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Dear Dr. Henry:

On August 20, 1997, EPA notified you of a proposed test program requiring health effects testing for 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] Two subsequent Federal Register notices[2] established an extended 120-day public comment period allowing interested parties to comment on the proposed requirements. This letter and its attachments are the final notification for this test program. This notice is directed to you specifically in your capacity as administrator and representative of the Section 211(b) Research Group (RG), the consortium of F/FA manufacturers organized by the American Petroleum Institute (API) to share compliance burdens and costs related to these test requirements.[3]

The 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 of conventional gasoline and various oxygenate-gasoline blends (collectively referred to here as "oxyfuels"),[4] and to guide future regulatory action pursuant to Section 211 of the Act. To adequately serve this purpose, the 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

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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] Proposed Alternative Tier 2 Requirements, 62 FR 47400, (September 9, 1997) and 62 FR 60675, (November 12, 1997).

[3] 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.

[4] 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.

effect endpoints, and addresses certain other endpoints not ordinarily included in standard Tier 2.[5]

In finalizing the Alternative Tier 2 testing requirements, EPA has placed a special emphasis in assuring that new testing protocols are properly developed beforehand, and properly implemented to assure that the best possible data will result. EPA has also placed special emphasis on the proper interpretation of the results of the testing. To this end, a rigorous peer review process, explained in further detail herein under the Study Protocols section 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 received comments from and 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.[6] Based on these discussions, EPA scientists developed a test regimen under the Alternative Tier 2 provisions to address the specific research needs associated with gasoline and oxyfuels. In a letter to API dated August 20, 1997, EPA proposed this test regimen noting that application of this 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.[7] Under the 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 proposed in the original notification and finalized below, one set of the Alternative Tier 2 requirements is specifically imposed for Baseline Gasoline and MTBE-gasoline, while a different set of requirements is imposed for the other identified oxygenate groups. 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

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

[6] EPA Public Air Docket number A-96-16.

[7] 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.

(TBA). If additional oxygenate-defined groups come into existence, then the Alternative Tier 2 requirements described below for "other" oxyfuel groups (i.e., other than MTBE) will likely be required for the new groups as well. This final notification, however, is limited to the Baseline Gasoline category and the Nonbaseline Gasoline oxyfuel groups just described.

The specific studies 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 Alternative Tier 2 testing program is necessary, describes the overall structure of the test regimen, describes the general nature of the requirements, discusses areas of testing not included in Standard Tier 2, discusses potential follow-up studies that may be required at the Tier 3 level, discusses the peer review process for developing study protocols, discusses the schedule associated with completing the testing and reviews the administrative aspects of the Alternative Tier 2 process. In the appropriate sections below, EPA discusses the comments received during the comment period, EPA's response to those comments and EPA's decisions on the health testing requirements which are finalized as part of this notification.

**The Necessity for the Alternative Tier 2 Testing Program.**

As was explained in the August 20, 1997 proposed test program notification, a number of recent expert analyses have demonstrated the necessity for the testing required by this final test program notification. 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.[8] 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

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[8] 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 focused 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.

using controlled human exposures; neurotoxic effects; neoplastic and non-neoplastic long-term effects; studies on the genotoxicity of MTBE; developmental effects; and assessment of potential contamination of drinking water with MTBE.[9] 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 exposure to MTBE and acute health effects; and potential for biodegradation of MTBE and other alkyl ether oxygenates in surface water, soil, and groundwater.[10]

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."[11] The expert analyses clearly demonstrate the necessity for testing focusing on acute health effects, carcinogenicity, neurotoxicity, developmental effects, exposure assessments, pharmacokinetic parameters, and potential exposures via drinking water.

**Tiered Requirements.**

The Alternative Tier 2 testing program is not intended to address every identified research need on baseline gasoline and the various oxyfuels. Rather, the 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 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

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[9]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).

[10]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).

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

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 final notification.

**Role of Evaporative and Combustion Emissions.**

Toxicologic studies included in the final Alternative Tier 2 regimen are based on animal inhalation exposures to evaporative emissions mixtures of the gasoline or oxyfuel in question. Thus, the 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 RG submitted for EPA's evaluation in August, 1996.[12] Prepared as a result of discussions held at an API-sponsored information meeting on December 11-12, 1995,[13] 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

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[12]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).

[13]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).

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.[14] 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 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. EPA received no public comments disagreeing with this proposed approach. EPA also recognizes 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 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.

**Alternative Evaporative Emissions Generation.**

The Research Group has developed and submitted to EPA, an alternative method for evaporative emissions generation for gasoline and gasoline oxygenate

[14]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.

mixtures[15] and has formally requested that it be used for all relevant toxicology testing under the Alternative Tier 2 requirements discussed herein. Known as the "stripper still" method, this method produces a light-end gasoline vapor fraction that is similar to headspace vapor from a vehicle fuel tank at near maximum in-use temperature. Among other things, the RG submission included light-end gasoline evaporative health effects testing which confirmed that this method produces a sufficiently concentrated and constant exposure sample during the course of a study.

In EPA's August 20, 1997 proposed test program notification, EPA proposed that the Agency consider approval of this alternative technique when individual protocols are submitted to the Agency for approval. The RG commented that it had previously submitted the details associated with this alternative technique including supporting documentation demonstrating that the technique meets the criteria set out in 79.57(f)(5).[16] The RG further argued that review and approval of this technique as part of a case-by-case review of protocols would cause an unnecessary delay.

EPA agrees with the comments of the Research Group. In development of the health effects testing regulations, EPA specifically promulgated criteria to use in judging the adequacy of alternative techniques for evaporative emissions generation. EPA scientists have reviewed the RG submission and have concluded that the "stripper still" method meets the criteria required under the regulation. EPA thus approves this method as an alternate evaporative emissions generation approach for all Tier 2 toxicology testing. Concurrent with the Federal Register notice announcing the finalization of testing requirements that are the subject of this letter, EPA will also announce, as required by 40 CFR 79.57(f)(5)(ii), the approval of this alternative method for evaporative emissions generation. A copy of the RG submission including a description of the alternative generation procedure[17] and EPA's evaluation of the submission[18] have been placed in the docket.

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[15]See memorandum from C.J. Henry, Ph.D., Director, Health & Environmental Sciences Department, American Petroleum Institute, Request for Alternative Evaporative Emissions Generation Method for Baseline and Nonbaseline Gasoline Groups, July 1, 1997. (Docket item A-96-16/II-D-2).

[16]See memorandum from C.J. Henry, Ph.D., Director, Health & Environmental Sciences Department, American Petroleum Institute, Comments to the 8-20-97 Alt Tier 2 Notification of Testing Requirements for Baseline and Nonbaseline Gasoline, December 23, 1997. (Docket item A-96-16/IV-D-1).

[17]See memorandum from C.J. Henry, Ph.D., Director, Health & Environmental Sciences Department, American Petroleum Institute, Request for Alternative Evaporative Emissions Generation Method for Baseline and Nonbaseline Gasoline Groups, July 1, 1997. (Docket item A-96-16/II-D-2).

[18]See memorandum from John Brophy, EPA, Fuels and Energy Division, Evaluation of the 211(b) Research Group's Alternative Evaporative Emissions Generator Method for the Alternative Tier 2 Health Effects Testing Regulations, June 18, 1998. (Docket item A-96-16/IV-B-1).

**Alternative Tier 2 Toxicity Testing Requirements.**

The Alternative Tier 2 testing regimen as proposed and finalized here 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 oxyfuels, 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 impose 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.

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

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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.

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

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.

Issues associated with scheduling of the Alternative Tier 2 testing requirements are discussed in the section below entitled “Schedules”.

**Pharmacokinetic Studies on "Neat" Oxygenates**

As explained in the August 20 proposed notification, EPA believes that an understanding of the pharmacokinetic (PK) characteristics of the oxygenates as pure compounds is important to our understanding of their relative toxicities when mixed in gasoline. Further, EPA believes that development of a data base on the disposition (uptake, distribution, metabolism, and elimination) of the neat oxygenates could provide the basis for a better understanding of mixtures that include oxygenates. This understanding, in conjunction with toxicity and mechanistic studies, would guide the choice of test levels to describe dose-response for future toxicity testing of mixtures.

Therefore, EPA proposed to require inhalation pharmacokinetic studies on ethyl alcohol, ETBE, TAME, DIPE, and TBA. These studies were proposed to be conducted in accordance with the applicable provisions of OPPTS Health Effects Test Guideline 870.7485. In addition, EPA proposed that the inhalation pharmacokinetic studies be

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

directed at the development of a physiologically based pharmacokinetic (PBPK) model for each additive.

The RG commented that ample PK data exist on ethyl alcohol, TAME and ETBE, and that such data "provide sufficient information to EPA and should enable the Agency to conduct route-to-route extrapolations on the neat oxygenates and thus provide *some* basis for comparative risk assessment." (emphasis supplied). The RG also commented that PK data on TBA has been developed because TBA is a metabolite of both MTBE and ETBE. Moreover, The Chemical Industry Institute of Toxicology (CIIT) is developing a PK model for TBA in rodents. Finally, the RG commented that the development of a PBPK model for each oxygenate is not appropriate in the context of the alternative Tier 2 health effects testing regimen. The RG stated that they are willing to perform the pharmacokinetic testing required by the Health Effects Text Guideline 870.7485, but have reservations regarding the viability of attempting to develop and validate a PBPK model under a compliance testing schedule such as found in Attachment E in the notification proposal.

In response to the RG's comments, EPA is unconvinced that the data and information identified by the RG in its comments satisfy the requirements of Test Guideline 870.7485 for each of the oxygenates. With respect to the PBPK modeling, however, EPA accepts the RG's comments and EPA will not require as part of the alternative Tier 2 testing regimen development of separate PBPK models for each oxygenate. EPA strongly encourages, however, the RG to separately develop PBPK models for these oxygenates, to further enhance the Agency's risk assessment capabilities and diminish the potential need for additional studies at the Tier 3 level.

PK Testing of Individual Oxygenates

The RG comments that extensive human and animal ethanol PK data have determined that the best biological indicator of ethanol toxicity is blood ethanol concentration (BEC), and that the only PK data necessary to assess the toxicity of ethanol inhalation exposures are data connecting exposure to BECs. The RG asserts that Pastino et al. (1997)[22] have developed the necessary data and models allowing prediction of BEC following inhalation exposure to ethanol, and that these models are appropriate for toxicity evaluation.

EPA does not agree that the data and models presented by Pastino et al. (1997) are sufficient to meet the objectives of the alternative Tier 2 test regimen. Pastino et al. (1997) describes a quantitative PBPK model for inhaled ethanol, which should be

[22]Pastino, G.M., Asgharian, B., Roberts, K., et al., (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. Pharmocol. 145:147.

acknowledged as an important contribution to our understanding of ethanol as a fuel additive. The paper does not, however, provide a complete listing of the metabolites of ethanol, nor report various other data which are required by 870.7485 (and thus required under this notification). These missing data include:

- Metabolite data;
- Tissue distribution time course;
- Plasma kinetics;
- An acknowledgment of enzyme induction: alcohols are known to induce the enzyme known as P4502E1 and the authors of the Pastino et al. paper themselves have stated that the in vivo role of P4502E1 is not clear, and there may be potential contributions of this enzyme at higher levels or chronic exposure. For such cases, the quantification and role of P4502E1 in the model may need to be taken into account;
- Animal body weights;
- Number of animals for inhalation exposure design and setup;
- Computer versions or copyright years for the computer software programs ACSL and Simusolv;
- Individual animal results (the manuscript figures presented averaged results).

To meet the alternative Tier 2 requirements for ethyl alcohol, the RG must submit data that are fully compliant with the 870.7485 Test Guideline requirements.

The RG comments that substantial PK data exists or is being developed on TAME, referencing Johanson et. al., 1997 and Sumner et al., 1997.[23] In addition, the RG points out that TAME PK data have been developed in rodents pursuant to a TSCA Section 4 consent agreement using EPA TSCA testing guideline 795.230.

The Johanson and Sumner papers are abstracts, not peer reviewed reports. These abstracts are not sufficient to meet the requirements of Test Guideline 870.7485. As provided in the proposed notification, in accordance with 40 C.F.R. § 79.53(b), existing PK data "providing data reasonably comparable to that which would result from the specified studies, may be submitted in lieu of conducting duplicative tests." Therefore, to the extent that the RG can demonstrate that the TSCA Test Guideline 795.230 data are "reasonably comparable" to the OPPTS Test Guideline 870.7485, the RG may submit them to satisfy the PK requirements for TAME. EPA will evaluate such data in accordance with the criteria set forth at 40 C.F.R. § 79.53(d).

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[23]Johanson, G., Lof, A., Nihlen, A. Pekari, K. and Rijhimki, V. (1997). Toxicokinetics of Ethers in Humans - A Comparison of MTBE, ETBE and TAME. Toxicologist 36, 339. Sumner, S.C.J., Asgharian, B., and Fennell, T.R., (1997). Blood Pharmacokinetics of Tertiary Amyl Methyl Ether in Male and Female Rats and Mice Following Inhalation Exposure. Toxicologist 36, 338.

The RG comments that absorption, disposition, metabolism, and excretion of ETBE have been observed in humans and rodents, referencing Lof et. al., 1997 and Borghoff et al., 1997.[24] In addition, the RG points out that ETBE PK data have been presented to EPA's TSCA Office.[25]

The Lof and Borghoff papers are abstracts, not peer reviewed reports. These abstracts are not sufficient to meet the requirements of Test Guideline 870.7485. As provided in the proposed notification, in accordance with 40 C.F.R. § 79.53(b), existing PK data "providing data reasonably comparable to that which would result from the specified studies, may be submitted in lieu of conducting duplicative tests." Therefore, to the extent that the RG can demonstrate that the TSCA Section 8(d) data are "reasonably comparable" to the OPPTS Test Guideline 870.7485, the RG may submit them to satisfy the PK requirements for ETBE. EPA will evaluate such data in accordance with the criteria set forth at 40 C.F.R. § 79.53(d).

The RG comments that TBA PK in humans has been characterized because TBA is a major metabolite of MTBE and ETBE. The RG also identifies a Poet, et al. (1997) study of TBA PK in rodents,[26] states that Arco Chemical Co. has completed a dermal absorption study of 14C-TBA in male rats (to be submitted to EPA's TSCA Office), and comments that CIIT is developing a TBA PK rodent model.

However, EPA believes that further work is needed to characterize the pharmacokinetics of TBA <u>as an oxygenate,</u> rather than as a metabolite of MTBE or ETBE. TBA's PK under these circumstances may differ from its PK via inhalation, oral, or dermal routes when it is <u>administered itself as an oxygenate</u>. While some parameters are likely to be similar, others may be dissimilar. Further, we are not convinced that models developed for other oxygenates are appropriate for TBA due to differences in certain physicochemical properties, such as TBA's water solubility and polarity. While a basic model may be applicable to the ether oxygenates, it is less likely to be true for TBA. Therefore, EPA is requiring data/information consistent with the requirements of Test Guideline 870.7485 for TBA.

EPA agrees with the RG that the PBPK model for MTBE (and TBA as a metabolite) currently being developed at the Chemical Industry Institute of Toxicology

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[24]Lof, A., Nihlen, A., and Johanson, G. (1997). Toxicokinetics of Ethyl t-Butyl Ether (ETBE) in Male Volunteers. Toxicologist 36, 339. Borghoff, S.J., Laethem, C.L., Turner, M., Robert, K., Asgharian, B., and Wright, G., (1997). Elimination of $^{14}$C Ethyl t-butyl Ether (ETBE)- Derived Radioactivity from Rats and Mice Following Single and Repeated Exposures. Toxicologist 36, 338.

[25]*Animal inhalation Toxicity Testing Results for Ethyl Tertiary Butyl Ether*, January 10 & 28, 1997, Reported to TSCA Section 8(d) under the requirements of 40 CFR 716.35.

[26]Poet, et al. (1997), Toxicology Letters 92: 179-186.

(CIIT), may prove to be responsive to the needs of the Agency.[27] Therefore, prior to submission, the RG should satisfy itself that, at a minimum, the CIIT modeling satisfies the requirements of Guideline 870.7485. However, as in the case of the other oxygenates, it would be useful if a formal, documented presentation of the MTBE PK data and model were provided.

The RG did not comment on PK testing of DIPE specifically, except to state that it believes that the data developed by testing in accordance with Test Guideline 870.7485 "will enable EPA to conduct route-to-route extrapolations for use in comparative risk assessments of the fuels." EPA agrees, and for purposes of the alternative Tier 2 testing regimen it is only requiring testing of DIPE under Test Guideline 870.7485, but again, encourages development of a PBPK model for DIPE or a basic model that extends to DIPE.

PBPK Modeling

At this time, EPA cannot specify *a priori* what would constitute an adequate and sufficient level of pharmacokinetic characterization for each oxygenate. Therefore, EPA is requiring only the work described in Test Guideline 870.7485. However, the Agency encourages the RG to go beyond 870.7485 to develop the PBPK models and to present the pharmacokinetic data, PBPK models, and interpretations and applications of the data and models in as scientifically rigorous a form as possible. The overall strategy reflected in the proposed Alternative Tier 2 requirements for oxyfuels other than MTBE and baseline gasoline is based, in part, on having pharmacokinetic data to help inform (a) decisions about whether or not further toxicity testing might be required under Tier 3 and (b) assessments (especially quantitative comparative assessments) of potential health risks associated with respective oxyfuels. Thus, more extensive pharmacokinetic characterization, e.g., quantitative PBPK modeling, would be advantageous to everyone concerned (including the RG), as it would reduce uncertainties and enhance confidence in the outcomes of both (a) and (b) above. By reducing uncertainties, it is less likely that further toxicity testing under Tier 3 would be required and that quantitative dose-response assessments would not have to be as conservative. Moreover, this approach (going beyond the minimal requirements) is already articulated in 870.7485 [sec. (g)(5)] and EPA strongly encourages the RG to consider it.

## Use of GFAP as a Neurobiomarker

[27] 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).

In the proposed notification, EPA stated that the use of the glial fibrillary acidic protein assay (GFAP) within the subchronic toxicity testing regimen in Alternative Tier 2 will be an important step in comparing the neurotoxic potential of different types of gasoline mixtures. EPA also added that the fact that GFAP concentrations appear to be restricted to regions of the brain specifically targeted by the neurotoxic test material is precisely why it has the potential to be an excellent biomarker of neurotoxicity that could be used in screening. Primary screens generally suffer from the lack of specificity, rather than too much specificity.

The RG, in its comments, argued that the requirement for the use of GFAP as a neurobiomarker should be eliminated because the database to evaluate the test is inadequate, that there are inconsistent effects seen with other known neurotoxic solvents, such as toluene, that there is an absence of established protocols, and that there is an inadequate number of testing laboratories available to conduct GFAP tests. EPA believes the justification for elimination of the requirement for GFAP lacks a strong factual basis and the requirement should be maintained. EPA's response to each justification for elimination provided by the RG is as follows:

(a) No further evaluation of the GFAP assay is necessary before it can be used in this testing program. GFAP has been used with a wide range of neuropathic agents selected to produce damage to neuronal cell bodies, axons or myelin. [28] [29] [30] Compounds producing reversible changes in the function of the nervous system in the absence of neuropathology have not increased the expression of GFAP. Therefore, GFAP may be sensitive to the potential neuropathic effects of any organic compound, but not to their reversible "pharmacological" actions.

(b) Insufficient information was provided to support the view that inconsistent effects result with the use of a known neurotoxic solvent, toluene. The work by Hugh Evans [31] that forms the basis of the RG's argument should not be used in this context, since it has not been published in the peer reviewed literature. In addition, exposure to toluene at the dose levels reportedly used in the experiments by Evans is not known to produce damage to nervous tissue such as would elicit a glial response, but rather toluene produces reversible changes in neural function in the absence of damage.

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[28] O'Callaghan, J.P. (1991a) The use of GFAP in first-tier assessments of neurotoxicity, J. Am Coll Toxicol 6: 719-726.

[29] O'Callaghan, J.P. (1992) Assessment of neurotoxicity using assays of neuron-and glia-localized proteins: chronology and critique, Target Organ Toxicology Series: Neurotoxicology.

[30] O'Callaghan, J.P. and Miller. (1993) Quantification of reactive gliosis as an approach to neurotoxicity assessment, NIDA Monograph 136, Assessing neurotoxicity of drugs of abuse, pp 188-212.

[31] Evans, H.L. (1997) Brain Glial Fibrillary Acidic Protein (GFAP) as a Marker of Neurotoxicity During Inhalation Exposure to Toluene. API publ. #4647, 22pp.

Therefore, it is consistent with the known sensitivity of GFAP assays that the reported exposure to toluene did not cause a clear increase in GFAP concentration in brain tissue. Similarly, the RG cites another unpublished report regarding developmental exposure to PCBs. Like toluene, developmental exposure to PCBs is not known to produce the type of neural injury which elicits a reactive astrocytic hypertrophy and the associated increase in GFAP. Therefore, neither of the examples cited by the RG is considered to provide evidence which questions the ability of GFAP assays to detect a response to chemically-induced injury.

(c) The RG provided insufficient information to support the point that the absence of standard protocols should preclude use of GFAP. The RG may wish to review O'Callaghan and Miller (1993).[32] A microliter plate assay employing both monoclonal and polyclonal antibodies to GFAP in a sandwich format has been documented[33] and has been in use for several years.

(d) The published literature indicates that there are a number of laboratories capable of performing this assay.[34] [35] [36] [37] We are aware that a number of industrial laboratories know how to do this assay because these laboratories are participating in a collaborative GFAP study coordinated by the National Institute of Occupational Safety and Health (NIOSH)/Centers for Disease Control and Prevention (CDC). The RG may wish to contact Dr. O'Callaghan, NIOSH/CDC for more information.

(e) The RG stated that increases in GFAP concentrations appear to be restricted to regions of the brain specifically targeted by the neurotoxic test material and this sensitivity is not useful for a primary screen. The sensitivity of the assay is precisely why GFAP has the potential to be an excellent biomarker of neurotoxicity that could be

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[32]O'Callaghan, J.P. and Miller. (1993) Quantification of reactive gliosis as an approach to neurotoxicity assessment, NIDA Monograph 136, Assessing neurotoxicity of drugs of abuse, pp 188-212.

[33]O'Callaghan, J.P. (1991b) Quantification of glial fibrillary acidic protein: Comparison of slot-immunobinding assays with a novel sandwich ELISA. Neurotoxicology and Teratology 13: 275-281.

[34]Aschner, M. Astrocytic functions and physiological reactions to injury: The potential to induce and/or exacerbate neuronal dysfunction - A forum position paper. Neurotoxicology 19: 7-18. 1998.

[35]Simpson, M.G., Wyatt, I., Jones, H.B., Gyte, A. J. Widdowson, P.S. and Lock, E.A. Neuropathological changes in rat brain following oral administration of 2-chloropropionic acid. Neurotoxicology 17: 471-480, 1996.

[36]Dey, P.M, Polunas, M.A., Philbert, MA and Reuhl, K.R. Altered expression of polysialylated NCAM in mouse hippocampus following trimethyl tin administration. Neurotoxicology 18: 633-644, 1997.

[37]Selvin-Testa, A., Capani, F., Loidl, C.F., Lopez, E.M. and Pecci-Saavedra, J. Prenatal and postnatal lead exposure induces 70 kDA heat shock protein in young rat brain prior to changes in astrocyte cytoskeleton. Neurotoxicology 18: 805-818, 1997.

used in screening. The concern that neuropathic lesions and the consequent increase in GFAP would be restricted to a small brain region, and therefore the increase in GFAP levels would not be detected in an assay of a large amount of brain tissue, can be minimized by dissecting the brain into a number of brain regions for GFAP analysis. In this way, the potential sensitivity of the GFAP assay is increased, as is the ability to localize the site of brain damage. The RG also expressed concern about the level of technical expertise needed to dissect brain regions; this concern is not supported by the published literature suggesting that a number of laboratories are capable of meeting the test rule requirements.

Finally, we wish to emphasize that EPA's neurotoxicity risk assessment guidelines [38] indicate that chemical-induced GFAP increases in the nervous system are to be understood as an indicator of a neurotoxic injury in the region where the GFAP occurs. These increases are not to be considered specific adverse effects themselves. Also, it is widely accepted that a finding of injury does not always lead to detectable pathology, and GFAP measures have been shown to be more sensitive on some occasions than routine neuropathological measures. [39] Therefore, routine neuropathology measurements cannot replace studies of GFAP.

For these reasons, EPA disagrees with the arguments put forth by the RG and the requirement in this notification for GFAP is maintained.

**Immunotoxicity Screening**

An immunotoxicity screen was proposed to be included in Alternative Tier 2 for both the baseline gasoline and MTBE-gasoline group as well as the nonbaseline gasoline oxyfuel groups (Attachments B and C).

The screen was proposed because EPA believes it would be desirable to obtain data on immunotoxicity soon, under Alternative Tier 2, rather than waiting for the completion of Alternative Tier 2 testing and then developing follow-up test requirements at the Tier 3 level. EPA argued that, first, and most importantly, under Alternative Tier 2, the data for this endpoint would become available to EPA in a relatively shorter period of time. Second, from the point of practicality, this screen can be feasibly incorporated into the Tier 2 subchronic toxicity test regimen.[40] Finally, if the screening

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[38] Final Guidelines for Neurotoxicity Risk Assessment, 63 Fed. Reg. 26926 - 26954 (May 14, 1998).

[39] O'Callaghan, J.P. (1992) Assessment of neurotoxicity using assays of neuron-and glia-localized proteins: chronology and critique, Target Organ Toxicology Series: Neurotoxicology.

[40] *See* Health Effects Test Guideline, 870.7800, published by OPPTS. (Docket item A-96-16/II-I-2)

does not show a reasonable need for further and more extensive investigation under Tier 3, a lower overall cost would be incurred by the RG.

The RG argued that the immunotoxicity testing should be eliminated from the Alternative Tier 2 provisions, since it is unwarranted and the data collected could be easily misinterpreted. The RG cites as a justification for eliminating this provision, previous toxicity testing of wholly vaporized gasoline that showed no suggestion of immunotoxicity, based on hematotoxicity testing.[41] However, if required, the RG stated it would support conducting the one required test (functional responsiveness to a T Cell-dependent antigen) specified in the recently finalized health effect guidelines for Immunotoxicity testing (OPPTS Health Effects Test Guideline 870-7800).

EPA believes that sufficient information has not been provided demonstrating that such testing should be eliminated. Past research[42] has shown that hematologic endpoints (i.e., white blood cell counts) are not good predictors of immunotoxicity. In an evaluation of over 50 chemicals, white blood cell counts were less than 50 percent accurate in predicting immunotoxicity. Hence the lack of hematotoxicity cannot be used to justify not testing for immunotoxicity.

Further, insufficient information is provided demonstrating that these data are more likely to be misinterpreted than data for other endpoints. There are numerous immunotoxic chemicals that produce good dose response curves even when using high doses. We know of no data that demonstrate immunotoxicity is any more vulnerable than other toxicity endpoints to this phenomenon.

EPA agrees that the immunotoxicity requirement can be modified to address only the assay that assesses the antibody response to a T-cell dependent antigen. Although the Luster papers cited above show a good correlation between phenotyping of splenic cells and immune suppression in mice, and other investigators[43] reported use of

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[41]MacFarland, HN, Ulrich, CE, Holdsworth, CE, (1984). A Chronic Inhalation Study with Unleaded Gasoline Vapor. J. Amer. Coll. Toxicol. 3: 231-248.

[42]Luster, MI, Portier, C., Pait, D.G., White, K.L., Jr., Gennings, C., Munson, A.E., and Rosenthal, G.J. (1992) Risk assessment in immunotoxicology I. Sensitivity and predictability of immune tests. Fundam. Appl. Toxicol. 18,200-210.

Luster, MI, Portier, C., Pait, D.G., Rosenthal, G.J. Germolec. D.R., Corsini, E., Blaylock, B.L., Pollock, P., Kouchi, Y., Craig, W., White, D.L., Munson, A.E., and Comment, C.E. (1993) Risk Assessment in Immunotoxicology II. Relationships Between Immune and Host Resistance Tests. Fundam. Appl. Toxicol. 21,71-82.

[43]Ladics, G.S., Loveless, S.E. Cell surface marker analysis of splenic lymphocyte populations of the CD rat for use in immunotoxicological studies. (1994) Toxicology Methods 4: 77-91.

Smialowicz, R.J., Riddle, M.M., Williams, W.C., and Diliberto, J.J. 1994. Effects of

lymphocyte phenotyping to detect immunotoxicity in rats, we agree rat lymphocyte phenotyping is not appropriate at this time.

Therefore, EPA is requiring immunotoxicity testing under Alternative Tier 2, but modifies the requirement to accept OPPTS guideline 870.7800.

**Reproductive and Developmental Toxicity Tests**

EPA's notification of proposed testing included neuropathologic endpoints in addition to the two-generation reproductive toxicity and two species developmental toxicity test regimen required under Standard Tier 2 for the baseline gasoline and MTBE-gasoline group (Attachment B). The RG noted that neuropathology and GFAP assays on pups at weaning is not technically possible within developmental toxicity tests since pups will not be born as part of that protocol and, therefore, will not reach weaning.

EPA agrees with the RG on this point and notes that the developmental guidelines (OPPTS Health Effects Test Guideline 870-3800) specify killing the pregnant dams on the last day of gestation, and, following those guidelines, the GFAP assays cannot be done at weaning. Therefore, Standard Tier 2 neuropathology tests no longer apply for the developmental toxicity regimen. EPA is requiring that the information provided by GFAP on weanlings is to be obtained within the multigeneration reproductive test regimen. The test will be performed on the first generation of pups no sooner than 21 days after birth and no later than 28 days. This would also require modifying the protocol, however, since it currently does not include neurotoxicologic endpoints within a two-generation reproductive study.

**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

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2,3,4,8-tetrachlorodibenza-p-dioxin (TCDD) on humoral immunity and lymphocyte subpopulations: Difference between mice and rats. Toxicol. Appl. Pharmacol. 124:248-256.

Smialowicz, R.J., Riddle, M.M., Luebke, R.W., Copeland, C.B., Andrews, D., Rogers, R.R., Gray, L.E., and Laskey, J.W. 1991. Immunotoxicity of 2-methoxyethanol following oral administration in Fischer 344 rats. Tox. Appl. Phar. 109:494-506.

purposes of comparative public health risk assessment. To address these data gaps, EPA requires that initial screening level population exposure studies be conducted for baseline gasoline and MTBE-gasoline.

Consistent with the general approach of the Alternative Tier 2 testing program, the 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, Alternative Tier 2 requires subject personal exposures to be quantified only in specified microenvironments representing the upper end of the frequency distribution of potential exposures.

The 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, perhaps, through the use of biomarkers.

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), 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 studies 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.

The RG has raised several issues for comment and clarification as to the above described EPA proposal for exposure research under the Alternative Tier 2 approach.

First, the RG commented that the preferred approach to screening high end exposures would be contracted technicians performing scripted behaviors to simulate worker activities in micro-environments where such activities are likely to be encountered is preferred to a conventional industrial hygiene design which monitors workers as they perform their daily tasks.

EPA agrees that scripted behavior microenvironmental measurement is acceptable for characterizing high end exposures, provided that appropriate scripted behaviors are used in the studies. It would be important to note, however, that there may be events where time resolution of the measurements should be finer than 1-hour averages. The draft protocols should include scripted behavior choices, source characterization, and measurements that would be peer reviewed.

The RG commented that it interprets the directive that the exposure studies are to occur in cities with “ongoing ambient monitoring programs” to mean cities with active criteria and organic compound monitoring networks for pollutants designated in the study guidelines.

EPA confirms that this interpretation is correct.

The RG commented that the upper bound of public health risks related to the exposures of interest should be defined in the exposure study requirements as occurring at approximately the 90th percentile of total population risk.

EPA recommends that the upper bound be defined in qualitative and subjective terms as occurring at the 99$^{th}$ percentile (and not the 90$^{th}$). It should be recognized that in the absence of a population distribution study, it will not be possible to tell exactly where the results fall on the distribution and thus setting a definitive level is not possible. Given that millions of people have potential inhalation exposures to MTBE, an assessment of potential chronic exposure to MTBE necessitates consideration of conditions approximating the 99$^{th}$ (and not just the 90$^{th}$) percentile.

The RG commented that the exposure study requirements should clarify whether comparisons are to be made between low MTBE octane fuels and high MTBE-based oxyfuels or between high gasohol and high MTBE-based gasolines.

EPA’s goal is to compare a high-MTBE use city with a low-MTBE use city.

The RG recommended that the biannual American Automobile Manufacturers Association (AAMA) gasoline survey (conducted on January 15 and July 15th of each year) documenting fuel specification parameters, including benzene and oxygenate compositions from major branded distributors in surveyed cities, be accepted as

fulfilling the requirement for documenting fuels used in the selected cities. Further, that the January/July sampling should be accepted as fulfilling the exposure study requirements for sampling throughout the year.

EPA believes that in addition to the AAMA gasoline survey, it would be advisable to collect a statistically significant sample supporting validation of the survey, and agrees with the RG that January and July studies can be used to provide an adequate measure of seasonal differences. Furthermore, it may be possible to also utilize, to augment or substitute for such additional validating survey data, statistically accurate Retail Gasoline Surveys which are performed by the Survey Association, an independent group organized by API to satisfy the requirements of the Reformulated Gasoline (RFG) regulations. Such data is collected according to an EPA-approved survey plan but is only applicable to cities covered by the RFG program. If use of such survey data is applicable to a city chosen by the RG, EPA would be willing to work with the Survey Association and the RG to attempt to assure that such data would be collected in a timely manner.

The proposed test program notification required the study to include sampling periods throughout the year. The RG recommended that EPA accept January/July sampling as fulfilling this requirement.

EPA confirms that January/July sampling will satisfy the requirement for sampling throughout the year.

The RG stated that it would utilize existing data on service station exposures and in-cabin driver exposures in developing the study protocols.

EPA considers it appropriate to utilize such existing data in the development of study protocols.

The RG recommended that only recent model, well-maintained vehicles should be used in the exposure studies since the focus of the study should be fuel emissions and not the state of repair of the vehicles using the fuel.

EPA disagrees. The use of only recent model well-maintained vehicles is not appropriate for providing reliable measurements representative of high-end exposures most likely to be found in vehicles with average maintenance records and which are representative of the current fleet. It is the intent of EPA that the exposure work characterize, among other things, the relationship between exhaust and combustion emissions and exposures to specific emissions products and transformation products as is experienced in a real-world scenario. The use of only well maintained vehicles would not, for the reasons cited above, simulate such a condition.

The RG stated that it would utilize existing data on service station and garage exposures of mechanics in developing the study protocols.

EPA considers it appropriate to utilize such existing data in the development of study protocols.

The proposed test program notification required an adequate number of test subjects to assure statistically valid results. The RG recommends, given the earlier recommendation for a scripted personnel approach, that “subjects” be redefined as micro-environmental and scripted personnel measurements.

EPA considers the proposed redefinition acceptable.

The RG commented that the ambient particulate matter ($PM_{2.5}$) measurements should be eliminated from the required pollutant monitoring list in the exposure study because automotive gasoline exhaust contains only modest amounts (<5 mg/mile) of particulate matter when compared to non-gasoline related sources (i.e., roadway dust). The RG commented further that, with regard to transformation products, tertiary butyl nitrate (TBN) and formic acid should only be measured, if at all, in urban ambient background samples. The added analytical burden in light of the questionable connection of these compounds to MTBE, rather than other organic species, is marginally justified.

EPA concurs that the requirement for $PM_{2.5}$ measurements may be eliminated for the Alternative Tier 2 requirements, due to the complexity of a study to address the question of whether changes in PM could occur with oxygenated-fueled vehicles relative to baseline gasoline. However, changes in PM associated with the use of oxyfuels have not been adequately described. In particular, we believe it is uncertain whether the increased aldehydes associated with oxygenated-fueled vehicles could lead to increased PM concentrations under both summer and winter conditions under a widespread number of atmospheric conditions. Aldehydes can lead to more rapid oxidation of nitrogen oxides (NOx) resulting in the formation of nitrate-containing PM. This matter may be considered under Tier 3.

With regard to measuring transformation products, EPA believes that the issue of where TBN and formic acid are to be measured is most appropriately addressed in the context of protocol development. Therefore, this question should be addressed by peer reviewers of the draft protocols. As discussed below, EPA will review the draft final protocols.

The proposed test program notification required periodic measurements of biomarkers of exposure. The RG recommended that such biomarker measurements not be required until Tier 3 because of “the relative complexity of implementing such invasive procedures.” Further, the RG recommended that such measurements be required at the Tier 3 level only if “risks of exposure to specific emission components quantified in Tier 2 are found to be of concern.” The RG notes that this approach would be consistent with the approach adopted by EPA in a 1997 draft of the *Research Strategy for Oxygenates in Water*. Lastly, the RG recommended that urine analyses

should be considered an acceptable alternative to blood and breath sampling when biomarker measurements are eventually required. Recent advances in measuring biomarkers in urine are advantageous due to the non-intrusive nature of the procedure as compared to blood sampling, the availability of a time-weighted measure for rapidly changing concentrations within the blood, and avoidance of biohazard precautions associated with handling blood.

EPA believes that biomarkers likely will be an important component of the personal exposure studies. The inclusion of biomarker measurements can potentially supply evidence of exposure that not only complements personal breathing zone measurements but may even be a more direct indicator of actual exposure dose than such environmental measurements. Some biomarker measurements are relatively noninvasive (e.g., breath), and others would likely be feasible if the studies employ paid technicians who follow scripted activity patterns. Furthermore, the inclusion of one or more biomarkers in these studies is not inconsistent with statements in the draft *Research Strategy for Oxygenates in Water*. In the latter document the focus is on potential population exposures to oxygenates in drinking water, for which data are limited at present. Therefore, a reasonable strategy in the case of drinking water would be to first determine whether, in fact, significant population exposure to oxygenates even occurs. In contrast, considerable evidence already indicates that populations are exposed by inhalation to oxygenates.[44] Therefore, it is not unreasonable to conclude that biomarkers can play an important role in the exposure studies specified in this notification.

However, due to the potentially complex protocols associated with the exposure work required herein, the Agency is not in this notification specifying the nature of biomarker use or the degree of biomarker inclusion in the exposure study. Instead, the Agency and the peer reviewers will consider the adequacy of the protocols developed, including the use of biomarkers, when those protocols are reviewed during the course of the studies. (The peer review and Agency review process is explained below.) The Agency expects the RG to include in the exposure protocols appropriate measurements of biomarkers to aid in the overall strategy reflected in the proposed Alternative Tier 2 requirements for oxyfuels and baseline gasoline. The resulting data would help inform (a) decisions about whether or not further exposure testing will be required under Tier 3 and (b) assessments (especially quantitative comparative assessments) of potential health risks associated with respective oxyfuels. Thus, the Agency believes that exposure information that includes biomarker measurements would be advantageous to

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[44]Buckley, T. J.; Prah, J. D.; Ashley, D.; Zweidinger, R. A.; Wallace, L. A. (1997) Body burden measurements and models to assess inhalation exposure to methyl tertiary butyl ether (MTBE). J. Air Waste Manage. Assoc. 47: 739-752.

Health Effects Institute. (1996) The potential health effects of oxygenates added to gasoline: a review of the current literature, a special report of the Institute's Oxygenates Evaluation Committee. Cambridge, MA, Health Effects Institute, Oxygenates Evaluation Committee.

the RG, to the Agency and to the general public, as it may reduce uncertainties regarding potential risks and may reduce the potential need for further exposure work under Tier 3.

Issues associated with scheduling of the Alternative Tier 2 testing requirements are discussed in the section below entitled "Schedules".

**Study Protocols and Related Reviews.**

In its proposal, EPA stated that development of detailed protocols for each required study is the responsibility of the Research Group. The protocols must be scientifically valid, responsive to the objectives of the 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,[45] and Vehicle Emissions Inhalation Exposures.[46]

Originally, EPA asserted that all draft protocols, including all toxicity and exposure tests, were to be peer reviewed by competent and impartial experts.[47] The RG commented that it is inappropriate and redundant to incorporate peer review into routine regulatory compliance toxicology studies,[48] since standard protocols exist and have recently undergone extensive review in the context of EPA's approval of protocols for use under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

EPA has reconsidered its proposal to generally require new protocol review for all aspects of the toxicology testing required under this notification. EPA agrees with the RG that peer review of standard protocols based on the established toxicology test guidelines specified in the rulemaking generally is unnecessary. However, should it be determined that any protocol mentioned in this testing notification deviates from the applicable EPA test guideline, EPA maintains the option of having the RG provide for an external peer review of the draft protocol. It is important to note that the specified and already approved EPA protocols are associated with the toxicology testing aspects of this notification and not the exposure assessment.

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[45]40 C.F.R. § 79.60.

[46]40 C.F.R. § 79.61.

[47]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.

[48]Health Effects Test Guidelines published by OPPTS.

For those testing requirements for which EPA protocols are not specified or used, the draft protocols undergoing peer review should fully describe all relevant important procedures, including those procedures pertaining to the control animals, the manner of exposure, exposure/dose levels, the test endpoints to be measured and the statistical methods. Draft protocols, where applicable, 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 Alternative Tier 2 testing program. The schedule for completion of the Alternative Tier 2 requirements (Attachment E) includes adequate time for protocol development, peer review if applicable, and EPA approval. Later protocol changes, if any, must also be approved in advance by EPA.

EPA proposed that, once any part of the Alternative Tier 2 research has been completed, including the results from any standard toxicology studies, a draft report will be submitted by the RG for external peer review by an independent set of reviewers selected by the RG.

The RG has expressed its concern with an incremental peer review approach to the draft reports as individual pieces of the testing requirements are completed. They argued that these reports already undergo rigorous technical review and an independent quality assurance/quality control audit at the contract laboratory and another review by the 211(b) Research Group Toxicology Committee, composed of a number of professional toxicologists with extensive experience in the design, implementation, and review of toxicology studies. Further, they believe that external peer review would cause compliance problems as final study report deadlines would be difficult to adhere to and proposed that an external review of the total, collective findings from the various studies would alleviate this problem.

Consistent with the Agency's recently issued Science Policy Council Peer Review Handbook,[49] EPA continues to believe that peer review of draft final reports of studies (including reports from standard toxicology studies) should be required. The purpose of peer review is to enhance the scientific credibility of the reported work. Therefore, this notification requires that the 211(b) Research Group (RG) ensure that each draft final report is peer reviewed.

It is our understanding that the 211(b) RG will have its own Toxicology Committee composed of toxicologists from member companies. The functions of the RG Toxicology Committee and the peer review process are consistent and mutually

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[49] Science Policy Council Peer Review Handbook Office of Science Policy/ Office of Research and Development, U.S.EPA, Washington, D.C., January, 1998.

supportive. We strongly encourage that provisions be made for having an adequate number of qualified experts who are recognized as independent and without any potential bias or conflict of interest take part in the peer review. To this end, EPA would be willing to facilitate the independent peer review by providing a list of candidate experts.

The use of independent expert peer reviewers need not require additional time beyond that which the RG Toxicology Committee would take to review the draft reports. The independent reviewers could be required by contractual or other agreement to provide their critical comments according to a prescribed deadline, so that the overall schedule for delivery of the final report could be met. Nevertheless, EPA believes that an appropriate administrative procedure should be provided to alleviate these concerns. Thus, these requirements will allow the RG to appeal to EPA should delays occur outside of the control of the RG. Should the RG appeal reasonably demonstrate that the delays were unavoidable and/or uncontrollable on the part of the RG, EPA will alter the remaining related milestones in the schedule (as finalized herein) to accommodate such delays. Any appeal in this regard should be addressed to the proper EPA contact found in the section below entitled “Administrative Procedures”. EPA will promptly notify the RG of any allowed schedule changes as a result of the appeal.

The RG also commented that it would be appropriate to utilize a peer review panel during the final stages of the Alternative Tier 2 program, in order to review, in total, the collective findings from the various studies that were conducted under Alternative Tier 2, and to make critical interpretations of the data and suggest any additional studies that may be needed. EPA agrees with the RG that a final interpretation of the results of the studies, including comparative risk assessments and risk evaluations should be performed and that recommendations for further study should be made. However, EPA does not agree that these final assessments should be considered or required as part of the Alternative Tier 2 data collection requirements. EPA will undertake at the appropriate time, working with peer reviewers and all interested parties, a process to produce final assessments of the data collected.

**Contingent Studies.**

As discussed above, the 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.

Also, as is mentioned above in the discussion of requirements on exposure monitoring, Tier 3 requirements may include an expansion of the exposure monitoring requirements to include monitoring of particulates and biomarkers associated with mobile sources emissions.

**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 currently exploring these issues in controlled exposure studies using human subjects. Thus, acute exposure studies are not included in the Alternative Tier 2 testing regimen. But, if the results of these studies 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.[50] 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 included as a requirement in Attachment D in the emissions measurement section of the exposure study requirements. Studies to explore the health effects of

[50]EPA understands that the ARCO Chemical Corporation has developed a method for measuring TBF in ambient air. However, limited attempts at detecting TBF in air samples taken from urban sites in which MTBE-gasoline is used have been unsuccessful. The results of a comparative study on the respiratory irritancy of TBF and other formates has been completed by ARCO and the document may be found in the docket (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.

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. An Agency-wide task force has recently published an external review draft of the "Research Strategy for Oxygenates in Water."[51] The Strategy identifies the current, or soon to be started, projects in the areas of environmental occurrence, source characterization, transport and transformation, exposure, toxicity, remediation, and other areas for fuel oxygenates such as MTBE. 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 to address MTBE water issues 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 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.

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[51]EPA/600/R-98/048, April, 1998. An external review draft of the Research Strategy can be obtained through the internet at http://www.epa.gov/ncea/oxywtr.htm.

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.

## Schedules

Toxicology and Pharmacokinetic Studies

In proposing a schedule in its August 20, 1997 notification EPA noted that the RG had indicated to EPA that, due to the availability of fuels for testing and other factors, some of these studies may have to be staggered. Although EPA's proposed schedule indicated what EPA believed to be appropriate durations of time to conduct specific studies, EPA noted that it would consider the need for staggering or otherwise changing the schedules in the final notification.

The RG's comments suggested a staggered timeline contingent on the assumption that the proposed alternative evaporative emissions generating procedure would be approved by EPA, that protocol review of standard EPA protocols would not be required, and that no peer or EPA review of draft toxicology reports from standard toxicology studies would be required. The first two assumptions are incorporated into this notification. However, as stated in this notification, EPA is requiring that study reports be peer-reviewed prior to submission to EPA. As stated in the above section on peer review, this notification requires that the peer review of these reports be performed by the RG's Toxicology Committee augmented by other external peer reviewers. EPA does not believe that the augmentation with external peer reviewers of the RG's already planned peer review (by the Toxicology Committee) should significantly extend the needed time for peer review.

Therefore, with a few exceptions, the schedule presented by the RG which includes staggering of studies is reasonable, based on considerations put forth by the RG. These include the efficient use of resources for management of contract laboratories, minimizing the use of multiple laboratory settings which would result in a potential for interlaboratory variability, and the necessarily sequential nature of some of the required toxicology testing (e.g., a subchronic study needs to be conducted prior to the initiation of the two-year carcinogenicity study). However, in the final testing schedule which is part of this notification, 60 days has been added to the animal toxicity testing schedule suggested by the RG to allow for review of draft reports by EPA, including peer review comments, and an additional 60 days for the RG to produce a final report after EPA has provided its comments to the RG.

For the purposes of simplicity, Attachment E should be used as a general overview of the types of tests required under Alternative Tier 2, the staggering of the

studies, and the relevant scheduling deadlines. For the complete set of toxicological testing endpoints actually required under Alternative Tier 2, Attachments B and C should be consulted.

Exposure Studies

The RG requested an extension of the schedule associated with exposure work due to the increased potential for difficulties associated with exposure studies. The RG cited several reasons for extending the proposed schedule for the exposure requirements.

Regarding protocol development the RG noted several issues: a lack of codified protocols for micro-environmental and personal exposure monitoring; the need for the development of methods associated with emerging personal monitoring technologies; the need to await reports due in 1998 on pilot study results of these new monitoring technologies in order to develop protocols. Based upon these comments, the RG recommended that protocol development not begin until 12 months after receipt of this notification and that 6 months (instead of 3) be allowed for the protocol development process.

The RG also pointed out that under the proposed schedule 15 months are allowed to conduct the actual field study and submit a report on the study. The RG argued that, since the multi-season field portion of the study will take at least 12 months, only 3 months would be left to assess the data, draft a report, peer review the report, respond to peer review comments and submit the report to EPA. The RG recommended that an additional 9 months be allowed (to replace the 3 months in the proposal), 6 of which would be utilized to assess the data collected and 3 months to review and respond to comments on the collected data. The RG also commented that, due to the potential for difficulties associated with utilizing new monitoring technologies, the RG should be allowed to request additional extensions should difficulties arise.

The RG thus envisioned a timetable of 52 months in length compared to the 28 months proposed in the Attachment E schedule of the draft notification letter.

EPA agrees, in part, with the RG's comments and believes that some additional time in the schedule is appropriate to adequately fulfill the Alternative Tier 2 exposure-related testing requirements described herein. The exposure testing table in Attachment E displays the revised schedule.

Six months have been added to the original schedule to allow for the results of ongoing monitoring studies to be incorporated into the process of drafting the peer-reviewed exposure protocols. The RG request for 12 additional months in order to view the results of pilot studies collected in 1998 was based on the date that the RG submitted comments, December 23, 1997. Since this notification is being issued well

past mid-year,1998, EPA believes that an extension of 6 months will be more than adequate to await test results finalized in 1998.

Due to the complexity of developing and incorporating new methodologies into the exposure study protocols, EPA has agreed with the RG in expanding to 6 months the time needed for protocol development. Thus, protocols must be submitted to EPA within 12 months after receipt of this notification.

EPA agrees with the RG concerns associated with the original 15 month proposal for actually conducting the studies and submitting the reviewed report on the studies. Thus, an additional nine months have been added to the original 15 months allowed to complete the research portion of the exposure studies, to develop a report, to peer review the report and to incorporate the peer review comments. Finally, two additional months have been added to allow for the RG to respond to the Agency's comments and revise the final report for submission to the Agency resulting in a final submission 48 months after receipt of the notification.

Finally, EPA recognizes that unforseen problems or emergency situations can create unavoidable delays in any large testing program especially when new technologies are being employed. Recognizing this, EPA will consider on a case-by-case basis, in such a situation, an appeal by the RG to alter test schedules or test protocols. If the situation is judged by EPA to have resulted from uncontrollable or unavoidable situations and if protocol or schedule alterations are judged by EPA as not detrimental to the quality of the results, EPA will consider granting an extension of associated deadlines.

**Comments by Parties Other than API**

EPA received comments on the proposed notification from two parties other than API. One commenter questioned the Agency's proposal to not require that comments be attributed to individual peer reviewers. The names and affiliations of each of the peer reviewers serving on an Alternative Tier 2 Peer Review Panel, along with their comments in complete form, will be public information and placed in the EPA docket. However, to assure that all peer reviewers will comment freely on draft protocols or study results, EPA will not require that the specific comments be attributed to specific peer reviewers. EPA believes that the expertise of the peer review panel can be judged by its membership and that individual comments, while required to be submitted in their entirety, should be judged on their own merit.

Several comments were also received from Ethyl Corporation, a member of the Research Group.[52] Ethyl's comments state that the proposed Alternative Tier 2 testing regimen "embodies a number of fundamental principles that govern <u>any</u> exercise by EPA of its Alternative Tier 2 authority."[53] Ethyl then purports to briefly outline these principles. As discussed below, to a great extent, the "fundamental principles" that Ethyl asserts are embodied in EPA's proposed test program notification are without basis either in law or in fact.

First, Ethyl mischaracterizes EPA's description of the Alternative Tier 2 testing program by asserting that "EPA has proposed a 'tiered approach' for test programs that implements a 'stepwise' research program 'not intended to address every research need.'"[54] Ethyl then "agrees that a 'stepwise' approach to the completion of research must be adopted whenever a rational basis can be articulated for such an approach."[55] Ethyl's characterization misplaces several concepts clearly and sequentially set forth by EPA at page 4 of the proposal and thus reflects a misunderstanding of EPA's description of how the Alternative Tier 2 program implements the F/FA testing regime set forth at 40 C.F.R. Part 79, Subpart F. This misunderstanding results in a conclusion that is incorrect.

EPA did not propose a "tiered approach" for the proposed Alternative Tier 2 test program implementing a "stepwise" research program "not intended to address every identified research need." To recapitulate EPA's discussion of the purpose and approach of the Alternative Tier 2 testing program:[56]

(1) the proposed alternative tier 2 test program is not intended to address every identified research need;

(2) the proposed alternative tier 2 test program is intended to "fill critical data gaps" and "act as a screen" to determine whether additional information is necessary for decisions concerning potential risks associated with the subject F/FAs;

(3) consistent with the "general strategy" of the F/FA testing program, set forth at 40 C.F.R., Part 79, Subpart F, "the proposed Alternative Tier 2 testing regimen <u>is</u>

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[52]Submission from Kevin L. Fast, Hunton & Williams, to Docket A-96-16 (January 7, 1998) [hereinafter Ethyl comments].

[53]Ethyl comments at 1 (emphasis in original).

[54]*Id.* at 1 (quoting the proposed test program notification at 4).

[55]*Id.*

[56]Proposed notification at 4.

part of a tiered approach which may also include Tier 3 test requirements in the future;"[57]

(4) such a stepwise approach, i.e., requiring specific health effects tests at the Alternative Tier 2 level to fill "critical data gaps" and to determine whether additional, more extensive, data and information (which would be obtained through Tier 3 testing) are necessary to enable the Agency to make regulatory determinations regarding these F/FAs, will help assure a wise investment of manufacturer and laboratory resources.[58]

Thus, the stepwise approach, as explained by EPA, consists of requiring and obtaining critical data at the Alternative Tier 2 stage and then, if necessary, obtaining additional information at the Tier 3 stage. EPA did not state that the instant Alternative Tier 2 test regimen reflects a "stepwise approach" in and of itself. Therefore, it is incorrect to conclude that a "stepwise approach" to testing at the Alternative Tier 2 level must be adopted.

Second, Ethyl asserts that "the mere identification of a 'research need' is not sufficient for imposition of research obligations under an Alternative Tier 2 program. Rather, EPA must first establish a record demonstrating why the research is essential to the exercise of its regulatory responsibilities under § 211."[59] EPA interprets this comment to assert that the Agency must first establish a record - as that term is understood in a rulemaking context - demonstrating why required research is essential to the exercise of regulatory authority under Section 211(b) of the Clean Air Act. EPA disagrees. Section 211(b)(2)(A) of the Clean Air Act provides: "For the purpose of registration of fuels and fuel additives, [EPA] may also require the manufacturer of any fuel or fuel additive - (A) to conduct tests to determine potential public health effects of such fuel or additive (including, but not limited to, carcinogenic, teratogenic, or mutagenic effects).[60] The plain language of the statute does not support the suggestion

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[57]Id. (emphasis supplied).

[58]As EPA stated in promulgating the F/FAs testing regulations, the alternative tier 2 option provides the Agency with necessary flexibility "when available information indicates that, in a specific case, another testing regimen is preferable to the standard set of Tier 2 tests." 59 Fed. Reg. at 33081. For example, where available information causes concern about potential health effects related to endpoints not specifically addressed in standard Tier 2, EPA may require additional studies targeted to the identified area of concern, notwithstanding that such studies are not normally included in Tier 2. This added flexibility enables EPA to obtain necessary health effects data earlier than would occur by submission of the standard Tier 2 data and subsequent Tier 3 requirements. "When the additional testing can be coordinated with the standard Tier 2 testing program, the alternative Tier 2 provision will also save costs relative to conducting the additional tests at a separate point in time.

[59]Ethyl comments at 1-2.

[60]42 U.S.C. 7545(b)(2)(A).

that EPA must establish a "record" that the required information is essential prior to requiring health effects testing under this provision.

Moreover, the legislative history of Section 211(e) - which expressly mandated that EPA promulgate regulations implementing the Section 211(b)(2)(A) authority - explicitly demonstrates that EPA need not establish such a record prior to imposition of testing obligations. Section 211(e) was enacted because Congress was concerned about the "unwarranted delay and gross inadequacy" of EPA's efforts to implement Section 211(b)(2)(A).[61] Prior to enactment of Section 211(e), EPA had indicated an intention to implement the Section 211(b)(2)(A) health effects testing requirement as follows: (1) "Selected fuel additives" would be tested by manufacturers on the basis of EPA's preliminary evaluation. (2) The preliminary evaluation would consist of EPA-sponsored research on the effects of additives. (3) "Where results of this research demonstrate a need, implementation of the health effects test protocols . . . will be sought.[62] Congress expressly disapproved of this approach:

> In the Committee's view, the approach suggested by the Agency for the implementation of section 211 of the Act is wholly unsatisfactory. The reasons are several. <u>First, the Agency's proposed approach requires it to prove a fuel or fuel additive probably harmful before the manufacturer would be required to test it. In the Committee's view, this approach fails to assure adequate protection of the public health</u> and continued effectiveness of emission control systems in which substantial investments have been made. The approach improperly shifts the burden of proof from the manufacturer to the Agency (and ultimately the public it is supposed to protect).[63]

Thus, Congress did not intend that EPA seek to establish a "record demonstrating why the research is essential to the exercise" of the Section 211(b) authority prior to requiring health effects testing of fuels and fuel additives.

Ethyl also states that the availability of a comprehensive set of health data for baseline gasoline is a prerequisite to the establishment of testing requirements for non-baseline products."[64] Ethyl apparently relies on juxtaposition of two separate statements by EPA that are separated by substantial intervening discussion concerning EPA's basis for distinguishing between baseline gasoline/MTBE-gasoline and the other

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[61]H. R. Rep. No. 294, 95th Cong., 1st Sess. 306 (1977).

[62]*Id.* at 307 (*quoting* letter from Acting Administrator John Quarles to Representative Paul G. Rogers (Feb. 28, 1977)).

[63]*Id.* (emphasis supplied).

[64]Ethyl Comments at 2.

oxyfuels. What EPA does not state, however, is that the existence of baseline gasoline data is a "prerequisite" to requiring health effect testing of nonbaseline products:

> [E]ven 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. 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.[65]

The Agency was not suggesting that testing on all non-baseline products (including atypical F/FAs not belonging to any oxyfuel group or to the baseline gasoline group) should wait until all testing of baseline gasoline is completed. The quote was clearly related to only the non-baseline oxyfuels groups and to the extent of testing initially required of the non-MTBE oxyfuels based upon bridging data that may serve to guide and limit the more extensive testing for these groups. The relevant proposal language not quoted by Ethyl demonstrates that the Agency proposed to require all the testing applied to baseline gasoline to the MTBE non-baseline group. This final notification does implement that requirement.

Ethyl asserts that "any other approach would be inconsistent with EPA's authority" under Section 211. Ethyl has provided no basis either in law or in fact for its claim that the approach suggested by Ethyl is the only approach that is consistent with Section 211.

Ethyl asserts that "health testing requirements cannot be imposed as part of an Alternative Tier 2 test program where exposure data obviate the need for such testing."[66] Ethyl's statement is based on the following purported statements from the proposed notification: (1) "'information on human population exposures to various evaporative and combustion emissions components . . . may change current perceptions about the continued need for, and specific targets of, future combustion

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[65]Proposed Notification at 8 (emphasis supplied).

[66]Ethyl Comments at 2.

emissions studies'" and (2) "'will have a strong influence on EPA's determination of whether additional studies are required.'"[67] EPA's actual statements are: (1) "We also recognize that the results of the [proposed Alternative Tier 2] 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."[68] (2) "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."[69] EPA has never stated that "health testing requirements cannot be imposed as part of an Alternative Tier 2 test program where exposure data obviate the need for such testing." (Emphasis supplied.) In effect, it would be impossible to assess the risk associated with the emissions of a F/FA in the absence of sufficient health effects data even if available exposure data indicated very low exposure levels.[70]

Ethyl points out that the Agency's "proposal acknowledges the necessity of engaging in 'reasonable extrapolation' of exposure data measured in one or more study areas to other areas not studied." (In fact, because only the MTBE oxyfuel was proposed to be utilized in the exposure testing, the proposal utilized the term "reasonable extrapolation" only in the following context: "Reasonable extrapolation to the expected emissions from other oxyfuels should also be possible.") The Agency agrees that exposure data cannot be collected in every area of the country and every environment where potential public exposure may occur, and, thus, extrapolation at some point is clearly necessary. However, as was pointed out in the proposal, the exposure data "should also identify what circumstances (e.g., climate, season, microenvironment, fuel type) are associated with increased health risk."[71] Although reasonable extrapolation is appropriate, when factors such as climate, season, and vehicle use are suspected to cause wide variations in exposure, significantly more areas and conditions may have to be studied to get an accurate exposure picture. Thus, Ethyl's assertion that any approach other than extrapolating from limited exposure data "would be contrary to law" is unfounded and incorrect.

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[67] *Id.* (purportedly quoting the Proposed Notification at 6 and 10).

[68] Proposed Notification at 6 (deletions emphasized).

[69] Id. at 10 (deletions emphasized). Also, the full set of factors explicitly identified by EPA as "likely" to a have a strong influence on the decision whether to require additional studies at the Tier 3 level include health effects data from the Alternative Tier 2 toxicity studies and other sources; and circumstances associated with increased health risk, e.g., climate, season, microenvironment, and fuel type.

[70] See, e.g., Guidelines for Reproductive Toxicity Risk Assessment, 61 Fed. Reg. 56274, 56276 (Oct. 31, 1996) (risk assessment comprises some or all of the following components: (1) hazard identification; (2) dose-response assessment; (3) exposure assessment; and (4) risk characterization).

[71] Id.

Ethyl also states that market penetration "is relevant to the scope of testing that can be imposed in an Alternative Tier 2 test program."[72] EPA's proposal did not present this view. The focus of the relevant discussion to which Ethyl refers is the scope of testing likely to be considered under Tier 3 as a result of increased market penetration for a non-MTBE oxyfuel:

> 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 reevaluation 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.[73]

This discussion is entirely prospective and does not concern the establishment of the Alternative Tier 2 testing regimen. Thus, EPA did not state or imply that "market penetration must be considered by EPA when establishing an Alternative Tier 2 program for a fuel product." Moreover, there is nothing in the statute, the legislative history, or the 40 C.F.R. Part 79 regulations that support such a claim.

Ethyl's references to certain caselaw to support other of its conclusions are also inapposite. First, Ethyl takes issue with EPA's statement that "Tier 3 combustion emission 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".[74] According to Ethyl, the Court of Appeals for the District of Columbia Circuit "recently characterized EPA's focus on potential 'concern' as a basis for regulatory action under § 211 to be a 'bizarre departure from existing practice . . . '"[75] In *Ethyl*, however, the Court of Appeals was characterizing an EPA regulatory action taken under Section 211(f)(4), not 211(b), and the Court's statement was specifically addressed to EPA's action under Section 211(f)(4). As explained below, neither the decision, nor the Court's analysis and characterization had anything to do with Section 211(b). Therefore, Ethyl's attempt to apply the Court's holding in the context of this Section 211(b) action is wholly inappropriate.

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[72]Ethyl Comments at 2.

[73]Proposed Notification at 15 (emphasis supplied).

[74]Ethyl Comments at 3 (referring to Proposed Notification at 12).

[75]*Id.* (*citing Ethyl Corp. v. EPA*, 51 F.3d 1053, 1063 (D.C. Cir. 1995)).

In *Ethyl*, a fuel additive manufacturer filed a fuel additive waiver application with EPA under Section 211(f)(4).[76] EPA determined that the manufacturer had demonstrated satisfaction of the 211(f)(4) emissions requirement. Nonetheless, EPA denied the waiver application on the basis that, inter alia, "there was a 'reasonable basis for concern about the effects on public health that could result if EPA were to approve use of'" the additive.[77] The D.C. Circuit held that EPA "violated the clear terms of section 211(f)(4)" by denying the manufacturer's waiver application on the basis of public health concerns. The court based its decision on the following factors: (1) EPA had "misconstrued" the language of Section 211(f)(4) - which only requires demonstration that a subject FA will not adversely effect emission control systems - and (2) in considering public health effects, EPA used a standard different from that previously used in Section 211(c)(1) actions.[78] The court characterized EPA's Section 211(f)(4) waiver decision as a "bizarre departure from existing practice, in complete defiance of the plain terms of the statutory criterion".[79]

The Court's sole focus was on EPA's action under Section 211(f)(4). Moreover, in analyzing the legislative history of Section 211, the Court explicitly recognizes that Congress distinguished actions under Section 211(f) with actions under other subsections of Section 211 (including Section 211(b)):

> While one finds numerous comments regarding public health in the legislative history surrounding the 1977 amendments to section 211, Congress did not specifically link such comments to the waiver provision. The EPA concedes this point. Brief for Respondent at 29. Congress did, however, link the consideration of public health to other provisions, stating "[t]he committee expects the Administrator to require manufacturers to test registered additives insofar as they affect health and public welfare under subsections (a), (b) and (c) of this section."[80]

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[76]Section 211(f)(4) authorizes EPA to waive the Section 211(f)(1) bans on F/FAs if the applicant has established that the F/FA and its emissions products "will not cause or contribute to a failure of any emission control device or system" to achieve compliance with specified emissions standards. 42 U.S.C. § 7545(f)(4).

[77]*Ethyl*, 51 F.3d at 1055 (*quoting* 59 Fed. Reg. 42,260).

[78]*Id.* at 1063 (Section 211(c) permits EPA to control or prohibit F/FA manufacture, use, etc., on the basis of health effects information obtained under Section 211(b) or otherwise, if EPA determines that F/FA may be anticipated to endanger the public health or welfare).

[79]*Id.*

[80]*Id.* at 1062-63 (*quoting* S. Rep. No. 95-127, 95th Cong., 1st Sess. at 91-92 (1977), *reprinted in* 1977 Clean Air Act Legislative History at 1465-1466)).

As discussed above, it is entirely appropriate and, indeed, wholly reflective of Congressional intent, for EPA to establish health effects testing requirements under Section 211(b) on the basis of potential public health concerns.

Ethyl also points to *Ethyl Corp. v. Browner*,[81] for support of its contention that EPA cannot require testing under Section 211(b) on the basis of "potential public health concerns." The discussion cited by Ethyl, however, appears in a section of the opinion entitled "*Issues Not Reached*." "There are a number of claims by Ethyl that we do not reach , either because our holding above makes their resolution unnecessary or because they are unripe."[82] This case does not support Ethyl's claim.

Ethyl also argues that EPA "misconstrues its authority under the Alternative Tier 2 program when it proposes to include exposure testing as part of the program."[83] EPA disagrees. EPA clearly intended that additional tests requirements could be imposed as part of the Alternative Tier 2 process. That, in fact, is the very basis for including Alternative Tier 2 in the health effects testing scheme: "In summary, the Alternative Tier 2 provision will give EPA the flexibility, when indicated, to prescribe <u>additional tests</u> to be performed along with the standard Tier 2 program, to <u>substitute different tests</u>, and/or to modify the underlying vehicle/engine specifications for Tier 2."[84] Nowhere do the Alternative Tier 2 regulatory provisions constrain EPA to a certain type of testing specifically related to the types of information collected under standard Tier 2 testing. Finally, given the overall purpose of the regulations, that being to more definitively evaluate the risk associated with the use of F/FAs, exposure testing is crucial to come to any conclusions. It would be unreasonable, and it is inconsistent with a plain reading of the regulations, to conclude that exposure tests are not allowed under the Alternative Tier 2 provisions.

Finally, Ethyl argues that EPA does not have the authority to require testing of neat oxygenates as opposed to combustion or evaporative emissions associated with the oxygenates. In appropriate circumstances, EPA can utilize Alternative Tier 2 requirements to mandate testing of "neat" substances in addition to or in lieu of the testing of whole emissions. However, in this instance EPA has not asserted the authority to test specific fuel additives or constituents which are not included in combustion or evaporative emissions. Rather, EPA proposed to test "neat" oxygenates because they are themselves a component of evaporative emissions.

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[81] 67 F.3d 941, 946 (D.C. Cir. 1995).

[82] *Id.* at 945.

[83] Ethyl's Comments at 3.

[84] 59 Fed. Reg. 33081 (emphasis supplied). Also, see additional discussion of this issue *supra* at footnote 7..

**Administrative Procedures.**

In accordance with the F/FA test program regulations, this letter constitutes the final notification of EPA's Alternative Tier 2 testing regimen and the schedule for completion and submission of such tests. Draft peer-reviewed testing protocols and results, including individual peer review comments, as well as requests for extensions or protocol alterations should be sent by certified mail to 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 final notification of Alternative Tier 2 requirements is being placed in Docket No. A-96-16.[85] 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.[86] In accordance with 40 C.F.R. § 79.57(f)(5)(ii), a Federal Register notice will also be published approving and announcing the availability of the alternative evaporative emissions generation procedure previously discussed in this notification.

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

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[85] 40 C.F.R § 79.58(c)(3).

[86] 40 C.F.R. § 79.58(c)(2).

# Attachment E. Alternative Tier 2 Testing Schedules

# 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: For purposes of emissions generations the “stripper still” alternative emissions generation procedure as proposed by the Section 211(b) Research Group in its July 1, 1997 letter from Dr. Carol Henry to Mr. Charles Freed, Director, Fuels and Energy Division, U.S. EPA. In accordance with 40 C.F.R. §79.57(f)(5), EPA has approved this alternative emissions generation procedure for purposes of the instant Alternative Tier 2 testing regimen.

## 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 F/FA program guidelines for Good Laboratory Practices (GLP), as provided at 40 C.F.R. § 79.60 shall be in effect for purposes of the entire testing regimen.

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, and, where applicable, 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, 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). Testing conducted in accordance with the relevant provisions of the OPPTS Health Effects Test Guidelines shall not require EPA approval. Note that a guideline may be in the process of re-evaluation and may be updated or revised as a result. New guidelines that are announced prior to the initiation of relevant testing 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 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. Where test protocols differ from already peer reviewed and EPA-approved protocols, specifically when they are not 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), 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. The peer review group may be composed of the RG's Toxicology Committee augmented by at least two additional external peer reviewers with appropriate expertise. 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 a 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. Included with the report, shall be any relevant comments and materials provided by the required independent QA/QC review.

   1. The draft final report shall be submitted in writing to a peer review 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. The peer review group may be composed of the RG's Toxicology Committee augmented by at least two additional external independent peer reviewers with appropriate expertise. 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. In accordance with 40 C.F.R. § 79.60(h)(3), documentation records, raw data, and applicable specimens shall be retained for no less than ten years. Documentation records and raw data must be 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 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 (section 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). Although not required under this notification, 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.

3. In addition to the measurements included in OPPTS 870.3800, the two-generation reproductive study shall include the Standard Tier 2 neuropathology and GFAP assessments (40 C.F.R. §§ 79.66-67) conducted on the first generation of pups no sooner than 21 days after birth and no later than 28 days.

B. Two-species Developmental Study

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.

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 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 immunotoxicity.

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 (section 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). Although not required under this notification, 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 may, in addition, develop and validate 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. 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 duplicate tests.[87]

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[87] 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 (i.e, exposures approaching the $99^{th}$ percentile), 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. The peer review group may consist of the RG Toxicology Committee augmented by at least two external independent peer reviewers. 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 ten 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 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 in the months of January and July and may include additional 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 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. The vehicle fleet used to generate the emissions resulting in the microenvironment concentrations shall reflect a range of model types and maintenance conditions representative of in-use vehicles.

- 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
    - MTBE, TBF, other emissions transformation products
    - Formaldehyde and acetaldehyde
    - Benzene, Toluene, Ethyl benzene, Xylene (BTEX)
    - 1,3-butadiene

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

## Animal Testing

| Test Group | Fuel Mixture | Toxicology Studies | Studies Initiation | Draft Report Due to EPA | Comments Due to RG | Final Report Due to EPA |
|---|---|---|---|---|---|---|
| Group A | Baseline Gasoline - Gasoline MTBE | Study Set 1<br>-Subchronic w/ Neurotoxicity, Immunotoxicity, and In Vivo/In Vitro Genotoxicity *<br>-Developmental Toxicity (Two Species) | 0 months | 26 months | 28 months | 30 months |
| | | Study Set 2<br>-Two Generation Reproductive Toxicity | 12 months | 36 months | 38 months | 40 months |
| | | Study Set 3<br>-Oncogenicity (One Species) | 12 months | 52 months | 54 months | 56 months |
| Group B | Gasoline Ethanol<br>Gasoline TAME<br>Gasoline ETBE | Study Set 4<br>-Subchronic w/ Neurotoxicity, Immunotoxicity, and In Vivo/In Vitro Genotoxicity *<br>-Developmental Toxicity (One Species) | 6 months | 32 months | 34 months | 36 months |
| | | Study Set 5<br>-One Generation Reproductive Toxicity | 18 months | 38 months | 40 months | 42 months |
| Group C | Gasoline DIPE<br>Gasoline TBA | Study Set 6<br>-Subchronic w/ Neurotoxicity, Immunotoxicity, and In Vivo/In Vitro Genotoxicity *<br>-Developmental Toxicity (One Species) | 18 months | 38 months | 40 months | 42 months |
| | | Study Set 7<br>-One Generation Reproductive Toxicity | 30 months | 50 months | 52 months | 54 months |
| Group D | EtOH, TAME, ETBE, DIPE, TBA | Study Set 8<br>-Neat Oxygenate PK (where applicable) | 6 months | 26 months | 28 months | 30 months |

* To include the in vivo micronucleus assay and the in vivo sister chromatid exchange assay, as well as the in vitro salmonella test specified in 40 CFR para. 79.68.

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

## Exposure Studies

| Exposure Assessment Task | Original Schedule | Revised Schedule |
|---|---|---|
| Incorporate results of ongoing monitoring studies | not considered | 6 months |
| API submits draft peer-reviewed protocol including individual peer review comments and disposition of comments | 3 months | 12 months |
| EPA provides comments on draft protocol to API | 5 months | 14 months |
| API submits revised draft protocol to EPA | 7 months | 16 months |
| EPA approves/disapproves revised draft protocol | 9 months | 18 months |
| API submits draft final report for review by EPA including individual peer review comments and disposition of comments | 24 months | 42 months |
| EPA provides comments on draft final report | 26 months | 44 months |
| API submits final report to EPA on results of testing | 28 months | 48 months |