

HEALTH RESEARCH DIRECTIONS FOR NEW FUELS.

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INTRODUCTION

Throughout much of the twentieth century, the general population has been routinely exposed to conventional gasoline and its evaporative and combustion emissions. However, with the introduction of new fuel additives and formulations, attention has become focused in recent years on the potential for public health impacts from widespread exposure to motor vehicle fuels. The Clean Air Act required the Administrator of the U.S. Environmental Protection Agency (EPA) to promulgate requirements for testing the health effects of evaporative and combustion emissions of fuels and fuel additives (F/FAs). The Fuels and Fuel Additives Rule, promulgated on May 27, 1994, established new health testing requirements for the registration of designated F/FAs, organized within a three-tier structure. Tier 1 requires F/FA manufacturers to perform a literature search on the health and welfare effects of F/FA emissions and to characterize F/FA emissions. Tier 2 requires toxicological testing by subchronic inhalation exposure and designated assays for specific health endpoints if adequate information is not already available. When necessary, Tier 3, which may include follow-up or additional studies, can be required.

Additionally, the rule includes a provision known as Alternative Tier 2, which gives EPA the flexibility to prescribe additional tests to be performed along with the standard Tier 2 program, to substitute different tests, and/or to modify the underlying vehicle/engine specifications for Tier 2. EPA may also use the Alternative Tier 2 authority to waive certain Tier 2 endpoint evaluations (generally on occasions when additional and/or more rigorous tests are being required for other Tier 2 endpoints). However, testing for Tier 2 endpoints may not be waived in the absence of adequate information or requirements for more rigorous testing.

At the time of this writing, EPA is about to issue proposed Alternative Tier 2 testing requirements for baseline (conventional) gasoline and various oxygenate-gasoline blends (collectively referred to here as "oxyfuels"), including methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), ethyl alcohol (EtOH), tertiary amyl methyl ether (TAME), diisopropyl ether (DIPE), and tertiary butyl alcohol (TBA). The primary objective of this testing program is to develop an information base that will support quantitative, comparative risk assessments of baseline and oxygenated gasolines. The risks, and benefits, of a given fuel are relative to its alternatives. Therefore, to determine whether a particular oxyfuel is better or worse than conventional gasoline or some other oxyfuel, comparable data must be available by which to evaluate the comparative risks. More extensive discussions of needed information and research relative to oxyfuels may be found in EPA's "Oxyfuels Information Needs" (U.S. Environmental Protection Agency, 1996) and other recent reports (e.g., Health Effects Institute, 1996; Interagency Oxygenated Fuels Assessment Steering Committee, 1996, 1997; National Research Council, 1996). This paper will explain the rationale underlying the proposed Alternative Tier 2 testing program and summarize key features of the program as an illustration of a scientifically sound and efficient approach for obtaining data needed for comparative risk assessment purposes.

RATIONALE FOR ALTERNATIVE TIER 2 TESTING REQUIREMENTS

To understand the rationale behind the Alternative Tier 2 testing program, one must first understand the purpose of standard Tier 2 requirements in the F/FA rule. Standard Tier 2 assessments include a basic subchronic inhalation toxicology study as well as tests to determine potential reproductive, developmental, neurotoxic, mutagenic, and carcinogenic effects (summarized in Table I). These assays, while sufficient for screening level evaluations of the toxicological effects of inhalation exposure to the emissions of designated F/FAs in test animals, were not intended necessarily to provide an adequate base for quantitative risk assessments. Rather, the intent was to provide for the collection of basic toxicological data that, along with information on exposure potential and other considerations, could guide decisions on whether or not more extensive toxicological evaluation would be required. If the results from standard Tier 2 assays indicated low toxicity for a particular F/FA and little potential for human exposure existed, then further testing would probably not be warranted. However, in the case of oxyfuels, a testing regimen that exceeds the standard screening requirements of Tier 2 is considered necessary and appropriate because of continuing uncertainties regarding the public health effects of gasoline and oxyfuels, and the widespread public exposure to these fuels and related emissions. This approach is clearly more cost-effective and time-efficient than simply requiring standard Tier 2 testing, waiting for the completion of such testing, and then

Table I. Fuel/Fuel Additive (F/FA) Rule Standard Tier 2 Tests

90-Day Subchronic Inhalation General Toxicity: Screening information on target organ toxicities and on concentrations useful for running chronic studies.

30 rodents per concentration per group (add specified numbers for different assessments combined with general toxicity); recovery group (N = 20) observed for reversible, persistent, or delayed effects

Observation (including body weight)

Clinical exams: hematology (e.g. Hct, Hb, RBC, DLC); clinical biochemistry (e.g., electrolyte balance, liver and kidney function, Ca-P-Cl-Na-K, glucose, BUN)

Ophthalmological exam

Urinalysis

Gross pathology

Histopathology (especially respiratory tract)

Fertility/Teratology: Information on potential health hazards to fetus and on gonadal function, conception, and fertility.

25 males, 40 females per group; mating after 9 weeks of exposure, then exposure of females continues through GD 15

Limit test (if no effects at highest concentration, then omit lower concentrations)

Observation for ≤ 13 weeks

Vaginal cytology

Mating and fertility

Gross necropsy (especially including reproductive organs)

Fetal anomalies, resorptions

Histopathology of reproductive organs

In Vivo Micronucleus: Detect damage to chromosomes or mitotic apparatus of cells (based on increase in frequency of micronucleated RBCs); provides information on potential carcinogenic and/or mutagenic effects.

5 females and 5 males per group

Positive control

In Vivo Sister Chromatid Exchange: Detect enhancement of exchange of DNA between two sister chromatids of a duplicating chromosome (using peripheral blood lymphocytes grown to confluence in cell culture); provides information on potential mutagenic and/or carcinogenic effects.

5 females and 5 males per group

Positive control

Neuropathology: Provides data on morphologic changes in central and peripheral nervous system.

N = 10 per group; N = 20 for reversible, persistent, or delayed effects

Positive control

Limit test (if no effects at highest concentration, then omit lower concentrations)

Observation (including body weight, movement disorders, etc.)

Brain size and weight; light (and possible electron) microscopy of sections

Peripheral nerve teasing

Glial Fibrillary Acidic Protein: An indicator of neurotoxicity associated with astrocytic hypertrophy at site of damage.

10 animals per group

Change in amount of GFAP for specific brain region as a function of treatment and dose

Salmonella Typhimurium Reverse Mutation: Microbial assay that measures histidine (*his*) reversions (*his⁻* to *his⁺*), which cause base changes or frame-shift mutations in the genome; provides data on mutagenicity.

Positive controls

Data presented as number of revertant colonies per plate, per kilogram (or liter) of fuel, and per kilometer (or mile) for each replicate and dose.

Source: U.S. Environmental Protection Agency (1996)

developing follow-up test requirements at the Tier 3 level that would be necessary to support quantitative, comparative risk assessments.

Although both evaporative and combustion emissions are encompassed by the F/FA rule, the proposed test program focuses on evaporative emissions of the fuels. A full discussion of the reasons for deferring combustion emissions testing is outside the scope of this paper, but the evaporative emissions amply serve to illustrate a testing strategy for new F/FAs. The proposed Alternative Tier 2 test program has been structured so as to obtain rather extensive data on baseline gasoline. Several considerations have led EPA to propose more extensive test requirements for baseline gasoline and MTBE-gasoline than for the other oxygenates. First, and most important, conventional gasoline and MTBE-gasoline predominate within the U.S. fuel marketplace, and thus present the highest potential for human and environmental exposures. A thorough understanding of the individual and comparative public health risks of these fuels thus constitutes a critical need. Second, the fact that nearly all fuels have some degree of toxicity means that the relative risk of different fuels is particularly important. Accordingly, a comprehensive database on baseline gasoline toxicity is vitally needed to provide a level basis for comparison with other F/FAs in the gasoline family. Similarly, since MTBE is the most frequently used oxyfuel, 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. For example, the comparative carcinogenic potential of baseline gasoline emissions relative to those of MTBE-gasoline emissions is an outstanding fundamental issue which must be evaluated in the context of long-term emission exposures. In addition, dose-response relationships for developmental, reproductive, and neurotoxic effects have not been adequately characterized. Fourth, even though each oxygenate has its own chemical characteristics and, perhaps, toxicological 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 for 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 illustrations help explain why the more extensive set of requirements are initially to 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 across the board to all registered oxyfuels.

SUMMARY OF ALTERNATIVE TIER 2 TESTING REQUIREMENTS

For baseline gasoline and MTBE-gasoline, the standard Tier 2 testing regimen is to be supplemented by (1) two additional neurotoxicity assessments, the functional observational battery and motor activity assessment; (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. (The two-generation reproductive study and two-species developmental study replace the Standard Tier 2 fertility/teratology combined screening assessment.) The testing requirements for the other oxyfuels are much less extensive, consisting 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 other 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.

The Alternative Tier 2 program also incorporates provisions for pharmacokinetic studies on "neat" oxygenates. An understanding of the pharmacokinetic characteristics of the oxygenates as pure compounds is important to our understanding of their relative toxicities when mixed in gasoline. Basic pharmacokinetic characterization (i.e., absorption, distribution, metabolism, and elimination of the pure inhaled oxygenates) can provide mechanistic information on disposition that will be useful to determining whether the toxicological testing results for one oxyfuel (e.g., MTBE-gasoline) can be compared with another. Such studies can also shed light on the relevance of animal-based study results to humans and help determine the extent to which effects by one route of exposure may be relevant to another route.

Comprehensive inhalation pharmacokinetic studies have already been conducted for MTBE; therefore, additional such testing is not required. But, the availability of inhalation pharmacokinetic data for the other oxygenates varies considerably. For example, pharmacokinetic studies are already underway for TAME. In addition, EPA has been informed that such testing on pure ETBE is being conducted by industry on a voluntary basis. To our knowledge, however, there are currently no

similar test plans for pure EtOH, DIPE, TBA, or other oxygenates. Consequently, the proposed Alternative Tier 2 test regimen for the oxygenates other than MTBE includes pure compound inhalation pharmacokinetic test requirements.

CONTINGENT STUDIES

As discussed above, the proposed Alternative Tier 2 testing program has been designed to fill critical data gaps and act as a screen to determine the need for additional studies. Thus, the results of the Alternative Tier 2 tests may indicate that additional studies are required at the Tier 3 level. In the case of baseline gasoline and MTBE-gasoline, follow-up tests may be required to further characterize significant unexpected positive findings. For example, mechanistic studies may be required to determine if positive results of concern in the Alternative Tier 2 animal studies were 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 that might be obtained on MTBE-gasoline, another oxygenate mixture, and/or baseline gasoline. Similarly, a two-year inhalation bioassay may be required if either positive results are obtained in the Alternative Tier 2 mutagenicity studies for a given oxyfuel or if significant unexpected results are 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. Other tests may also 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 of concern. Such additional tests could include evaluation of acute health symptoms in humans.

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