("October Waiver Decision").[3] First, it partially approved Growth Energy's waiver request to allow the introduction of E15 into commerce for use in MY2007 and newer light-duty motor vehicles, subject to several conditions. The October Waiver Decision was based on a determination that E15 will not cause or contribute to a failure of MY2007 and newer light-duty motor vehicles to achieve compliance with the emissions standards to which they were certified under section 206 of the CAA over their useful lives. Second, the Agency denied the waiver request for MY2000 and older light-duty motor vehicles, heavy-duty gasoline engines and vehicles, highway and off-highway motorcycles, and other nonroad engines, vehicles, and equipment. The Agency also deferred making a decision on the waiver request for MY2001–2006 light-duty motor vehicles to await the results of additional testing being conducted by the Department of Energy (DOE). On January 21, 2011, EPA partially approved Growth Energy's waiver request to allow the introduction of E15 into commerce for use in MY 2001-2006 light-duty motor vehicles after receiving and analyzing the completed DOE test data ("January Waiver Decision").[4] The January Waiver Decision noted that additional steps must be completed before E15 may be distributed or sold. The January Decision stated: "These steps include but are not limited to submission of a complete E15 fuels registration application by the fuel and fuel additive manufacturers who wish to introduce E15 into commerce, and EPA review and approval of the application, under the regulations at 40 CFR Part 79".[5]

Section 211 of the Act requires registration of designated motor fuels and fuel additives (F/FAs). Registration requirements at 40 CFR 79 include emissions speciation testing and a literature search of the associated emissions (Tier 1 testing) and animal testing of exposure to emissions for purposes of determining health effects

[1] The information in the submissions discussed below was evaluated by EPA's Office of Air and Radiation and Office of Research and Development. This document was prepared utilizing input from EPA's Office of Air and Radiation, Office of Research and Development and Office of General Counsel.
[2] Since E15 has greater than 2.7 wt% oxygen content, E15 needs a waiver under CAA section 211(f)(4).
[3] 75 FR 68094 (November 11, 2010).
[4] 76 FR 4662 (January 26, 2011).
[5] 76 FR 4663 (January 26, 2011).

(Tier 2 testing). In certain cases, a small business is exempt from some or all of this testing.

On February 18, 2011, October 19, 2011, and February 3, 2012, the Renewable Fuels Association (RFA) and Growth Energy (GrE) submitted Tier 1 and Tier 2 data and analyses which would, if approved by EPA, partially satisfy the requirements for a fuel or fuel additive manufacturer's registration of E15 under 40 CFR Part 79. For any potential registrant with over $50 million in annual revenue, Tier 1 and Tier 2 requirements must be met before registering E15. Specifically, RFA/GrE submitted Tier 1 emissions speciation testing and for Tier 2 testing provided an analysis making the case that Alternative Tier 2 testing previously conducted for E10 and baseline gasoline should be sufficient for testing that would otherwise be required under the regulations for E15. An evaluation of the RFA/GrE submissions for fulfillment of these Tier 1 and Tier 2 testing requirements is the subject of this document.

As explained in detail below, based on our evaluation, we conclude that the Tier 1 and Tier 2 data and testing requirements for an E15 registration application would be fulfilled by the RFA/GrE submissions. It is important to note, however, that the RFA/GrE submissions do not constitute a complete application nor do they meet all of the requirements for a complete application. Under the Clean Air Act and EPA's fuel registration regulations, every fuel or fuel additive manufacturer that intends to introduce E15 into commerce must submit for EPA approval a fuel registration application for E15. With RFA/GrE's permission, E15 fuel/fuel additive manufacturers may rely on RFA/GrE submissions for completing the Tier I and Teir 2 portions of their applications. To submit a complete application, each E15 fuel manufacturer must also submit specified information about its company. EPA will act on complete applications as they are received. An E15 fuel manufacturer may introduce E15 into commerce only after EPA has approved its registration application and the fuel manufacturer meets the misfueling mitigation conditions.

Tier 1 Requirements

The Tier 1 registration regulations at 40 CFR 79.52 require a characterization of the emission products which are generated by evaporation and combustion of E15 in a motor vehicle. Combustion testing must be conducted with and without aftertreatment of exhaust emissions. A literature search for information on the potential toxicological, environmental, and other public welfare effects is required for emission products, with the exception that it is not required for those emission products which are the same as the emission products of baseline gasoline (E0). A test group organized by the American Petroleum Institute tested E0 and E10 and conducted the literature search for E0's and E10's emission products.

RFA contracted to have the Tier 1 testing performed by the Southwest Research Institute (SwRI) in San Antonio, Texas. The evaporative emissions report and the combustion emissions report for E15 have been reviewed and would meet the test

requirements required for registration. As noted above, the combustion testing required testing the engine with and without exhaust aftertreatment (i.e., the catalytic converter). In all, 21 new emission products were found compared to E0. In the catalyzed exhaust, only ethanol, 2-methyl hexane, and methacrolein were new. Seventeen of the new products were in the untreated exhaust. Ethane, propane, propyne, and ethanol were new products in the E15 evaporative emissions, although ethane, propane and propyne were found in the E0 combustion emissions. In all, nine new emission products were found compared to E10. In the catalyzed exhaust, only 2-methyl hexane was new. All nine were in the untreated exhaust. Ethane, propane, and propyne were new products in the E15 evaporative emissions compared to the E10 evaporative emissions, but were found in the E10 combustion emissions. RFA/GrE submitted the literature search for all of these new products on October 19, 2011. It has been reviewed and would meet the applicable regulatory requirements.

Tier 2 Requirements

The Tier 2 registration regulations at 40 CFR 79.53 require health effects testing, and the Alternative Tier 2 testing provisions can be found at 40 C.F.R. § 79.58(c). In an August 20, 1997 notification, EPA issued Alternative Tier 2 testing requirements for baseline gasoline and various oxygenated gasolines including E10.[6] This was based upon EPA's determination that alternative test procedures would yield more useful data, as described below EPA required alternative testing for baseline gasoline and various oxygenated gasoline groups, including E10. The health studies for E10 that were required under the notification have now been completed and approved. The studies were undertaken by the Section 211(b) Research Group.

The primary difference between the testing for E10 under the Alternative Tier 2 requirements and Standard Tier 2 requirements is that the Alternative Tier 2 testing focuses on the health effects of evaporative emissions. Alternative Tier 2 testing for E10 and other fuel/fuel additives did not include examination of combustion emissions due to methodological complications caused by carbon monoxide (i.e., carbon monoxide in the exhaust gases would kill the test animals before any useful information could be developed). In the notification requiring Alternative Tier 2 testing for baseline gasoline and several oxygenated fuels, including E10, EPA explained the rationale for focusing on evaporative emissions and why the combustion emissions studies would likely not produce useful information. Since then, there have been advances in potential combustion emission testing techniques that hold promise for effectively addressing the methodological complications caused by carbon monoxide. Additionally, newer vehicles have lower carbon monoxide emission rates. EPA will continue to monitor and assess these advancements and consider the usefulness of requiring such testing under additional (Tier 3) testing provisions for any appropriate gasoline test groups that are or might become a significant part of the U.S. gasoline market.

In their Tier 2 submissions, RFA/GrE state that there is no need for Tier 2 testing of E15 because the emissions species are so similar to emission species of E10 and

[6] The notification can be found at http://www.epa.gov/otaq/consumer/fuels/mtbe/fnlno19a.pdf.

baseline gasoline that the Alternative Tier 2 testing for those fuels should satisfy the Tier 2 health testing requirements for E15. In part, RFA/GrE states:

> Section 211(e)(3)(C) gives EPA authority to "exempt any person from such regulations with respect to a particular fuel or fuel additive upon a finding that any additional testing of such fuel or fuel additive would be duplicative of adequate existing testing." 42 U.S.C. §7545(e)(3)(C). EPA has interpreted this authority to allow fuel or fuel additives "that are similar in composition and usage to those already on the market *to group with* [emphasis added] those similar F/FAs and complete the testing with the other F/FAs in their group." 59 Fed. Reg. at 33,051. "EPA interprets section 211(e) and (b) to give it authority to require any necessary health or welfare effects information for F/FAs that are *significantly different* in composition or usage from currently registered products." *Id.* (emphasis added). EPA focuses on "potential emissions-based effects." *Id.*
>
> In its submission regarding Tier 2 testing for E15, RFA/GrE also states that:
>
> *Tier 2 Testing [for E15] is not necessary, as E15 should fall under the approved Alternative Testing already completed for E10:* EPA's Tier 1 and Tier 2 testing is intended to identify compounds for potential regulation under the Act. 59 Fed. Reg. at 33,042. Based on the analysis above, there are no new compounds that have not already been detected, tested, or analyzed under the fuels already registered and approved under section 211(b)(2). The attached comparison of E10 Tier 1 speciation data and E15 data, shows that E10 Tier 2 health effects data are applicable to E15. A review of the compounds for which the Research Group conducted health effects testing further supports this determination. The 12 species that were both found in E15 and which were tested in GEVC[7] constitutes approximately 95% by weight of E15 emissions. This evidence indicates that E15 should have similar health effects with registered fuels, and do not "pose new or different health risks to the public." *Id.* at 33,050.

Although the grouping approach adopted in the regulations and mentioned by RFA/GrE in its statement above relies heavily on similarity in composition of emissions, E15 cannot be grouped with E10 for purposes of Tier 1 or Tier 2 testing requirements under the regulations.[8] This is because the regulations were finalized with the recognition that the same oxygenate added to fuels at a higher level might produce different emissions and health effects than when added at a lower level.[9] Therefore, under the testing schemes reflected in the regulations, a fuel with 15 volume percent (vol%) ethanol would generally require its own Tier 1 and Tier 2 testing. However, with regard to Tier 2 health testing, the regulations recognize that, in certain cases, for one or more reasons, previous testing might provide results which would be reasonably

[7] GEVC refers to vapor condensate of baseline gasoline with ethanol (Gasoline Ethanol Vapor Condensate) used in the Alternative Tier 2 health effects studies by the Section 211(b) Research Group, the organization which conducted the Tier 2 testing on E10.

[8] 40 CFR § 79.56(e)(4)(ii)(A)(*1*)(iii).

[9] This is discussed in the preamble of the final testing rule found at 59 FR 33058-33061.

comparable to the results of E15 testing were it to be completed ("reasonably comparable" analysis). The Agency has discretion to decide whether such previous testing could be substituted for the Tier 2 testing requirements for E15. This provision of the regulations is found at 40 CFR 79.53(d) and states that:

> After submission of all information and testing, EPA in its judgment shall determine whether previously conducted tests relied upon in the registration submission are adequately performed and documented and provide information reasonably comparable to that which would be provided by the tests described herein. Manufacturers' submissions shall be sufficiently detailed to allow EPA to judge the adequacy of protocols, techniques, experimental design, statistical analyses, and conclusions. Studies shall be performed using generally accepted scientific principles, good laboratory techniques, and the testing guidelines specified in these regulations.

EPA discussed these issues with RFA/GrE, and RFA/GrE submitted an addendum to its previous submissions providing further support for its position regarding the reasonably comparable analysis. EPA has reviewed RFA/GrE's submissions regarding Tier 2 testing for E15, and we agree with RFA/GrE's basic analysis and conclude that replicating E10 Alternative Tier 2 health effects testing for E15 evaporative emissions would yield reasonably comparable results. This analysis considers the emissions data submitted by RFA/GrE as well as the emissions and Alternative Tier 2 testing data from the 211(b) Research Group that evaluated exposure to baseline gasoline and GEVC. A summary of our findings is below.

"Reasonably Comparable" Review

The Alternative Tier 2 testing program evaluated baseline gasoline vapor condensate and fuels with six different oxygenates, including ethanol at 10% volume, or gasoline ethanol vapor condensate (GEVC). The testing considered subchronic inhalation exposure and effects on reproduction and development in rats exposed to baseline gasoline and GEVC at three dose levels (2,000 mg/m^3, 10,000 mg/m^3, and 20,000 mg/m^3) and a clean air control. Health outcomes evaluated for baseline gasoline and GEVC include genotoxicity, development, neurotoxicity, immunotoxicity, and reproduction. Following exposure to GEVC, the following adverse health outcomes were identified: 1) decreased body weight at the high dose level; 2) neurotoxicity in male rats at all three exposure levels; and 3) immunotoxicity in male and female mice at the high dose. These results are explained in more detail in the following sections.

Prior to discussing the adverse health outcomes in more detail, we first compare the emissions speciation of the baseline gasoline vapor condensate and the GEVC. The 211(b) health effects test reports provide speciated data for 18 compounds that comprised approximately 97 percent of the vapor condensate that the animals were exposed to. See Table 1, columns A and B. (The comparison to RFA/GrE data in columns D-F, related to the generation of the fuel, is discussed in Section IV.) Data were reported on an area-percent basis, and the 18 target hydrocarbons were selected

based on their relative abundance and as being representative of distinct hydrocarbon classes (e.g., paraffins, olefins, aromatics).[10] The primary difference between the baseline gas and GEVC speciation profiles is the presence of ethanol.[11] There are minor differences in the hydrocarbon proportions, but these are most likely impacts caused by the addition of ethanol and do not indicate any actual quantitative change in the absolute levels of these hydrocarbon constituents. Therefore, any differences in health effects observed between the baseline gasoline and the GEVC fuel blend can reasonably be attributed to the presence of ethanol.

[10] "Gasoline Vapor Condensate Characterization Final Report. Study number 167490. Completed March 18, 2009. Performed for API at ExxonMobil Biomedical Sciences, Inc.

[11] The proportional concentrations of 2-methylpropane (isobutane) and 2-methylbutane (isopentane) are higher in more saturated non-oxygenated hydrocarbon portion of the E10 and E15 (and even E0) used in the RFA/GrE speciation than they were in GEVC. This issue is discussed later in this document.

Table 1. Proportional Composition of Fuel Vapors

COMPOUND	A 211(b) Baseline Gasoline Vapor Condensate (%)[5]	B 211(b) Gasoline Ethanol Vapor Condensate - E10 (%)[6]	C RFA/GrE E15 (%)[7]	D RFA/GrE E10 (%)[7]	E RFA/GrE E0 (%)[7]
BUTANE[1]	16.1	11.6	*6.3*	*6.4*	15.2
2-METHYLPROPANE (ISOBUTANE)	2.8	2.2	16.3	16.8	18.0
ETHANOL	NA	13.3	**12.4**	**7.7**	0.0
2-METHYLBUTANE (ISOPENTANE)	34.0	34.0	50.9	53.8	49.9
PENTANE[2]	10.8	10.2	*6.5*	*6.5*	6.4
TRANS-2-PENTENE	2.5	2.1	*0.0*	*0.0*	0.0
2,3-DIMETHYLBUTANE	3.2	2.3	*0.8*	*0.9*	0.8
2-METHYLPENTANE	7.6	5.1	*0.9*	*1.1*	0.9
3-METHYLPENTANE	4.8	2.9	*0.4*	*0.5*	0.4
HEXANE[3]	2.4	2.4	*0.5*	*0.6*	0.5
METHYLCYCLOPENTANE[4]	1.3	1.2	*0.1*	*0.1*	0.1
2,4-DIMETHYLPENTANE	0.9	1.0	*0.1*	*0.2*	0.2
BENZENE	2.1	1.6	*0.0*	*0.0*	0.0
2-METHYLHEXANE	1.3	1.1	*0.0*	*0.1*	0.2
2,3-DIMETHYLPENTANE	1.1	1.1	*0.0*	*0.0*	0.0
3-METHYLHEXANE	1.7	1.2	*0.0*	*0.1*	0.1
2,2,4-TRIMETHYLPENTANE (ISOOCTANE)	1.2	1.3	*0.5*	*0.6*	1.0
TOLUENE	3.0	2.4	*0.0*	*0.0*	0.0
SUM	96.6	97.0	95.8	95.4	93.9

Bold indicates compounds values that merit further evaluation. *Italics* indicates the proportion is less in the modern fuel than in the 211(b) fuel.

1 - 211(b) data were reported as n-butane; RFA/GrE data were reported as butane. We assume these are equivalent categories.

2 - 211(b) data were reported as n-pentane; RFA/GrE data were reported as pentane. We assume these are equivalent categories.

3 - 211(b) data were reported as n-hexane; RFA/GrE data were reported as hexane. We assume these are equivalent categories.

4 - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds in the RFA/GrE data.

5 - These data are from the 211(b) Gasoline Vapor Condensate Characterization Report. The values are a calculated average of the three baseline gasoline vapor condensate (BGVC) fuels analyzed in this study and found in Table 6 of the report: MRD-00-674, MRD-00-695, MRD-03-747. The values are derived as an area-percent basis.

6 - These data are from the 211(b) Gasoline Vapor Condensate Characterization Report, Table 6 (MRD-00-714). The values are derived as an area-percent basis.

7 - These data are from the Evaporative Emissions Characterization of E0, E10, and E15 in Support of the Fuel and Fuel Additive Registration of E15 prepared by SwRI for RFA/GrE in February 2011. They are a subset of information found in Table 5. Average values were used.

I. Body Weight

As noted above, in a 1997 notification, EPA issued Alternative Tier 2 testing requirements for baseline gasoline and various oxygenated gasolines including E10.[12] With regard to fuel oxygenates other than MTBE, the testing program was intended to provide a screening assessment of the potential toxicological effects in test animals of inhalation exposure to the evaporative emissions of oxygenate gasoline fuel formulations including baseline gasoline plus ethanol vapor condensates(GEVC). The assessments were to be conducted in accordance with the relevant provisions of the Health Effects Test Guideline (870 series) published by EPA's Office of Prevention, Pesticides and Toxic Substances (OPPTS). Among the provisions of the guidelines was that the highest concentration tested should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation (e.g. Health Effects Test Guidelines OPPTS 870.3465 90-Day Inhalation Toxicity EPA 712–C–98–204 August 1998). Among the observations from the 90 day inhalation toxicity study of GEVC were lower mean body weight and lower body weight gain in animals exposed to 20,000 mg/m3, which was the highest concentration tested.[13] Decreased body weight gain was also observed in male rats exposed to 20,000 mg/m3 GEVC during the pre-mating period in a study of reproductive toxicity. In addition, decreased body weight gain was observed in pregnant dams exposed to 20,000 mg/m3 GEVC in a study of developmental toxicity.

For comparison, animals exposed to baseline gasoline vapor condensate without ethanol did not show statistically significant changes in body weight parameters in rats exposed for up to 20,000 mg/m3 in a 90-day subchronic inhalation study. However, in a chronic study including 512 exposure days, male rats exposed to 10,000 and 20,000 mg/m3 had significantly lower body weights than did clean air controls throughout the study. There were also decreases in body weight gain in F0 female rats and in F1 male rats exposed to 20,000 mg/m3 baseline vapor condensate in a two-generation reproduction study. Finally, a significant decrease of mean fetal body weight was observed in both sexes and all dose groups relative to controls in the study of baseline gasoline vapor concentrate, although in this study the pup body weights of the air control group were high relative to historic controls.

A reduction of body weight or lower body weight gain at 20,000 mg/m3 indicates that the highest dose tested achieved an observable toxic effect in accordance with the requirements of the test rule. This provision was intended to assure that the test program did not miss any potential adverse outcomes by examining too low a range of concentrations. However, lower body weights, or a reduction in body weight gain, were observed in several studies following exposure to baseline gasoline vapor condensates both with and without added ethanol vapors, suggesting the observed effects on body weight parameters were not uniquely associated with the ethanol component of the gasoline vapor mixture. Because weight loss was not uniquely associated with the

[12] The notification can be found at http://www.epa.gov/otaq/consumer/fuels/mtbe/fnlno19a.pdf.
[13] The studies discussed in these sections are the Alternative Tier 2 test reports performed under the 211(b) requirements discussed earlier and can be found in the docket at EPA-HQ-OAR-2003-0065.

ethanol component of GEVC, this outcome was not considered to be a critical factor in evaluating the potential for adverse health consequences associated with an increased exposure to ethanol vapors if changing from E10 to E15.

II. Neurotoxicity

In our judgment, the 211(b) Research Group's Alternative Tier 2 tests[14] performed for E10 generally did not indicate any potential neurotoxicity concerns at environmentally relevant doses. Although the test results were inconclusive on potential neurotoxicity using GFAP (Glial Fibrillary Acidic Protein, a marker of potential neurotoxicity) as an indicator, in our judgment the GFAP effects seen in these tests are not toxicologically significant. Several factors mitigate potential concern based on these results:

- Whereas statistically significant increases in GFAP concentrations were found for all tested concentrations of E10 in some brain regions of male rats, unsystematic, non-monotonic increases were seen in other brain regions, and no significant changes were observed in females.

- Tests of brain regions that showed statistically significant changes were those in which GFAP values for the control group (exposed to clean air) were low relative to the control values from tests of E0 and the six other fuel oxygenate blends that were evaluated.

- The number of significant differences found among the large number of statistical tests applied to these data (2 sexes x 9 brain regions x 7 fuels x 3 comparisons per fuel = 378 tests) may represent the expected number of false positives at a 5% statistical significance level.

- No significant effects of treatment were observed in neuropathological evaluations, brain weight, brain size measurements or in neurobehavioral assessments (functional observational battery and motor activity) for animals exposed to GEVC. Had such effects been observed, they could have corroborated the GFAP increases.

- Whereas others have reported that repeated exposure to ethanol increases GFAP in rats, the internal blood ethanol concentrations required to produce those effects were much higher than those likely achieved in the 211(b) studies. For example, Udomuksorn et al. (2011)[15] reported significant increases in GFAP in rat brains after oral doses in the range of 2 – 5 g/kg, which produced peak blood

[14] Alternative Tier 2 test reports performed under 211(b) can be found in the docket at EPA-HQ-OAR-2003-0065.

[15] Udomukorn, W., W. Mukem, E. Kumarnsit, S. Vongvatcharanon, and U. Vongvatcharanon. 2011. "Effects of alcohol administration during adulthood on parvalbumin and glial fibrillary acidic protein immunoreactivity in the rat cerebral cortex." *Acta Histochemica* 113:283-289.

ethanol concentrations (BECs) between 30 and 150 mg/dL. In contrast, the highest air concentration of E10 used in the 211(b) studies was 20,000 mg/m^3, for which we can assume that approximately 10% (or 2,000 mg/m^3) was ethanol. Using a pharmacokinetic model for inhaled ethanol in rats,[16] we estimate that the peak blood concentrations achieved by exposure to 2,000 mg/m^3 ethanol vapor for 6 hr would be approximately 0.6 mg/dL. Thus, the BECs achieved in the 211(b) inhalation studies are roughly 50 - 250 times lower than those reported to increase GFAP after oral exposure.

Thus, these considerations suggest that the increases in GFAP observed in the 211(b) studies do not indicate potential health concerns at environmentally relevant doses, and if performed for E15, results would likely be negative.

III. Immunotoxicity

As discussed in more detail below, the 211(b) Alternative Tier 2 tests suggest immunosuppressive effects attributable to the ethanol in the GEVC. Therefore, we consider the potential impact on adverse health outcomes of the additional ethanol in E15 compared to E10.

A. Results of the 211(b) tests

In the Alternative Tier 2 study (White, 2010)[17] of GEVC, a statistically significant impairment of immune function was observed in female rats exposed to 20,000 mg/m^3. Immune function was not evaluated in male rats. At the dose of 20,000 mg/m^3, the primary Immunoglobulin M (IgM) antibody response to immunization was decreased by 85%, relative to controls. This level of immunosuppression is likely to increase vulnerability to infectious diseases, particularly those caused by common bacteria and viruses. The average IgM response appeared to be reduced in animals exposed to 10,000 mg/m^3, but the results were not significantly different from clean air controls. Exposure to 2,000 mg/m^3 GEVC similarly did not cause a statistically significant impairment of immune function. No adverse effects on immune system function were observed in the Alternative Tier 2 study of baseline gasoline vapors alone. The major difference between the composition of the baseline gasoline vapor condensate and the GEVC was the ethanol content, suggesting that immunosuppression was caused either by the ethanol component or by ethanol plus gasoline vapors in the GEVC mixture. Gasoline blended with DIPE and ETBE additives also caused immune suppression, suggesting that other fuel oxygenates in gasoline could produce similar effects.

---

[16] Pastino GM, Asgharian B, Roberts K, Medinsky MA, Bond JA. (1997). A comparison of physiologically based pharmacokinetic model predictions and experimental data for inhaled ethanol in male and female B6C3F1 mice, F344 rats, and humans. Toxicol Appl Pharmacol, 145, 147-57.

[17] White KL., Immunological evaluation of gasoline ethanol vapor condensate in female Sprague Dawley rats using the plaque forming cell assay (2nd Audited Draft Report). Submitted to EPA as a component of: Study No 00-6127 Gasoline ethanol vapor condensate: a 13-week whole-body inhalation toxicity study in rats with neurotoxicity assessments and 4-week in vivo genotoxicity and immunotoxicity assessments, (Final Report). 27 January 2010.

B. E10 and E15 Comparisons

Because it appears that the immunosuppressive effects of GEVC exposure may be attributed to the ethanol component of the mixture or to ethanol plus gasoline vapors, the implications of increasing ethanol concentration from E10 to E15 are discussed in detail. This section focuses on the E10 and E15 evaporative emissions speciation characterization submitted by RFA/GrE[18]. We note that the RFA/GrE's emissions characterizations used a slightly different gasoline hydrocarbon base fuel stock.[19]

As expected, ethanol content (both absolute mass and percent composition) is approximately 1.5 times higher in E15 than E10 (Table 2).

To confirm that ethanol is still the only compound that varies substantially between the various fuel blends, as was the case in the 211(b) baseline gasoline and GEVC fuel blends, we also examined the other speciated constituents in RFA/GrE's data. We found four other hydrocarbon constituents were higher on an absolute or percentage basis with E15 than E10 (Table 2). They include 1) ethane; 2) trans-2-butene; 3) unidentified C7 compounds; and 4) unidentified C9-C12+ compounds. The reported increases were very small, changing by 0.5 mg or less and 0.1% or less. These changes are likely to be random fluctuations (i.e., "noise") in the data. No reliable evidence was presented that vapor components of evaporative emissions other than ethanol increased from E10 to E15 for these fuels. Even if it were assumed that the four non-ethanol components reliably increased in E15 evaporative emissions, the small magnitude of the increases makes it unlikely that these changes reflect a credible concern for adverse health effects.

In addition to the increases in certain hydrocarbons, there were five compounds that were present in the E10 evaporative emissions but not in the E15 evaporative emissions. There were also 22 compounds that decreased in the E15 average test results compared to the E10 average test results.

Table 2: Emission component measurements that were higher in RFA/GrE's evaporative emissions speciation for E15 than E10.

	Absolute Mass (mg)		**Percent Composition (% of total)**	
Compound	**E10**	**E15**	**E10**	**E15**
ETHANE	1.5	1.7	0.1	0.2
TRANS-2-BUTENE	0.0	0.2	0.0	0.0

[18] Southwest Research Institute Final Report 03.15812, "Evaporative Emissions Characterization of E0, E10, and E15 in Support of the Fuel and Fuel Additive Registration of E15", February, 2011, pp. 12-21.

[19] Base fuel stock is the hydrocarbon portion of the gasoline fuel to which the ethanol was added to make the ethanol gasoline blend.

ETHANOL	91.1	136.7	7.7	12.4
UNIDENTIFIED C7	0.5	1.0	0.0	0.1
UNIDENTIFIED C9-C12+	1.0	1.1	0.1	0.1

C. Implications of Increased Ethanol in E15

As discussed above, ethanol or ethanol combined with gasoline constituents is the cause of the adverse immunotoxicity result in the 211(b) GEVC study. Since the ethanol component of E15 vapors would be greater than that from the same concentration of GEVC or E10, it is reasonable to conclude that the immunotoxicity of exposure to E15 would be greater than that from the same concentration of E10. In addition, the threshold concentration for producing immune suppression would be expected to be lower for exposure to E15 than for GEVC or for E10.

It is possible to approximate the doses of E15 that would be associated with immune suppression based on the ethanol concentration of the vapors and in comparison to the results of the 211(b) Alternative Tier 2 GEVC study. Since the GEVC was composed from gasoline plus 10% ethanol, we can approximate the ethanol concentration of the vapor to be roughly 10% of the total vapor, and likewise we can approximate the ethanol component of E15 vapor to be approximately 15% of the total vapor (Table 3).

Table 3. Approximation of ethanol content of vapors and anticipated results of immunotoxicity testing given that ethanol concentration

Material	**Exposure concentration (mg/m^3)**	**Estimated Ethanol Concentration (mg/m^3)**	**211(b) Tier 2 Immunotoxicity results**	**Presumed outcome if E15 were tested based on ethanol concentration**
E15	20,000	3,000		Effect level
GEVC	20,000	2,000	LOAEL	
E15	10,000	1,500		Uncertain
GEVC	10,000	1,000	NOAEL	
E15	2,000	300		No effect
GEVC	2,000	200	No effect	

Table 3 is ordered from top to bottom based on the estimated concentration of the ethanol component of the exposure vapor. For example, if E15 vapor is assumed to be 15% ethanol then the ethanol concentration of 20,000 mg/m^3 would be 3,000 mg/m^3 (0.15 x 20,000). The lowest observable adverse effect level (LOAEL) and no observable adverse effect level (NOAEL) results of the Tier 2 testing for GEVC are listed from the White 2010 study report. Based on the estimated ethanol concentration of the vapor, therefore, E15 at 20,000 mg/m^3 is anticipated to cause some immunosuppression since the ethanol concentration of the total vapor is higher than that of GEVC at 20,000 mg/m^3. Exposure to E15 vapor at 2,000 mg/m^3 would be expected to have no significant effect since the estimated ethanol concentration in the

vapor is lower than that from GEVC at 10,000 mg/m^3. The likely effect of exposure to 10,000 mg/m^3 E15, however, cannot be precisely estimated from these data because the ethanol component of vapor falls between the LOAEL and NOAEL of the GEVC studies.

In summary, these analyses suggest that (a) the ethanol component of the emissions is likely contributing to suppressing immune function, (b) the ethanol component of E15 vapor is higher than that from E10 or GEVC, (c) the immunotoxicity of E15 vapor therefore would likely be somewhat greater than the same concentration of E10 vapor were such testing to be conducted, and (d) the LOAEL/NOAEL for E15 causing immune suppression would likely be between 2,000 and 20,000 mg/m^3, but cannot be specified to greater precision from the data available.

The importance of these differences depends on several factors, such as whether these levels of exposure are environmentally relevant. Based on data available from the 211(b) exposure study[20], ethanol concentrations in the U.S. appear to be far below even the lowest estimated concentrations of ethanol that may cause immunotoxicity. For example, the highest peak concentration of ethanol recorded in the 211(b) exposure study in Chicago was less than 120 parts per billion by volume (ppbv). By comparison, the lowest level that could be associated with adverse health effects observed in the 211(b) Alternative Tier 2 testing is greater than 1,000 mg/m^3 of ethanol (see Table 3). As a conservative illustration, this NOAEL of 1,000 mg/m^3 is equivalent to 530,600 ppbv[21], which is three orders of magnitude above the measured peak ethanol exposure. We note that the results discussed here are based only on the health effects observed in the 211(b) Alternative Tier 2 testing for GEVC.

We note here that the proportional concentrations of 2-methylpropane (isobutane) and 2-methylbutane (isopentane) are higher in more saturated non-oxygenated hydrocarbon portion of the E10 and E15 (and even E0) used in the RFA/GrE speciation than they were in GEVC. This is a reasonable consequence of lower levels of some constituents, specifically aromatics, which necessarily increases the proportion of other remaining constituents that constitute the whole mixture. Fuels utilized in the 211(b) toxicity testing were produced using gasoline specifications more common in the early 1990s when the testing was designed. These fuels were relatively high in aromatic hydrocarbons and relatively lower in saturates. Although the emissions of E15 utilized for speciation purposes is higher in isobutane and isopentane than the GEVC, this is not due to the addition of ethanol, and the levels of these emittants would vary from gasoline to gasoline around the country and even from batch to batch within a refinery. Thus, we do not consider the significance of the increase in these two compounds in our discussion of immunotoxicity above.

---

[20]Zielinski, Barbara and Eric M. Fujita, John C., Sagebiel and David E. Campbell, Desert Research Institute,Section 211(B) Tier 2 High End Exposure Study of Conventional and Oxygenated Gasoline, Compiled Final Report, March 25, 2009, EPA Docket Number EPA-HQ-OAR-2003-0065-0673.

[21] The equation used to convert the exposure concentration in mg/m^3 to ppm is: ethanol molecular weight * ppm = 24.45 * mg/m^3, or ppm = (24.45*1,000) / 46.08. The resulting ppm was multiplied by 1000 to convert to ppb.

IV. Conclusion

For a fuel registration application to be complete without Tier 2 test results for the fuel being registered, the regulations at 40 CFR 79.53(d) require a showing that replicating E10 Alternative Tier 2 health effects testing for E15 evaporative emissions would yield reasonably comparable results. For the reasons mentioned above, we conclude that, for the immunotoxicity testing, the results for E15 may or may not be identical to those for E10, but they would be in the same range of exposures and would be reasonably comparable. We also conclude that the results for E15 would be reasonably comparable for the other health effects discussed above.

Future Considerations

In the future, EPA may consider the potential need for testing, under Tier 3, to evaluate developmental neurotoxicity. Developmental neurotoxicity is the most sensitive adverse outcome from ethanol ingestion, but there is a lack of comparable data following developmental exposure to ethanol by inhalation. Current EPA research is investigating this gap by evaluating developmental neurotoxicity associated with inhalation exposure to evaporative emissions from various gasoline/ethanol blends. At this point, it is unlikely that EPA would recommend further developmental toxicity testing, but we reserve final judgment until the completion of our ongoing research. Similarly, EPA may consider further immunotoxicity testing if environmental exposures increase substantially. If after the completion of our ongoing research such testing is considered, it would likely be in the context of Tier 3[22] requirements for ethanol fuels generally.

Additionally, EPA will continue to consider the possibility of a fuller examination of speciated combustion emissions and exposures to help refine our understanding of potential risks associated with the use of E15 or ethanol gasolines generally. EPA will consider testing which might include a further examination of speciated combustion emissions including speciation of particulate matter (PM) and semi-volatile organic compounds (SVOCs), more robust vehicle testing of emissions (more vehicles, more tests and broader conditions), and modeling of potential environmental exposures associated with emissions from selected E15 use scenarios (e.g., near roadways). EPA and DOE have ongoing research in these areas that might further inform the need for additional studies. For example, EPA is developing new analytical methods for PM and organic compounds.

Such testing might also include health effects testing if, in the future, EPA determines that exposure of test animals to combustion emissions may yield useful results which would not be compromised by exposure to carbon monoxide emissions. If and when such testing is required, EPA would likely also consider such testing for any other gasoline fuels with significant market share, including E10.

[22] 40 CFR 79.54.

Conclusion

Our evaluation therefore concludes that RFA/GrE has submitted data and analysis that would satisfy the Tier 1 and Tier 2 testing requirements for registration.