August 20, 1997

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Dear Dr. Henry:

The purpose of this letter and its attachments is to notify you of a test program which the Environmental Protection Agency (EPA) proposes to require for the Baseline Gasoline and certain Nonbaseline (oxygenated) Gasoline groups, in accordance with the Alternative Tier 2 provision of the fuels and fuel additives (F/FA) health effects testing regulations.[1] This notice is directed to you specifically in your capacity as administrator and representative of the "Section 211(b) Research Group," the consortium of F/FA manufacturers organized by the American Petroleum Institute (API) to share compliance burdens and costs related to these test requirements.[2]

The proposed Alternative Tier 2 testing regimen is required pursuant to sections 211(b)(2) and 211(e) of the Clean Air Act. It is designed to provide information for identifying and evaluating the potential adverse effects and risks associated with conventional gasoline and various oxygenate-gasoline blends (collectively referred to here as "oxyfuels"),[3] and to inform future agency decision making pursuant to Section 211 of the Act. To adequately serve this purpose, the proposed Alternative Tier 2 test program includes most of the standard Tier 2 test requirements, requires more definitive testing related to some standard Tier 2 health effect endpoints, and addresses certain other endpoints not ordinarily included in standard Tier 2.[4]

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[1] The F/FA health effects testing program regulations are codified at 40 CFR part 79, subpart F. The Alternative Tier 2 provisions appear at 40 C.F.R. § 79.58(c).

[2] Such grouping and cost sharing arrangements are authorized by section 211(e) of the Act and are specified at 40 C.F.R. § 79.56. In addition, this notification is being sent to other applicable manufacturers that are not members of the Consortium.

[3] The blends of interest contain at least 1.5 weight percent oxygen and are categorized as "nonbaseline" under § 79.56(e)(3)(i)(B). Such blends include wintertime oxygenated fuels and reformulated gasolines.

[4] See Fuels and Fuel Additives Registration Regulations, 59 FR 33042, 33081 (June 27, 1994) (discussing appropriate use of the Alternative Tier 2 requirements).

In proposing Alternative Tier 2 testing requirements, the EPA has placed a special emphasis in assuring that the testing protocols are properly developed beforehand, and properly implemented to assure that the best possible data will result. To this end, a rigorous peer review process, explained in further detail under the section Study Protocols, and in Attachment A, has been set in place.

In view of the continuing uncertainties regarding the public health effects of gasoline and oxyfuels, and the nearly universal public exposure to their emissions, a testing regimen which exceeds the standard screening requirements of Tiers 1 and 2 is necessary and appropriate for these F/FA groups. EPA has had ongoing consultations with individual fuel and additive manufacturers, API and other trade organizations, state environmental departments, toxicologists, and other scientific and policy experts, to identify specific gaps in the information currently available for characterizing the risks related to the use of these fuels, and to establish relative priorities among the identified research areas. Based on these discussions, EPA scientists have developed a test regimen under the Alternative Tier 2 provisions to address the specific research needs associated with gasoline and oxyfuels.[5] Application of this testing regimen is clearly more appropriate than waiting for the completion of standard Tier 2 and then developing follow-up test requirements at the Tier 3 level.[6] Under the proposed Alternative Tier 2 testing regimen, critical test data which meet and exceed the standard Tier 2 requirements should become available in a relatively shorter period of time and at lower overall cost.

As previously mentioned, the Alternative Tier 2 requirements proposed in this letter pertain to F/FAs which meet the regulatory criteria for classification into the Baseline Gasoline category,[7] or into a Nonbaseline Gasoline group defined by oxygenate content.[8] It is my understanding that, within the Section 211(b) Research Group which you represent, various F/FA manufacturers have enrolled products in the Baseline Gasoline group and in the following Nonbaseline oxyfuel groups: methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), ethyl alcohol (EtOH), tertiary amyl methyl ether (TAME), diisopropyl ether (DIPE), and tertiary butyl alcohol (TBA). As described below, one set of Alternative Tier 2 requirements is proposed for Baseline Gasoline and MTBE-gasoline, while a different set of requirements is proposed for the other identified oxygenate groups. If additional oxygenate-defined groups come into existence, then the Alternative Tier 2 requirements described below

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[5] Our intent to require special testing under the Alternative Tier 2 provision, and to provide a suitable schedule for its completion, was previously communicated to you in my letters of November 23, 1995, January 11, 1996, and October 28, 1996. (Docket items A96-16/I-C-1, A96-16/I-C-2, and A96-16/I-C-3).

[6] As EPA stated in promulgating the F/FA registration regulations, use of the alternative Tier 2 provisions “can facilitate earlier and potentially more efficient acquisition of the required data” than use of standard Tier 2 testing and subsequent Tier 3 testing. 59 Fed. Reg. at 33081.

[7] 40 C.F.R. § 79.56(e)(3)(i)(A).

[8] 40 C.F.R. § 79.56(e)(3)(i)(B).

for "other" oxyfuel groups (i.e., other than MTBE) would probably be required for the new groups as well. This notification, however, is limited to the Baseline Gasoline category and the Nonbaseline Gasoline oxyfuel groups described above.

The specific studies proposed to be required for these F/FA groups under Alternative Tier 2 are set forth in the attachments. Inhalation toxicology studies are described in Attachments A through C, population exposure studies in Attachment D, and the schedule for completion of these requirements in Attachment E. The remainder of this letter explains why the proposed Alternative Tier 2 testing program is necessary, describes the overall structure of the proposed test regimen, describes the general nature of the requirements, discusses potential follow-up studies that may be required at the Tier 3 level, discusses the proposed peer review process for developing study protocols, and reviews the administrative aspects of the Alternative Tier 2 process.

The Necessity for the Proposed Alternative Tier 2 Testing Program.

A number of recent expert analyses have demonstrated the necessity for the proposed testing. A committee of the National Science and Technology Council reviewed published and unpublished reports made available since 1990. This committee identified the following areas as requiring additional research: human exposures; pharmacokinetics of MTBE; acute health effects related to oxygenates; mechanisms of carcinogenicity; and dose-response relationships between exposure to oxygenates and risk of carcinogenicity.[9] Similarly, the Health Effects Institute Oxygenates Evaluation Committee conducted an "intensive review" of the existing oxygenates health effects database, EPA risk assessments, and health effects of new oxygenates as they relate to other pollutants whose emissions are altered by use of oxygenates. The Oxygenates Evaluation Committee identified the following outstanding research needs: personal exposures to oxygenates using standard protocols; metabolism of MTBE; pharmacokinetics of other ethers; short-term effects using controlled human exposures; neurotoxic effects; neoplastic and nonneoplastic long-term effects; studies on the genotoxicity of MTBE; developmental effects; and assessment of potential contamination of drinking water with MTBE.[10] A committee of the National Research Council reviewed the Interagency Assessment and identified the following research needs: representative personal exposure monitoring of MTBE in the exposed population; toxicokinetic data of MTBE and other oxygenates; study of

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[9] National Science and Technology Council Committee on Environment and Natural Resources, Interagency Oxygenated Fuels Assessment Steering Committee, Interagency Assessment of Potential Health Risks Associated with Oxygenated Gasoline, (February 1996 - draft, July 1997 - final) [hereinafter Interagency Assessment] (Docket items A-96-16/II-A-1 & II-A-6). The Interagency Assessment focussed on inhalation exposures. The 1997 document specifically stated that "Because of the very limited data set for fuel oxygenates in drinking water, it is not possible to characterize human exposure from consumption of contaminated drinking water." page v - executive summary.

[10] Health Effects Institute, Oxygenates Evaluation Committee, The Potential Health Effects of Oxygenates Added to Gasoline, (April 1996). (Docket item A-96-16/II-A-2).

exposure to MTBE and acute health effects; and potential for biodegradation of MTBE and other alkyl ether oxygenates in surface water, soil, and groundwater.[11]

As EPA concluded in a review of the Interagency Assessment and the Health Effects Institute review: "It is quite evident, however, that a consistent theme in all of the reports is the need for more information on the exposure and health aspects of conventional and oxygenated fuels."[12] The expert analyses clearly demonstrate the necessity for testing focussing on acute health effects, carcinogenicity, neurotoxicity, developmental effects, exposure assessments, pharmacokinetic parameters, and potential exposures via drinking water.

Tiered Requirements.

The proposed Alternative Tier 2 testing program is not intended to address every identified research need on baseline gasoline and the various oxyfuels. Rather, the proposed testing is intended to fill critical data gaps and act as a screen to determine the need for additional information that may be necessary to enable the Agency to make decisions concerning the potential risks associated with these F/FA's. Thus, consistent with the general strategy of the F/FA testing program, the proposed Alternative Tier 2 testing regimen is part of a tiered approach which may also include Tier 3 test requirements in the future. Such a stepwise approach will help assure a wise investment of manufacturer and laboratory resources. It will also allow the Alternative Tier 2 results to influence the objectives and design of any necessary follow-up studies at the Tier 3 level. Changes in F/FA usage patterns over time may also alter future research priorities. Furthermore, some information gaps may be filled by other studies currently being conducted; conversely, research work which EPA currently understands to be ongoing or planned may not be done after all, may be inadequately performed, or may raise important new concerns that must be evaluated.

Thus, the Alternative Tier 2 requirements set forth in this notification must be regarded as first steps in a test regimen which may encompass one or more additional steps at the Tier 3 level. Later sections of this letter identify some of the Tier 3 studies which, at this time, appear likely to receive our future consideration. Some of these studies are discussed as "contingent" studies - i.e., generally dependent upon outcomes of the Alternative Tier 2 tests required under this notification. Others could be required in the wake of external events or information which highlight new sources of concern. It should be clearly understood, however, that EPA cannot foresee every eventuality, and that any actual Tier 3 requirements could include areas of investigation not discussed in this letter.

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[11]National Research Council, Committee on Toxicological and Performance Aspects of Oxygenated and Reformulated Motor Vehicle Fuels, Toxicological and Performance Aspects of Oxygenated Motor Vehicle Fuels., National Academy Press, Washington, DC, (June 19,1996). (Docket item A-96-16/II-A-3).

[12]EPA, Oxyfuels Information Needs, EPA/600/R-96/069 (May 1996). (Docket item A-96-16/II-A-4).

Role of Evaporative and Combustion Emissions.

Toxicologic studies included in the proposed Alternative Tier 2 regimen are based on animal inhalation exposures to evaporative emissions mixtures of the gasoline or oxyfuel in question. Thus, the proposed Alternative Tier 2 testing regimen contrasts with standard Tier 2 requirements which may require testing of both evaporative and combustion emissions.

The decision to omit combustion emissions exposure studies from the current set of requirements was based in part on the peer-reviewed "white paper" which the 211(b) Research Group submitted for EPA's evaluation in August, 1996.[13] Prepared as a result of discussions held at an API-sponsored information meeting on December 11-12, 1995,[14] the white paper summarized certain gasoline exhaust emission toxicology studies reported in the scientific literature, and compared them to the test requirements included in the standard Tier 2 screening regimen. It also presented an analysis intended to demonstrate that the relatively high concentration of carbon monoxide (CO) in gasoline exhaust imposes a practical limit on achievable exposures to hydrocarbon (HC) exhaust components. The paper stated that the amount of exhaust gas dilution required to avoid CO toxicity of animal subjects would bring the concentration of HCs in the exposure chamber below the no-effect level. The paper concluded that further exhaust emission toxicology tests of gasoline-based F/FAs would not provide meaningful health effects data.

EPA scientists who reviewed the white paper generally concurred that further inhalation toxicology testing of gasoline-based combustion emissions, if conducted using the approach prescribed in the F/FA rule, seemed unlikely to provide additional useful data for comparative risk assessment.[15] Their concurrence was based on the likelihood that, at the exhaust dilution ratios necessary to avoid acute CO toxicity, the effects of the inhaled combustion emissions mixture would be dominated by exposure to CO and/or oxides of nitrogen (NOx) rather than by the HCs of primary interest. This conclusion did not imply that the existing test data cited in the white paper were judged sufficient to resolve the uncertainties about either the cancer or non-cancer health risks

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[13]Barter, Robert A., et al., The Utility of Gasoline Engine Exhaust Emission Toxicology Testing, August 1, 1996. (Docket item A-96-16/II-D-1).

[14]Participants in this API-sponsored meeting included inhalation toxicology experts, industry representatives, and state health officers, in addition to API and EPA staff. The meeting record, and the presentation of Dr. Robert Drew, are available as Docket items A-96-16/II-I-8 and A-96-16/II-I-9, respectively. Subsequent written comments received by EPA from meeting participants are summarized in a memorandum to the F/FA Workgroup from Charles M. Auer, Office of Prevention, Pesticides and Toxic Substances, Comments on Section 211 Testing Table, March 25, 1996. (Docket item A-96-16/II-C-1).

[15]See memorandum from Mike Davis, National Center for Environmental Assessment, to Judy Gray, Office of Mobile Sources, Comments on Gasoline Combustion Emissions White Paper, October 7, 1996. (Docket item A-96-16/II-C-2). While EPA scientists did not agree with many of the central arguments and conclusions of the white paper, these are not at issue here, and do not alter the fact that its conclusions regarding inhalation toxicology testing appear valid.

of gasoline (or oxyfuel) combustion emissions. On the contrary, the reviewing EPA scientists recommended continued evaluation of other approaches for investigating gasoline exhaust toxicity, such as the use of synthesized surrogate exhaust mixtures, the use of different exposure routes, and/or the development of analytic models to assess comparative risks.

EPA believes, however, that the public interest would be best served by timely initiation of appropriate toxicity testing on the evaporative emissions of gasoline and oxyfuels while the Agency continues to evaluate the complex issues surrounding exhaust emissions testing. We also recognize that the results of the evaporative emissions tests, together with information on human population exposures to various evaporative and combustion emissions components (discussed below), may change current perceptions about the continued need for, and specific targets of, future combustion emissions studies. For these reasons, the proposed Alternative Tier 2 requirements only include inhalation toxicity tests of evaporative emissions. Potential requirements to investigate the toxicity of combustion emissions will be reconsidered at the Tier 3 level.

Generation of Evaporative Emissions.

To generate evaporative emissions for use in inhalation toxicology testing, the F/FA Program regulations specify that an "Evaporative Emissions Generator (EEG)" apparatus shall be developed and used unless EPA has approved an alternative emissions generation method.[16] To receive approval, an alternative method must meet the following criteria: 1) be reliable and safe; 2) generate an emissions mixture which is reasonably similar to the equilibrium composition of the vapor which occurs in the head space of a vehicle fuel tank under near-maximum in-use temperature conditions; 3) generate an emissions mixture that is sufficiently concentrated to provide adequate exposure levels for the required tests; and 4) ensure that the emissions delivered to the biologic exposure chamber provide reasonably constant exposure.[17] The request must also provide information on the safety and reliability of the alternative method.

EPA has been informed that, over the past several months, API scientists associated with the Section 211(b) Research Group have been developing an alternate emissions generation approach which they believe to be more feasible and more fuel efficient than the EEG approach specified in the regulations. EPA scientists consulted during this development period have also expressed positive opinions about the procedure. Therefore, it is possible that this alternative emissions generation method may be approved for use in the Alternative Tier 2 testing program.[18] This alternative

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[16] 40 C.F.R. § 79.57(f).

[17] 40 C.F.R. § 79.57(f)(5)(I).

[18] In accordance with Section 79.57(f)(5)(ii), if EPA approves the alternate method for generating evaporative emissions, then all associated technical documentation will be placed in the public docket and made available for use by other manufacturers and groups.

emissions generation approach may be formally submitted as part of the protocols described in the section of this notification entitled “Study Protocols and Related Reviews” and EPA will consider approval of this approach as a part of the overall protocols.

Alternative Tier 2 Toxicity Testing Requirements.

The proposed Alternative Tier 2 testing regimen includes two separate sets of toxicity test requirements. The first set of toxicity requirements - set forth in Attachment B - applies to evaporative emissions of the Baseline Gasoline group and (separately) the MTBE-gasoline group. This testing program includes most of the basic standard Tier 2 testing regimen (subchronic toxicity, carcinogenicity, mutagenicity, teratogenicity, and neurotoxicity (absent the fertility assessment)). In addition, Alternative Tier 2 requires (1) additional neurotoxicity assessments; (2) a two-generation reproductive study; (3) a two-species developmental study; (4) a two-year carcinogenicity study; and (5) a screening panel for immunological effects.[19]

The second set of toxicity requirements - set forth in Attachment C - applies to evaporative emissions of each of the other oxyfuels and is much less extensive. This testing program consists of the Standard Tier 2 requirements modestly expanded to include a screening panel for immunological effects and certain histopathological requirements. Because there is a paucity of inhalation toxicity data on these oxygenates, the screening level studies required in Standard Tier 2 are appropriate for determining whether additional studies are necessary. The results of these studies will determine whether additional studies are required at the Tier 3 level.

Several considerations have led EPA to propose more extensive test requirements for Baseline Gasoline and MTBE-gasoline than for the other oxygenates:

First, and most important, conventional gasoline and MTBE-gasoline predominate within the U.S. fuel marketplace, and thus present the highest potential for human and environmental exposures. A thorough understanding of the individual and comparative public health risks of these fuels thus constitutes a critical need.

Second, the fact that nearly all fuels have some degree of toxicity means that the relative risk of different fuels is particularly important . Accordingly, a comprehensive database on Baseline Gasoline toxicity is vitally needed to provide a level basis for comparison with other F/FAs in the gasoline family. Similarly, since MTBE is the most frequently used oxygenate, comprehensive data on MTBE-gasoline is needed not only in comparison with Baseline Gasoline but also to provide an additional reference point for evaluating the relative toxicity of other oxyfuels.

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[19]The two-generation reproductive study and two-species developmental study replace the Standard Tier 2 fertility/teratology combined screening assessment.

Third, previous scientific work on conventional gasoline and on MTBE has identified specific information gaps which cannot be satisfactorily addressed by the short-term screening tests required under Standard Tier 2.[20] For example, the comparative carcinogenic potential of Baseline Gasoline emissions relative to those of MTBE-gasoline emissions is an outstanding fundamental issue which must be evaluated in the context of long-term emission exposures. In addition, dose-response relationships for developmental, reproductive, and neurotoxic effects have not been adequately characterized. To fully address these questions, studies of appropriate duration are required.

Fourth, even though each oxygenate has its own chemical characteristics and, perhaps, biological potencies, the test results obtained on one such fuel can still help to inform the Agency's decision making about potential testing needed on other oxyfuels. For example, if certain test results for baseline gasoline and MTBE-gasoline are negative, this may support the validity of negative results obtained from analogous screening tests on other oxyfuels.[21] On the other hand, a positive result obtained on MTBE-oxyfuel under relatively rigorous study conditions may indicate that comparative results are needed for the other oxyfuels. These are merely considerations, not hard and fast rules. Nevertheless, they provide another valid reason why the more extensive set of requirements should initially be applied on a selective basis to baseline gasoline and MTBE-gasoline, rather than applying the same, relatively stringent set of Alternative Tier 2 requirements to all registered oxyfuels.

Pharmacokinetic Studies on "Neat" Oxygenates

An understanding of the pharmacokinetic characteristics of the oxygenates as pure compounds is important to our understanding of their relative toxicities when mixed in gasoline. EPA believes that development of a data base on the disposition (uptake, distribution, metabolism, and elimination) of the neat constituents could provide the basis for simulations of mixture disposition. These simulations, in conjunction with toxicity and mechanistic studies, would inform the choice of test levels to describe dose-response for future toxicity testing of mixtures.

In the case of EtOH, inhalation pharmacokinetic studies could help determine the extent to which the extensive database on ingested EtOH is relevant to inhaled EtOH. Depending on the results, the need for additional inhalation studies on EtOH or EtOH-oxyfuel might be greatly diminished.

Comprehensive inhalation pharmacokinetic studies have already been conducted for MTBE; therefore, additional PK testing is not required. But, the availability of inhalation pharmacokinetic data for the other oxygenates varies

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[20] These data gaps are discussed above at pages 3-4.

[21] It should also be noted that, as discussed in the next section, the pharmacokinetic studies proposed for the other oxygenates can aid the interpretation of toxicity studies and may provide insights into the mode of action.

considerably. For example, pharmacokinetic studies are already underway for TAME under a consent order pursuant to the Toxic Substances Control Act (TSCA).[22] In addition, EPA has been informed that such testing on pure ETBE is being conducted by industry on a voluntary basis. To our knowledge, however, there are currently no similar test plans for pure EtOH, DIPE, TBA, or other oxygenates. Consequently, the proposed Alternative Tier 2 test regimen for the oxygenates other than MTBE includes pure compound inhalation pharmacokinetic test requirements.[23]

Population Exposure Studies.

As discussed above, each of the expert panels recommended that additional data and information be generated on population exposures to oxyfuels. This is consistent with EPA's own determination that the quantitative data currently available on personal exposures to gasoline and oxyfuel vehicle emissions is inadequate for purposes of comparative public health risk assessment. To address these data gaps, EPA proposes that initial screening level population exposure studies be conducted for baseline gasoline and MTBE-gasoline.

Consistent with the general approach of the proposed Alternative Tier 2 testing program, the proposed population exposure studies (1) focus initially on baseline gasoline and MTBE-gasoline; (2) require screening level studies of the most-exposed population; and (3) recognize that additional studies may be required at the Tier 3 level, if the data indicate that these are necessary because specific concerns are identified and an accurate quantitative estimate of the related public health risk is appropriate. Thus, the Alternative Tier 2 proposal requires subject personal exposures to be quantified only in specified microenvironments representing the upper end of the frequency distribution of potential exposures.

The proposed Alternative Tier 2 exposure studies (see Attachment D) are to take place in cities which have ongoing ambient monitoring programs and are located in different parts of the country. Cities with and without reformulated gasoline and winter oxyfuel (MTBE-gasoline) programs are to be sampled. Sampling will be conducted at intervals throughout the year to ensure that different meteorological and seasonal conditions are encountered. Within microenvironments representing the highest potential vehicle emission exposure scenarios, a number of key variables will be measured in ambient air and in subjects' personal breathing zones and blood.

As is discussed below in the section on “Atmospheric Transformation Products”, concerns have been raised about the potential toxicity of the atmospheric transformation products of vehicle emissions such as tertiary butyl formate (TBF),

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[22] *Testing Consent Order for Tertiary Amyl Methyl Ether*, 60 Fed. Reg. 14910 (March 21, 1995).

[23] In accordance with the F/FA testing regulations, results of adequately performed and documented previous testing may be submitted to comply with these requirements if such testing is comparable to the guidelines specified in Attachment C. *See* 40 C.F.R. § 79.53(b). EPA will review any such submission in accordance with the criteria set forth at 40 C.F.R. § 79.53(d).

isobutylene, tertiary butyl nitrite, and formic acid. In order to begin to address the relevance of these materials to human exposure scenarios, the measurement of atmospheric transformation products is included as a requirement in Attachment D in the microenvironment section of the exposure study requirements.

The results of these microenvironmental studies should enable estimation of the upper end of the frequency distribution of annual average inhalation exposures to evaporative and combustion emissions of gasoline and MTBE-oxyfuels. Reasonable extrapolation to the expected emissions from other oxyfuels should also be possible. In conjunction with health effects data from the Alternative Tier 2 toxicity studies and other sources, this information should help determine whether such exposures represent a significant cause for public health concern. It should also identify what circumstances (e.g., climate, season, microenvironment, fuel type) are associated with increased health risk. In addition, the study should provide data for determining the relative proportion of evaporative vs. combustion emissions in ambient and breathing zone air. All of these factors likely will have a strong influence on EPA's determination of whether additional studies are required at the Tier 3 level.

Study Protocols and Related Reviews.

Development of detailed protocols for each required study is the responsibility of the Research Group/[manufacturer] (see Attachment A). The protocols must be scientifically valid, responsive to the objectives of the proposed Alternative Tier 2 requirements (as stated in the attachments), and consistent with any specific guidelines specified for the study. Unless otherwise approved by EPA, the protocols must also conform to the F/FA program guidelines on Good Laboratory Practices,[24] and Vehicle Emissions Inhalation Exposures.[25]

Draft protocols must be peer reviewed by competent and impartial experts.[26] Draft protocols shall be revised as may be indicated by the recommendations of the peer review. Thus, individual reviewer comments, along with a statement of the disposition of such comments, are to accompany the protocol versions submitted to EPA. EPA will respond in writing, either approving the protocol, or describing necessary modifications. EPA will make the final determination of whether protocols are acceptable for purposes of the proposed Alternative Tier 2 testing program. The proposed schedule for completion of the Alternative Tier 2 requirements (Attachment E) includes adequate time for protocol development, peer review, and EPA approval. Later protocol changes, if any, must also be approved in advance by EPA.

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[24] 40 C.F.R. § 79.60.

[25] 40 C.F.R. § 79.61.

[26] While the Research Group/ [manufacturer] will be responsible for selecting an appropriate and balanced slate of reviewers, EPA is willing to engage in prior consultation with the Research Group/[manufacturer] on potential candidates.

The final Alternative Tier 2 notification will name an EPA contact person who will be available to discuss problems which might arise in regard to the Alternative Tier 2 requirements. As needed, this individual may also refer such issues to other EPA technical, scientific, or administrative persons for satisfactory resolution. EPA encourages the Research Group/[manufacturer] to organize a Technical Advisory Panel to help resolve technical issues which arise before and during the performance of the Alternative Tier 2 test regimen.

Contingent Studies.

As discussed above, the proposed Alternative Tier 2 testing program has been designed to fill critical data gaps and act as a screen to determine the need for additional studies. Thus, the results of the Alternative Tier 2 tests may indicate that additional studies are required at the Tier 3 level. Potential Tier 3 study requirements that may result from the Alternative Tier 2 results include (but are not necessarily limited to) the following:

Further Evaporative Emissions Toxicology Testing

In the case of Baseline Gasoline and MTBE-gasoline, follow-up tests may be required to further characterize significant unexpected findings. For example, mechanistic studies may be required to determine if positive results in the Alternative Tier 2 animal studies are applicable to humans.

In the case of the other oxyfuels, additional testing may be required for a particular gasoline-oxygenate mixture, not only to explicate Alternative Tier 2 positive results on the mixture in question, but also to resolve uncertainties created by positive results which may be obtained on MTBE-gasoline, another oxygenate mixture, and/or Baseline Gasoline. For example, a two-generation reproductive study and/or two-species developmental study may be required on an oxyfuel to follow up on one or more of the following findings:

- Positive results in fertility/teratology screening test(s) for the oxyfuel in question.

- Adverse effects in the second generation of the MTBE-gasoline two-generation reproductive study, when such effects could not be expected on the basis of the first generation results.

- Adverse effects in the "other" species tested in either the MTBE-gasoline or Baseline Gasoline two-species developmental studies.

Similarly, a two-year inhalation bioassay may be required, not only to follow up on positive results obtained in the Alternative Tier 2 mutagenicity studies for a given oxyfuel, but also because of significant unexpected results obtained in the cancer bioassay conducted for Baseline Gasoline and/or MTBE-gasoline. Additional contingent tests for the oxyfuels may be required to further characterize other significant unexpected positive findings in the Alternative Tier 2 test battery.

Toxicology Testing of Combustion Emissions

For Baseline Gasoline and MTBE-gasoline (and for any other oxyfuel experiencing significant market growth), the results of the Alternative Tier 2 exposure study are expected to be an important consideration in determining the need for combustion emissions toxicology testing. Thus, Tier 3 combustion emissions toxicology testing may be indicated if the exposure study were to show that:

- Upper-end (highest) personal exposures to total vehicle emissions are sufficiently high to cause potential public health concerns, and

- Fuel combustion (as opposed to evaporative processes) contributes significantly to vehicle-related emission exposures.

For the other oxyfuels, combustion emissions toxicology testing would likely be contingent on the same Alternative Tier 2 exposure study outcomes, along with other considerations. For example, either of the following conditions may indicate a need for combustion emission testing of the other oxyfuels:

- Tier 3 (or other) toxicology testing on combustion emissions of Baseline Gasoline and/or MTBE-gasoline (or any other oxygenated gasoline) yields findings that would not be predicted by the test results obtained on evaporative emissions of the fuel in question.

- Combustion products of the oxyfuel include chemical species (other than the oxygenate itself) that differ significantly from those produced by combustion of Baseline Gasoline or MTBE-gasoline.

The types of combustion emissions toxicology tests to be required of Baseline Gasoline or any of the oxyfuels would likely be similar to the battery of tests required under Alternative Tier 2 (and Tier 3) for the evaporative emissions of the particular fuel in question. In view of the difficulties discussed earlier concerning the development of methods for generating an appropriate gasoline exhaust (or surrogate) exposure atmosphere, however, the underlying approach to these studies cannot be specified at this time.

Additional Exposure Testing

As previously discussed, population-based personal exposure monitoring studies could be required at the Tier 3 level, if the high-end microenvironmental exposure levels determined under Alternative Tier 2, combined with emission toxicology test results, indicate that there is significant reason for health concerns. The primary purpose of such studies would be to determine the entire frequency distribution of average annual personal exposures to gasoline and oxyfuel emissions. Accordingly, the study subjects would be selected based on probability sampling of the entire target population. Other study variables (locations, seasons, measurement variables) would be similar to those specified for the Alternative Tier 2 exposure study.

Other Possible Tier 3 Requirements.

In addition, other tests may be required at the Tier 3 level, based on data from ongoing studies not related to the Alternative Tier 2 testing regimen, or to fill other existing data gaps. Such additional tests may include (but are not limited to) the following:

Acute Health Effects

In response to substantial public concerns which arose after the introduction of MTBE-oxyfuels in 1992, numerous acute exposure studies using human volunteers were undertaken by government, industry, and academia. To date, no clear association has been demonstrated between exposure to ambient MTBE levels and acute health effects. Nevertheless, some uncertainty remains that certain susceptible subpopulations might be prone to the acute symptomatology and/or that exposure to MTBE-gasoline emissions rather than pure MTBE emissions might elicit acute health effects. EPA understands that the Environmental and Occupational Health Sciences Institute (EOHSI), affiliated with Rutgers University in New Jersey, is planning to explore these issues in controlled exposure studies using human subjects. Thus, acute exposure studies are not included in the proposed Alternative Tier 2 testing regimen. But, if the expected (or similar) studies do not go forward, or if the results demonstrate the need for additional study, such additional work may be required at the Tier 3 level. Furthermore, if positive results are obtained with MTBE, then studies to explore the potential of other oxyfuels to cause acute symptoms may also be required.

Atmospheric Transformation Products

Questions have been raised concerning the potential toxicity of the atmospheric transformation products of vehicle emissions. For example, tertiary-butyl formate (TBF) is a respiratory irritant gas which, in photooxidative chamber studies, has been shown to be the major transformation product of MTBE. While no TBF has been detected from MTBE gasoline combustion during preliminary measurements of the exhaust stream (Docket number A96-16/II-A-5), the extent to which TBF exposure under ambient conditions is an important factor in the toxic effect of oxyfuel emissions has not been fully explored.[27] Questions have also been raised regarding other atmospheric transformation products of vehicle emissions such as isobutylene, tertiary butyl nitrite, and formic acid. In order to begin to address the relevance of these materials to human exposure scenarios, the measurement of atmospheric transformation products is

[27] EPA understands that the ARCO Chemical Corporation is currently developing methods for measuring TBF in ambient air, and will try to detect TBF in air samples taken from urban sites in which MTBE-gasoline is used. The results of a comparative study on the respiratory irritancy of TBF and other formates has been recently completed by ARCO and the document may be found in docket number A96-16/II-I-10. The findings demonstrated that TBF is capable of causing pulmonary irritation in mice at doses of 500 parts per million or higher. While these results translate to only a potentially mild irritant to humans, it is still uncertain whether TBF, or other atmospheric transformation products, would be considered irritants, or cause other adverse health effects to humans at ambient levels of exposure.

included as a requirement in Attachment D in the microenvironment section of the exposure study requirements. Studies to explore the health effects of transformation products may be covered under future Tier 3 requirements should the exposure studies under Alternative Tier 2 microenvironmental studies indicate that exposures to these materials are significant.

MTBE Water Pollution

Concerns about oral exposure to MTBE have arisen with the finding of MTBE contamination in groundwater, drinking wells, and surface water. Moreover, each of the expert panels that reviewed the oxygenate and oxyfuel toxicity database recommended generation of additional data and information related to MTBE contamination of drinking water. The EPA has recently formed an “MTBE-Water” research task group coordinated by the Office of Research and Development. The group will identify the current, or soon to be started, projects in the areas of environmental monitoring/occurrence, source characterization, transport and fate, exposure, toxicity, remediation, and others. The identified research will help provide the necessary information to better understand the health effects related to MTBE in water, to further our knowledge on remediation techniques, and to direct future research planning towards the areas of highest priority.

The most appropriate testing to be required under the F/FA health effects testing regulations would be research related to the air deposition products associated with vehicle emissions. The U. S. Geological Survey (USGS), responsible for assessing the status of, and trends in the nation’s ground and surface water resources, is heavily involved in MTBE research, including air deposition studies. As a current project in Glassboro, New Jersey, the USGS is studying MTBE air deposition, transport, and environmental fate to determine to what extent, ambient MTBE adds to water as a product of non-point source contamination. In view of these ongoing efforts, the proposed Alternative Tier 2 requirements do not include studies on these issues. But, if the expected (or similar) studies do not go forward, or if their results raise further significant questions regarding potential impacts on the public health or the environment, then related studies might be required at the Tier 3 level.

Changes in Oxygenate Usage Patterns

A significant upswing in the market penetration of an oxyfuel which has been categorized here as one of the "other oxyfuels" would likely prompt a re-evaluation of the testing needed for that oxyfuel. With the increased potential for population exposure to emissions of the oxyfuel, a test regimen that is as comprehensive and rigorous as that required for MTBE-gasoline would probably be considered under Tier 3. The focus of Tier 3 population exposure studies (if any) may also be expanded or otherwise altered as a result of such market changes.

Follow-up of Health Effects Information Obtained from Other Sources

If test results and/or other information that become available from sources other than the test program described here raise new concerns or uncertainties, that also may result in follow up study requirements at the Tier 3 level.

Administrative Procedures.

In accordance with the F/FA test program regulations, this letter constitutes notification of EPA's proposed Alternative Tier 2 testing regimen and the proposed schedule for completion and submission of such tests.[28] You have sixty (60) days from receipt of this notification to comment on the proposed Alternative Tier 2 testing regimen and the proposed schedule. Your comments should be sent by certified mail to Mr. Charles N. Freed, Director, Fuels and Energy Division, Office of Mobile Sources, U.S. EPA (6406J), 401 M. Street, S.W., Washington, DC 20460.

As required, a copy of this notification of proposed Alternative Tier 2 requirements is being placed in Docket No. A-96-16.[29] A Federal Register notice will be issued, announcing EPA's intent to require special testing in lieu of or in addition to the standard Tier 2 testing for the Baseline Gasoline and Nonbaseline (oxygenated) Gasoline groups, and reporting the availability of this notification letter in the public docket.[30] The general public shall have at least 30 days after the notice appears in the Federal Register to submit its comments on the Alternative Tier 2 proposal.[31]

Copies of all timely comments, together with EPA's summary and analysis of the comments, shall be placed in the docket. You will be notified by certified mail of the final Alternative Tier 2 requirements. The final notification will also be placed in the public docket, and a Federal Register notice will be issued to announce its availability.[32]

We look forward to receiving your comments on these proposed Alternative Tier 2 requirements.

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[28] 40 C.F.R. § 79.58(c)(1).

[29] *Id.*

[30] 40 C.F.R. § 79.58(c)(2).

[31] *Id.*

[32] *Id.*

Sincerely yours,

Margo T. Oge
Director, Office of
Mobile Sources

Attachments:

Attachment A: General Requirements for Alternative Tier 2 Toxicology Testing of Baseline Gasoline and Nonbaseline (Oxygenated) Gasolines

Attachment B. Alternative Tier 2 Toxicology Test Requirements for the Baseline Gasoline and MTBE-Gasoline Groups

Attachment C. Alternative Tier 2 Toxicology Test Requirements for Nonbaseline (Oxygenated) Gasoline Groups other than MTBE-Gasoline

Attachment D. Alternative Tier 2 Exposure Study Requirements

Attachment E. Alternative Tier 2 Testing Schedule

cc (w/att): Charles Auer
J. Michael Davis
Stan Durkee
William Farland
Charles Freed
John Hannon

Attachment A

Fuels and Fuel Additives (F/FA) Health Effects Testing Program: General Requirements for Alternative Tier 2 Toxicology Testing of Baseline Gasoline and Nonbaseline (Oxygenated) Gasolines

Overview

Attachment A discusses the substances to be tested, testing procedures, the procedure for development of protocols, and the reporting requirements.

I. Test Substances

A. Group Representatives

1. In accordance with 40 C.F.R. § 79.56(e)(4)(I)(A), the Baseline Gasoline group is to be represented by the Gasoline Base Fuel specified in 40 C.F.R. § 79.55(b).

2. Unless otherwise specified, each oxygenate-gasoline group is to be represented by a formulation comprised of the oxygenate in question (chemical-grade or better) mixed in Gasoline Base Fuel (as specified in Section 79.55(b)) to achieve the following volume percent:

Methyl Tertiary Butyl Ether	15 vol %
Ethyl alcohol (EtOH)	10 vol %
Ethyl tertiary butyl ether (ETBE)	17 vol %
Tertiary amyl methyl ether (TAME)	17 vol %
Di-isopropyl ether (DIPE)	17 vol %
Tertiary butyl alcohol (TBA)	12 vol %

3. Upon request, EPA will specify the appropriate formulation to represent other oxygenate-gasoline fuels which manufacturers may wish to test.

B. Exposure Atmosphere: The provisions at 40 C.F.R. § 79.57 shall be in effect for purposes of emissions generation. For each group, the animal subjects are to be exposed to a test atmosphere generated in accordance with the regulations at 40 C.F.R. § 79.57(f)(1)-(4) unless otherwise approved by EPA as provided in Section 79.57(f)(5).

II. Conduct of Studies

A. The provisions at 40 C.F.R. § 79.53(c)(1) shall be in effect for purposes of conducting the inhalation exposure studies.

B. The provisions at 40 C.F.R. § 79.60 shall be in effect for purposes of conducting the inhalation exposure studies.

C. The provisions at 40 C.F.R. § 79.61 shall be in effect for purposes of conducting the inhalation exposure studies.

III. Study Protocols

A. A detailed written peer-reviewed protocol shall be approved by EPA prior to the initiation of any Alternative Tier 2 study. The protocols shall include detailed descriptions of the study design, technical procedures, statistical methods, Quality Assurance/Quality Control (QA/QC) procedures, and documentation. Where applicable, the objectives and methods for conducting particular assessments shall be in accordance with the relevant provisions of the Health Effects Test Guideline (870 series) published by the Office of Prevention, Pesticides and Toxic Substances (OPPTS) (Docket items A-96-16/II-I). Note that several of these guidelines are currently being evaluated and may be updated or revised in the coming months. New guidelines that have been announced must be incorporated into any applicable protocol designs.

B. In accordance with Section 79.60(g)(1)(I), the protocol must provide detailed technical descriptions of the planned experimental design, apparatus, procedures, analytic methods, and documentation.

1. Each protocol shall be consistent with all applicable provisions of the Good Laboratory Practice (GLP) and Vehicle Emissions Inhalation Exposure guidelines of the F/FA Health Effects Testing Program regulations (Sections 79.60 and 79.61), including (but not limited to) provisions regarding fuel handling and other safety measures; exposure chamber equipment, conditions, and quality assurance; exposure interruptions; number, selection, and care of animals; number and levels of dosages (emission concentrations) and control requirements; and record-keeping requirements.

2. Each protocol shall also be consistent with the objectives and guidelines specified for the specific test in question. In the instance that a specified test guideline is found to be inconsistent with the provisions of the GLP and/or the inhalation exposure guidelines, then the provisions of the GLP and inhalation exposure guidelines prevail unless otherwise specified or approved by EPA.

3. To facilitate comparisons of results for different fuels, study protocols (and performance) shall be standardized to the extent possible.

C. The draft protocols shall be submitted in writing to a group of independent and impartial peer reviewers who possess the appropriate expertise and relevant cross-section of practical experience to provide a useful technical critique of the

stated objectives and methods. While EPA is willing to suggest candidate reviewers, the Research Group/[manufacturer] has responsibility for achieving a rigorous peer review. Once finalized, the list of selected peer reviewers and copies of the documents sent for their review shall be supplied contemporaneously to EPA.

D. The draft protocols shall be revised as may be indicated by the results of the peer review, and then submitted to EPA for final review and approval. Individual reviewer comments (which may be unattributed), along with a statement of the disposition of the comments, should accompany this submission. EPA will respond in writing, either approving the protocols as submitted, or describing any required changes along with a timetable for protocol modification.

E. After protocol approval, the studies shall be conducted in accordance with the approved protocols unless a variance is requested in writing and approved in advance by EPA. In unusual circumstances, if an immediate protocol variance is needed to maintain or safeguard the overall integrity of the study, then such action may be taken without prior EPA approval. EPA must be notified of the change in protocol immediately after the event, including a description of the critical need that required taking the unapproved action and its expected impact on the overall study design and results.

III. Reporting Requirements

A. All reporting requirements applicable to standard tier 2 tests at 40 C.F.R. § 79.59(c) and (e) shall be in effect.

B. Brief status reports shall be submitted to EPA at six-month intervals while the work continues. The purpose of the status reports is to keep EPA informed of important events, developments, problems encountered or expected, and/or milestones achieved, and should be no longer than necessary to serve this practical purpose. At EPA's option, EPA staff may visit and inspect the laboratory or other facility where the Alternative Tier 2 work is being done.

C. At the conclusion of each study, a comprehensive report shall be prepared, including descriptions of the hypotheses tested QA/QC procedures, the statistical analyses conducted to meet the study objectives, and interpretations of the findings. Such reports shall conform with the general specifications of 40 C.F.R. § 79.60(h) as well as the reporting requirements included within the particular study protocol.

1. The draft final report shall be submitted in writing to a group of independent and impartial peer reviewers who possess the appropriate expertise and relevant cross-section of practical experience to provide a useful technical critique of the performance of the study and the interpretation of its results. While EPA is willing to suggest candidate reviewers, the Research

Group/[manufacturer] has responsibility for achieving a rigorous peer review. Once finalized, the list of selected peer reviewers and copies of the documents sent for their review shall be supplied contemporaneously to EPA.

2. The draft report shall be revised as may be indicated by the results of the peer review, and then submitted to EPA for final review and approval. Individual reviewer comments, along with a statement of the disposition of the comments (which may be unattributed), should accompany this submission.

D. The original experimental data shall be retained for no less than ten years and provided to EPA upon request, in printed and electronic format.

E. In accordance with the F/FA testing regulations, results of adequately performed and documented previous testing may be submitted to comply with these requirements if such testing is comparable to the guidelines specified in Attachments B, C, and D. *See* 40 C.F.R. § 79.53 (b). EPA will review any such submission in accordance with the criteria set forth at 40 C.F.R. § 79.53 (d).

Attachment B

Fuels and Fuel Additives (F/FA) Health Effects Testing Program: Alternative Tier 2 Toxicology Test Requirements for the Baseline Gasoline and MTBE-Gasoline Groups

Overview

Attachment B describes the specific requirements of the proposed Alternative Tier 2 Testing program for the Baseline Gasoline and MTBE-Gasoline groups. It identifies the objectives of the testing program for these groups, and identifies the specific testing requirements - including the Standard Tier 2 tests that have been retained, the Standard Tier 2 tests that have been deleted, and the test requirements that are in addition to the Standard Tier 2 requirements.

A. General objectives:

1. Develop a comprehensive characterization of the toxicological effects in test animals of inhalation exposure to the evaporative emissions of Baseline Gasoline and (separately) MTBE-gasoline.

2. Determine potential dose-response relationships and No Observed Adverse Effects Levels (NOAELs) for specific toxicologic endpoints.

3. Together with information from related studies on human population exposure levels, this information should permit accurate quantitative comparisons of the relative toxicologic risks of baseline gasoline and MTBE-oxyfuels, as well as providing solid bases for comparison with other oxygenate-gasoline fuel formulations.

B. The required assessments include basic inhalation toxicology in the context of a subchronic exposure, as well as tests to determine potential reproductive, developmental, neurotoxic, immunotoxic, mutagenic, and carcinogenic (chronic exposure) effects.

C. The requirements in Attachment A apply .

D. Together with information from related studies on human population exposure levels, these characterizations should permit accurate quantitative comparisons of the relative toxicologic risks of baseline gasoline and MTBE-oxyfuels, as well as providing solid bases for comparison with other oxygenate-gasoline fuel formulations.

Specific Requirements

I. Subchronic Inhalation Toxicity Study with Specific Health Effect Assessments:

A. The objectives and methodology of the standard Tier 2 tests in 40 C.F.R. § 79.62 apply, including the specific health assessments in Section 79.62(a)(2), except the Fertility assessment/Teratology study in Section 79.62(a)(2)(I).

B. In accordance with 40 C.F.R. § 79.62(c), one or more of the required specific health assessments may be combined with the general subchronic toxicity study, "as long as none of the requirements of any study are violated by the combination." These studies may also be conducted separately, as specified in the following standard Tier 2 guidelines:

- In vivo micronucleus assay - Section 79.64
- In vivo sister chromatid exchange assay - Section 79.65
- Neuropathology assessment - Section 79.66
- Glial fibrillary acidic protein assay - Section 79.67

C. **The following changes and additions to the standard Tier 2 subchronic study are required**:

1. Histopathology

   a. Preparation of the animals targeted for pathologic examination of the lungs as required by Section 79.62(d)(1)(ii)(A) and (d)(5)(iii) shall include inflation of the lungs with fixative. This will permit later examination of the lung tissues by electron microscopy, if follow-up to light microscopy is indicated.

   b. Respiratory tract histopathology shall be conducted in accordance with the applicable provisions of Health Effects Test Guideline, 870.1350 (sec. 11: I - iv), published by OPPTS (Docket item A-96-16/II-I-1).

2. Immunotoxicity Screening

   a. This is to be included in the subchronic inhalation toxicity study as an additional "special health assessment", but is to be performed at the end of 28 days of exposure. A satellite group of animals may be required.

   b. The immunotoxicity screening shall be conducted in accordance with the applicable provisions of Health Effects Test Guideline, 870.7800, published by OPPTS (Docket item A-96-16/II-I-2). Applicable provisions are those which describe the performance and analysis of the required primary antibody response (IgM) to sheep red blood cell antigen by either the Jerne and Nordin splenic antibody plaque forming cell assay or by an enzyme-linked immunosorbent assay (ELISA). Optional tests described in the guideline include flow cytometric analysis of phenotypic markers on peripheral blood lymphocytes and an NK cell activity assay. Included are situations when these optional tests may be performed.

3. Additional Neurotoxicity Assessments

   In addition to the required Standard Tier 2 neurotoxicity assessments (40 C.F.R. §§ 79.66 and 79.67), a Functional Observational Battery and Motor Activity assessment shall be performed. These assessments are to be conducted in accordance with the applicable provisions of Health Effects Test Guideline, 870.6200, published by OPPTS (Docket item A-96-16/II-I-3). These assessments may be done in conjunction with, or separately from, the general subchronic toxicity study.

II. Studies Requiring Other Exposure Regimens:

A. Two-Generation Reproductive Study

1. Together with the Developmental Study listed below, this study is to be conducted in lieu of the Standard Tier 2 combined Fertility/Teratology assessment.

2. The two-generation reproductive study is to be conducted in accordance with the applicable provisions of Health Effects Test Guideline, 870.3800, published by OPPTS (Docket item A-96-16/II-I-4). The study shall be done with rats.

B. Two-species Developmental Study

1. The two-species developmental study is to be conducted in accordance with the applicable provisions of Health Effects Test Guideline, 870.3600, published by OPPTS (Docket item A-96-16/II-I-5). One of the two required species shall be rats.

2. In addition to the measurements included in OPPTS 870.3600, the two-species developmental study shall include the Standard Tier 2 neuropathology and GFAP assessments (40 C.F.R. §§ 79.66-67) conducted on the pups at weaning.

C. Carcinogenicity Study

The carcinogenicity study is to be conducted in accordance with the applicable provisions of Health Effects Test Guideline, 870.4200, published by OPPTS (Docket item A-96-16/II-I-6).

a. Only **one** species will be required. The test species shall be rats.
b. The test substances shall be delivered by the **inhalation** route.

Attachment C

Fuels and Fuel Additives (F/FA) Health Effects Testing Program: Alternative Tier 2 Toxicology Test Requirements for Nonbaseline (Oxygenated) Gasoline Groups other than MTBE-Gasoline

Overview

Attachment C describes the specific requirements of the proposed Alternative Tier 2 Testing program for the Nonbaseline (Oxygenated) Gasoline Groups other than MTBE. It identifies the objectives of the testing program for these groups, and identifies the specific testing requirements - including the Standard Tier 2 tests that have been retained, the Standard Tier 2 tests that have been deleted (at the tester's option), and the test requirements that are in addition to the Standard Tier 2 requirements.

A. General objectives:

1. Provide a screening assessment of the potential toxicologic effects in test animals of inhalation exposure to the evaporative emissions of oxygenate-gasoline fuel formulations (other than MTBE-gasoline).

2. Identify the associated hazards and, where possible, determine potential dose-response relationships and No Observed Adverse Effects Levels (NOAELs) for specific toxicologic endpoints.

3. Determine the inhalation pharmacokinetic characteristics of each in its pure state.

4. The results of these studies should be useful in assessing the potential toxicities of the various oxyfuels individually, and in comparison with each other and Baseline Gasoline.

B. In the overall context of a 90-day exposure regimen, the required toxicologic assessments are intended to screen for general subchronic (including respiratory tract) effects, fertility and developmental effects, neurotoxicity, mutagenicity, and immunotoxicty.

C. The requirements in Attachment A apply.

D. The results of these studies should be useful in assessing the potential toxicities of the various oxyfuels individually, and in comparison with each other and Baseline Gasoline.

Specific Requirements

I. Subchronic Inhalation Toxicity Study, with Specific Health Effect Assessments:

A. The objectives and methodology of the standard Tier 2 tests in 40 C.F.R. § 79.62 apply, including the specific health assessments in Section 79.62(a)(2).

B. In accordance with 40 C.F.R. § 79.62(c), one or more of the required specific health assessments may be combined with the general subchronic toxicity study, "as long as none of the requirements of any study are violated by the combination." These studies may also be conducted separately, as specified in the following standard Tier 2 guidelines:

- Fertility/Teratology assessment - Section 79.63
- In vivo micronucleus assay - Section 79.64
- In vivo sister chromatid exchange assay - Section 79.65
- Neuropathology assessment - Section 79.66
- Glial fibrillary acidic protein assay - Section 79.67

C. At the tester's option, a standard reproductive study (one-generation) and a standard developmental study (one-species) may be conducted, in lieu of the Tier 2 combined Fertility/Teratology assessment (Section 79.63). In this instance, study protocols should be developed in accordance with OPPTS Health Effects Test Guidelines 870.3800 (through weaning of F1 offspring), and 870.3600 (in rats only) (Docket items A96-16/II-I-4 & A96-16/II-I-5).

D. **The following changes and additions to the standard Tier 2 subchronic study are required**:

1. Histopathology

   a. Preparation of the animals targeted for pathologic examination of the lungs as required by Section 79.62(d)(1)(ii)(A) and (d)(5)(iii) shall include inflation of the lungs with fixative. This will permit later examination of the lung tissues by electron microscopy, if follow-up to light microscopy is indicated.

   b. Respiratory tract histopathology shall be conducted in accordance with the applicable provisions of Health Effects Test Guideline, 870.1350 (sec. 11: I - iv), published by OPPTS (Docket item A-96-16/II-I-1).

2. Immunotoxicity Screening

   a. This is to be included in the subchronic inhalation toxicity study as an additional "special health assessment", but is to be performed at the end of 28 days of exposure. A satellite group of animals may be required.

b. The immunotoxicity screening shall be conducted in accordance with the applicable provisions of Health Effects Test Guideline, 870.7800, published by OPPTS (Docket item A-96-16/II-2). Applicable provisions are those which describe the performance and analysis of the required primary antibody response (IgM) to sheep red blood cell antigen by either the Jerne and Nordin splenic antibody plaque forming cell assay or by an enzyme-linked immunosorbent assay (ELISA). Optional tests described in the guideline include flow cytometric analysis of phenotypic markers on peripheral blood lymphocytes and an NK cell activity assay. Included are situations when these optional tests may be performed.

II. Inhalation Pharmacokinetic Studies

A. The test substance shall be the pure oxygenate compound in a vapor state. The study objectives and protocol shall conform to the applicable provisions of the Health Effects Test Guideline, 870.7485, Metabolism and Pharmacokinetics, published in public draft by OPPTS (Docket item A-96-16/II-I-7), and in addition be directed at development and validation of a physiologically-based pharmacokinetic (PBPK) model to quantitatively describe test substance disposition (uptake, distribution, metabolism and elimination). Such models account for fundamental physiological and biochemical parameters and processes such as blood flows, ventilatory parameters, and renal clearance tailored by the physicochemical (e.g., blood:air and tissue:blood partitions) and toxicokinetic properties (e.g., binding, depletion of cofactors) of the test substance in question. The use of an existing PBPK model structure as a template can greatly reduce the effort required for model development of analogous compounds, and this approach is likely applicable to MTBE and the other oxygenates. Although the development of a full PBPK model can involve greater effort than other methods using pharmacokinetic data, the application of PBPK models affords the flexibility required to simulate the disposition of test substance after various potential exposure conditions and provides considerable improvement in the reliability of extrapolation across species and routes.

B. Subject to advance approval by EPA, existing pharmacokinetic testing, adequately performed and providing data reasonably comparable to that which would result from the specified studies, may be submitted in lieu of conducting duplicative tests.[33]

[33] In accordance with the F/FA testing regulations, results of adequately performed and documented previous testing may be submitted to comply with these requirements if such testing is comparable to the guidelines specified in Attachment C. *See* 40 C.F.R. § 79.53(b). EPA will review any such submission in accordance with the criteria set forth at 40 C.F.R. § 79.53(d).

Attachment D

Fuels and Fuel Additives (F/FA) Health Effects Testing Program: Alternative Tier 2 Exposure Study Requirements

Overall Goal of the Study:

- To provide information on personal exposures to gasoline and oxyfuel emissions which, together with toxicologic data, will permit quantification of the upper bound of public health risks related to these exposures.

Study Objectives:

- Quantify personal exposures to motor vehicle gasoline and MTBE-oxyfuel emissions (both evaporative and combustion-related) in microenvironments which represent the upper end of the frequency distribution of such exposures.

- Determine the quantitative relationship between the personal exposures measured in the selected microenvironments, fixed site measurements in these microenvironments, and available ambient emission measurements.

- Determine how the high-end personal exposures differ in cities and seasons of the year in which MTBE-oxyfuel is used (MTBE-containing reformulated gasoline (RFG) or wintertime oxygenated gasoline) as compared with cities and seasons in which oxyfuels are typically not used.

- Determine the relative contributions of fuel combustion vs. evaporation as the source of personal exposures to gasoline and oxyfuel emissions.

- Provide sufficient information to serve as a baseline for extrapolation to other sites and, if possible, other oxygenated fuels.

Study Protocol and Reporting Requirements:

- Before the exposure study is initiated, a detailed protocol shall be developed, peer-reviewed, and submitted to EPA for approval.

- The protocol must include detailed descriptions of the study design, technical procedures, analytic methods, and documentation. These plans must be consistent with the objectives and guidelines provided herein.

- The draft protocol shall be submitted to a group of independent and impartial peer reviewers who possess the appropriate expertise and cross-section of

practical experience to provide a useful technical critique of the study plan. While EPA is willing to suggest candidate reviewers, the Research Group/[manufacturer] has responsibility for achieving a rigorous peer review. Once finalized, the list of selected peer reviewers and copies of the documents sent for their review shall be supplied contemporaneously to EPA.

- The draft protocol shall be revised as may be indicated by the results of the peer review, and then submitted to EPA for final review and approval. Individual reviewer comments, along with a statement of the disposition of the comments, should accompany this submission.

- After protocol approval, the study shall be conducted in accordance with the approved protocol unless a variance is requested in writing and approved in advance by EPA. In unusual circumstances, if an immediate protocol variance is needed to maintain or safeguard the overall integrity of the study, then such action may be taken without prior EPA approval. However, EPA must be notified of the change in protocol immediately after the event, including a description of the critical need that required taking the unapproved action and its expected impact on the overall study design and results.

- Brief status reports shall be submitted to EPA at six-month intervals while the work continues. The status reports shall describe the progress of the study, indicate whether it is proceeding on schedule, discuss any major problems encountered or anticipated. The reports should be no longer than required to serve the practical purpose of keeping EPA informed of the status of the study.

- At the conclusion of the study, the Research Group/[manufacturer] shall prepare a comprehensive report, including hypotheses tested, description of the statistical analyses that have been done to meet the study objectives, and interpretations of the findings.

  - The draft report shall be submitted to a group of independent and impartial peer reviewers who possess the appropriate expertise and cross-section of practical experience to provide a useful critique of the study. While EPA is willing to suggest candidate reviewers, the Research Group/[manufacturer] has responsibility for achieving a rigorous peer review. Once finalized, the list of selected peer reviewers and copies of the documents sent for their review shall be supplied contemporaneously to EPA.

  - The draft report shall be revised as may be indicated by the results of the peer review, and then submitted to EPA for final review and approval. Individual reviewer comments, along with a statement of the disposition of the comments, should accompany this submission.

- The original data shall be retained by the Research Group/[manufacturer] for no less than five years, and provided to EPA upon request.

Study Design Guidelines:

A. Site Selection

- The study shall be conducted in three large cities, representing the following fuel use patterns:

	RFG*	Winter Oxyfuel*
City 1	No	No
City 2	No	Yes
City 3	Yes	No

* MTBE-containing fuels

- Since MTBE can be used for octane enhancement, the City 1 selection should be chosen where current automotive fuel has very little, to no, MTBE.

- The selected RFG city (City 3) shall be in a relatively warm climate, while the selected Winter Oxyfuel city (City 2) in a relatively cold climate. All selected cities must have an ongoing ambient monitoring program.

- Due to the variability of MTBE concentrations in all fuels (particularly non-oxyfuel areas), we are requiring that all fuels used in the study be documented and reported to the EPA.

B. Seasons and Durations

- Because potential exposures can be influenced by seasonal differences in fuel content, human activity in key microenvironments, and meteorology, the study must include sampling periods throughout the year.

- Details regarding sampling periods, days per sampling period, samples per city, and the like should be specified in the exposure protocols sent to the EPA.

- Meteorological data, e.g., data on mixing heights, stability classes, and surface roughness, are to be provided to EPA, to permit better extrapolation of data to urban locations with different climatology.

C. Microenvironment Selection

- Microenvironments shall be selected based on their association with relatively high personal exposures to motor vehicle emissions, including both combustion

and evaporative emissions. The identification of specific microenvironmental sites shall be based on defensible reasons, including pilot study measurements.

- Key microenvironments are likely to include the following:

  - Gas station: fill-up, in-car, and ambient air scenarios

  - Sidewalk next to high-volume traffic: freeway, major intersection, and urban street canyon scenarios

  - Parking garage: above- and below-ground

  - In-cabin: commuter travel, professional driving (e.g., taxi driver or delivery person), stop-and-go traffic scenarios

  - Auto repair facility

  - Interior of homes and other buildings, especially those with attached garages

  - Roadside workers, e.g., toll attendants, traffic police, auto tunnel workers

D. Subjects

- An adequate number of subjects shall be enrolled in the study to assure statistically robust results

- Scripted personnel may be used, i.e., personnel who perform or simulate the performance of characteristic activities associated with the selected microenvironments. The scripted behaviors must be based on prior activity studies, and appropriate quality assurance measures must be in place to ensure strict adherence to the behavior script.

E. Emission Measurements

- With the broad range of fuels currently in use, and the continuing changes in fuel composition, a methodology is desirable which includes measurement of a sufficient number of evaporative and exhaust emission constituents so that, when such fuel changes occur, the results of the microenvironmental exposure study can be adjusted retrospectively and used to estimate the potential new exposures without repeating the study.

- In addition, a sufficient numbers of emission components should be measured to permit emission apportionment between fuel combustion and evaporative sources.

- In each selected microenvironment, measurements shall be taken both in the subjects' personal breathing zones and at a fixed "ambient" site within the microenvironment.

- These measurements shall include (but not necessarily be limited to) the following emission chemicals:

    - Total VOC, CO, $PM_{2.5}$
    - MTBE, TBF, other emissions transformation products
    - Formaldehyde and acetaldehyde
    - Benzene, Toluene, Ethyl benzene, Xylene (BTEX)
    - 1,3-butadiene

- In addition, biomarkers of exposure (e.g. blood carboxyhemoglobin, metabolites, and breath concentrations of compounds of interest) shall be measured periodically.

Attachment E
CAA - 211 (b) Alternative Tier II - Health Effects Testing Schedules

I. MTBE Gasoline and Baseline Gasoline Testing: [a,b]

Animal

A. Testing Schedule for 90 Day Subchronic Inhalation Toxicity (including histopathology and immunotoxicity tests)

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	3 months
EPA provides comments on draft protocol to API	5 months
API submits revised draft protocol to EPA	7 months
EPA approves/disapproves revised draft protocol	9 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	21 months
EPA provides comments on draft final report	23 months
API submits final report to EPA on results of testing	25 months

B. Testing Schedule for Neurotoxicity (including FOB and Motor Activity tests)

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	3 months
EPA provides comments on draft protocol to API	5 months
API submits revised draft protocol to EPA	7 months
EPA approves/disapproves revised draft protocol	9 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	21 months
EPA provides comments on draft final report	23 months
API submits final report to EPA on results of testing	25 months

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a Schedule commences upon issuance of final requirements by EPA.

b The Section 211 (b) Research Group has indicated to EPA that, due to availability of fuels for testing and other factors, some of these studies may have to be staggered. EPA will consider comments on this issue in formalizing a final notification.

C. Testing Schedule for Developmental Toxicity - 2 Species (in lieu of fertility/teratology tests and to include neuropathology and GFAP)

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	3 months
EPA provides comments on draft protocol to API	5 months
API submits revised draft protocol to EPA	7 months
EPA approves/disapproves revised draft protocol	9 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	21 months
EPA provides comments on draft final report	23 months
API submits final report to EPA on results of testing	25 months

D. Testing Schedule for Reproductive Toxicity - 2 Generation (in lieu of fertility/teratology tests) [c]

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	12 months
EPA provides comments on draft protocol to API	14 months
API submits revised draft protocol to EPA	16 months
EPA approves/disapproves revised draft protocol	18 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	36 months
EPA provides comments on draft final report	38 months
API submits final report to EPA on results of testing	40 months

E. Testing Schedule for Carcinogenicity - 1 Species [c]

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	12 months
EPA provides comments on draft protocol to API	14 months
API submits revised draft protocol to EPA	16 months
EPA approves/disapproves revised draft protocol	18 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	58 months
EPA provides comments on draft final report	60 months
API submits final report to EPA on results of testing	62 months

c Exposure levels should be based on an analysis of the results of the 90 Day Subchronic Inhalation Study.

Exposure

F. Testing Schedule for Microenvironments & Exhaust Studies

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	3 months
EPA provides comments on draft protocol to API	5 months
API submits revised draft protocol to EPA	7 months
EPA approves/disapproves revised draft protocol	9 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	24 months
EPA provides comments on draft final report	26 months
API submits final report to EPA on results of testing	28 months

II. Nonbaseline Gasoline Groups Other than MTBE Gasoline Testing: [a,b]

Animal

A. Testing Schedule for 90 Day Subchronic Inhalation Toxicity (including histopathology, and immunotoxicity tests)

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	3 months
EPA provides comments on draft protocol to API	5 months
API submits revised draft protocol to EPA	7 months
EPA approves/disapproves revised draft protocol	9 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	21 months
EPA provides comments on draft final report	23 months
API submits final report to EPA on results of testing	25 months

a Schedule commences upon issuance of final requirements by EPA.

b The Section 211 (b) Research Group has indicated to EPA that, due to availability of fuels for testing and other factors, some of these studies may have to be staggered. EPA will consider comments on this issue in formalizing a final notification.

B. Testing Schedule for Developmental Toxicity - 1 Species (optional - in lieu of fertility/teratology tests)

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	3 months
EPA provides comments on draft protocol to API	5 months
API submits revised draft protocol to EPA	7 months
EPA approves/disapproves revised draft protocol	9 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	21 months
EPA provides comments on draft final report	23 months
API submits final report to EPA on results of testing	25 months

C. Testing Schedule for Reproductive Toxicity - 1 Generation (optional - in lieu of fertility/teratology tests) [c]

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	12 months
EPA provides comments on draft protocol to API	14 months
API submits revised draft protocol to EPA	16 months
EPA approves/disapproves revised draft protocol	18 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	30 months
EPA provides comments on draft final report	32 months
API submits final report to EPA on results of testing	34 months

D. Inhalation Pharmacokinetics Studies (oxygenates in neat form only)

API submits draft peer-reviewed protocol including individual peer review comments (unattributed if desired) and disposition of comments	3 months
EPA provides comments on draft protocol to API	5 months
API submits revised draft protocol to EPA	7 months
EPA approves/disapproves revised draft protocol	9 months
API submits draft final report for review by EPA including individual peer review comments (unattributed if desired) and disposition of comments	21 months
EPA provides comments on draft final report	23 months
API submits final report to EPA on results of testing	25 months

c Exposure levels should be based on an analysis of the results of the 90 Day Subchronic Inhalation Study.