

March 29, 2001

Dr. Elizabeth Moran  
Manager, Olefins Panel  
American Chemistry Council  
1300 Wilson Blvd.  
Arlington, VA 22209

Dear Dr. Moran:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the C5 Noncyclics category, dated November 6, 2000. I commend the ACC Olefins Panel for their commitment to the HPV Challenge Program and encourage you to take appropriate steps to make your submission a successful contribution.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Chemical RTK HPV Challenge Program website EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

The Panel presents a category approach for eleven substances and mixtures. EPA has reservations about the Panel's approach to a health effects category because of the absence of a well-defined mechanistic framework that would allow hazard characterization of such a diverse set of substances. As detailed in the attached Comments, EPA believes that the Panel needs to elaborate its approach in such areas as supporting the category with available data, metabolism discussions, and selection of test substances. The Panel also needs to explain the basis for the category for ecological and environmental fate endpoints. EPA disagrees with part of the Panel's plan to characterize ecological effects using modeled rather than experimental data to define the 100% isoprene stream. EPA also recommends neohexene as a representative test substance for ecological effects.

Consistent with our goal to maintain a quality standard for information obtained under this program, EPA prefers measured physicochemical property data to estimated data. For example, measured data are likely to be available for many of the chemicals in the C5 streams.

The sponsor needs to address deficiencies in 13 health robust summaries and the inadequacy of one summary. Summaries for available data cited in your submission (Table 4) may be needed where those data are used to support the category.

As with other submissions where the available data are either inadequate or insufficiently documented, this case will remain open until adequate documentation is in hand.

EPA will post this letter and the attached Comments on the Chemical RTK web site within the next few days. As noted in the comments, we ask that the Panel advise the Agency, within 90 days of the posting on the Chemical RTK website, of any modifications to its submission.

If you have any questions about EPA's response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-260-3470. Submit general questions about the HPV Challenge Program through the Chemical RTK web site comment button or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at [tsc-hotline@epa.gov](mailto:tsc-hotline@epa.gov).

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

Oscar Hernandez, Director  
Risk Assessment Division

Attachment

cc: W. Sanders  
C. Auer  
M. E. Weber  
A. Abramson

## EPA COMMENTS ON CHEMICAL RTK HPV CHALLENGE SUBMISSION:

### C5 NONCYCLICS

#### SUMMARY OF EPA COMMENTS

The sponsor, the ACC Olefins Panel, submitted a Test Plan, Category Justification, and Robust Summaries to EPA dated November 6, 2000 for the C5 Noncyclics category. EPA posted the submission on the ChemRTK Web site on December 1, 2000. The proposed information-gathering plan is for eleven substances and mixtures (describable by 16 CAS numbers) considered by the sponsor to constitute a category.

EPA has reviewed this submission and has reached the following conclusions:

1. Physicochemical and Environmental Fate Data. a) The sponsor needs to explain the basis for the category for the environmental fate endpoints. b) The Test Plan for physicochemical properties suggests that all data on these properties will be derived from EPIWIN calculations. EPA prefers that measured physicochemical property data be provided, both to characterize a substance and to provide inputs to transport-distribution models. The use of estimated values introduces uncertainties that then become magnified in modeling applications. Measured data should be available from published sources on many of the category mixture components.
2. Health Endpoints: a) The proposed category approach for health endpoints is not convincing. b) Basing the proposed category analysis on the genotoxicity of isoprene may not apply to the systemic, developmental or reproductive toxicity of this group of substances. c) One acute toxicity robust summary is inadequate for the purposes of the U.S. HPV Challenge Program. In addition, the sponsor needs to address deficiencies in 13 robust summaries; see "Specific Comments on Robust Summaries."
3. Ecotoxicity. (a) The sponsor needs to explain the basis for the category for ecological effects. (b) EPA disagrees with the sponsor's plan to characterize the category using mostly modeled data to define the 100% isoprene stream and test data to define the low- and mid-isoprene ranges of the isoprene-containing fractions. Experimental values are needed to establish the boundaries or other anchor points of a category for read-across purposes. (c) EPA recommends neohexene as a representative test substance, since it may be the most toxic category member; see Test Plan section below. (d) All tests should follow the Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, June 2000—available on the OECD website at <http://www.oecd.org/ehs/test/monos.htm>).

EPA is requesting that the Sponsor advise the Agency within 90 days of any modifications to its submission.

#### EPA COMMENTS ON THE C5 NONCYCLICS CHALLENGE SUBMISSION

##### Category Definition

The C5 Noncyclics Category includes eleven substances associated with ethylene processing that are described by 16 CAS numbers. The submitter notes that any one stream can be properly defined with multiple CAS numbers, and one CAS number can be used to identify more than one stream (Table 1 in submission). The streams contain considerable amounts of isoprene and other C5 or C6 alkenes and alkanes. The sponsor defines the category on the basis of process and toxicological criteria. The category name notwithstanding, six streams do include cyclic compounds.

Of the eleven substances, eight are refinery stream mixtures that will vary considerably with the source

crude oil and refinery process. The other three—*isoprene*, *2-methyl-2-butene* and *neohexene*—are essentially single-component substances, the first two of which occur in many of the other streams. However, *neohexene* is not a component of any other stream. For this reason, and because it does not contain any *isoprene*, it appears not to meet the stated category criteria. The sponsor needs to explain the inclusion of *neohexene* in the category, especially with respect to health effects.

### **Category Justification**

The submitter suggests that these streams are toxicologically similar. For health effects, they anticipate that *isoprene*, and to a lesser extent *2-methyl-2-butene*, will dominate and determine the streams' toxicity profiles. Three of the streams (*2-Methyl-2-butene*, *Metathesis Byproduct*, and *Neohexene*) do not contain *isoprene*, but the sponsors suggest that these streams will have a toxicology profile similar to that of the 2% *isoprene* stream.

EPA has reservations about the sponsor's discussion. The test plan states that the existing data for these streams show that *isoprene* and *2-methyl-2-butene* are the most biologically active chemicals, and that genotoxicity is the endpoint of concern. Basing the health analysis on the genotoxicity of *isoprene* may not be adequately account for systemic, developmental, or reproductive toxicity. The sponsor's Table 4 identifies the existing test data for individual chemicals in the streams. However, there is no specific discussion of these data in the test plan or in the robust summaries. Without such discussion it is difficult to evaluate the toxicological basis for grouping these substances into a category.

In addition, there is no discussion of chemical structure and its place in the development of the category. The spectrum of substances and mixture components from alkanes (e.g., *pentane*) through alkenes (*isopentene*) to conjugated dienes (*isoprene*) suggests paying closer attention to the differences in their known or expected metabolism and the potential impacts on the category design. A case for a category of olefin-containing streams supported by a discussion of known aspects of metabolism and structure-activity relationships within the category would probably be more persuasive than one based on a single endpoint such as genotoxicity, and might better support the inclusion of substances such as the sponsor's *isoprene*-free streams. A matrix ordered by such a mechanistic principle could also improve the ability to read across to substances in the matrix lacking test data, a major goal of category approaches that is not well served in this proposal.

In most of the submission, the basis of the category for ecological effects appears to be *isoprene* and *2-methyl-2-butene* content and toxicity (for example, see the Plain Language and Executive Summaries). However, on page 6, the sponsor states (with little supporting discussion) that these two chemicals are expected to display toxicity similar to each other AND to the other category members. Yet the two test substance mixtures selected are characterized in terms of their *isoprene* content; it is not apparent why this is mentioned if the toxicities are expected to be similar for all components. Furthermore, Table 3, which summarizes the test plan, is organized according to the discussion for health effects, with a separate section for non-*isoprene*-containing streams, forcing the other endpoints into the same procrustean matrix. This makes it difficult to evaluate the test plans for the non-health endpoint groups. The sponsor needs to consider how to present the information for the non-health endpoints in a more comprehensible and helpful way, such as an array based on octanol-water partition coefficient ( $\log P$ ) values for ecological toxicity.

### **Test Plan**

The sponsor proposes that Pyrolysis C5s, Hydrotreated C5s, Isoprene, and 2-Methyl-2-butene be the representative test substances for the category. EPA has some overall concerns with the choice of test substances. Five of the eleven streams—*Piperylene Concentrate*, *Isoprene Concentrate*, *Isoprene Purification Byproduct*, *Metathesis Byproduct*, and *Neohexene*—contain components not present in any of the four streams designated for testing (see Sponsor's Table 2). Two of these five have a high

percentage of components not present in the streams to be tested. Thus, 47% of the Metathesis Byproduct stream (8% 3-hexene, 24% methyl-2-pentenes, and 15% 2-hexene) and 98.5% of the Neohexene stream (1.5% 2,3-dimethyl-1-butene and 97% 3,3-dimethyl-1-butene) are not test substance components.

EPA's remaining test plan comments are presented by endpoint type.

#### Chemistry (melting point, boiling point, vapor pressure, water solubility, and partition coefficient).

The sponsor plans to generate estimates on individual mixture components, using EPIWIN, and provide ranges of values for the different streams. However, EPA emphasizes that measured data should be provided for this purpose. With chemicals such as these mixture components that are well characterized or are being characterized in OECD SIDS and other programs, acceptable literature data should be available for many physicochemical endpoints and should be included wherever possible (generally, the log P value can be calculated for chemical classes that have been validated for the calculation). The use of estimated values introduces uncertainties that then become magnified in modeling applications. The sponsor will need to provide a Robust Summary for each endpoint for which it reports or estimates data.

#### Fate (photodegradation, stability in water, biodegradation, and transportation/distribution).

*Photodegradation* – The sponsor's approach is reasonable, but the substances chosen for the calculations should be identified.

*Stability in water* – The sponsor's approach is reasonable.

*Biodegradation* – The sponsor proposes testing pyrolysis C5s and the hydrotreated C5s along with 2-methyl-2-butene but gives no rationale for the choice. Biodegradation is known to be relatively rapid with straight chain molecules, but slow with cyclic aliphatics and tertiary carbon-containing acyclic aliphatics. The sponsor needs to explain its selection of test substances for this endpoint, as well as how tests on mixtures will be interpreted if components are degrading at different rates.

*Chemical Transport and Distribution in the Environment* – The sponsor plans to run an EQC Level 1 fugacity model using the properties calculated by the EPIWIN suite of programs. The sponsor, however, does not state which compounds will be used in the model nor how combinations of chemicals will be characterized. It is anticipated that if the sponsor calculates transport and distribution values for all of the chemicals present in the streams and listed in Table 2 of the submission, an adequate understanding of the behavior of these streams could be developed. However, a Level 3 model should be used rather than the proposed Level 1, because of the additional information that is developed. For inputs to the model, adequate measured values are preferred to calculated values (see Chemistry, above).

In order to estimate environmental fate endpoints EPA recommends using the EQC level III model from the Canadian Environmental Modeling Centre at Trent University. This model can be found at the following Web address: <http://www.trentu.ca/academic/aminss/envmodel/>.

#### Health Effects

The sponsor suggests that the genotoxicity of isoprene will be the principal biological activity in the streams in this category. The testing strategy is to use the existing data on isoprene, and to conduct full SIDS human health test batteries (except for acute inhalation toxicity) on mid-range (Pyrolysis C5s) and low-range (Hydrotreated C5s) isoprene content streams. In addition, a full SIDS human health test battery (except for acute toxicity) will be conducted for 2-methyl-2-butene, a chemical that has been

shown to have genetic activity, and is present at high levels (93%) in one stream that contains no isoprene. Two category members (metathesis byproduct and neohexene) do not contain either isoprene or 2-methyl-2-butene. The sponsor notes that the existing acute toxicity and genotoxicity data on neohexene support the conclusion that the low isoprene stream testing will characterize the toxicity of this stream. However, this assumption is not supported because the streams have no components in common.

There are three streams that do not contain isoprene: 2-Methyl-2-butene, Metathesis Byproduct, and Neohexene. The sponsor plans to evaluate 2-Methyl-2-butene stream by conducting a full SIDS human health test battery. The Metathesis Byproducts stream is proposed to be characterized by data on the low isoprene stream and data from the testing of 1-hexene and C6-C8 internal olefin stream, which are being sponsored in the OECD SIDS program by the American Chemistry Council Higher Olefins Panel. It is not logical to have a low-isoprene stream, a C6-C8 stream, or 1-hexene represent the Metathesis Byproduct stream because the latter contains: (1) no isoprene; (2) only C5 and C6 components; and (3) only 4% 1-hexene.

Finally, the neohexene stream is characterized according to the sponsor by existing data on the acute toxicity and genotoxicity of neohexene and its expected similarity with the Hydrotreated C5s stream. Neohexene, however, is not present in any of the other streams, including those proposed for testing, and the two endpoints evaluated, acute toxicity and genotoxicity, are poor predictors of other systemic toxicologic effects (e.g. specific target organ toxicity, reproductive or neurologic effects). The conclusion that neohexene will be toxicologically similar to the other alkenes in the two streams that are being tested appears to have little supporting toxicologic data.

### Ecological Effects

EPA disagrees with the sponsor's approach to determining the ecological hazard of the C5 Noncyclics. The sponsor proposes to characterize the category using test data to define the low- and mid-isoprene ranges of the isoprene-containing fractions and mostly modeled data to define the 100% isoprene stream. For a single-chemical submission, the predicted value using ECOSAR is acceptable for determining the hazard associated with a substance provided that a robust summary of experimental data for an appropriate analog chemical is submitted to support the value (see guidance at (<http://www.epa.gov/opptintr/chemrtk/sarfinl1.htm>)). However, for establishing the boundaries or other anchor points of a category for read-across purposes, all values should be experimental values. In this case EPA suggests that isoprene be tested in fish and Daphnia instead of applying a QSAR model.

The basis for test substance selection for ecological effects is unclear. In any event, EPA recommends neohexene as a test substance; on the basis of its predicted log P value, neohexene is likely to exhibit higher ecotoxicity than the sponsor's choice of 2-methyl-2-butene or any other of the category's constituent chemicals.

All tests should use closed systems and mean measured concentrations. Testing of these chemicals should follow the Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, June 2000-available on the OECD website at <http://www.oecd.org/ehs/test/monos.htm>).

## **SPECIFIC COMMENTS ON ROBUST SUMMARIES**

### **Chemistry**

No robust summaries were provided for these endpoints.

### **Fate**

EPA evaluated the one robust summary provided (isoprene biodegradation). A mixed culture obtained from environmental media and sewage treatment plants was used as the seed. Only 2% degradation was noted in the 28-day BOD test. While it appears that standard manometric techniques were used, it is not stated what, if any, procedures were used to minimize volatilization of the test substance. If the test substance volatilized significantly during manipulation and introduction into the BOD bottles, the actual concentration of the test substance would be lower and may partially explain the low BOD. While EPA finds the robust summary to be adequate, the sponsor needs to indicate whether volatilization was a factor in the results of the 28-day BOD test.

### **Health Effects**

EPA evaluated 28 health endpoint robust summaries and found one of them (acute toxicity of isoprene) to be inadequate for the purposes of the U.S. HPV Challenge Program and 13 of them to be deficient in some manner. Although the acute toxicity study is considered inadequate, EPA believes that the knowledge base for isoprene toxicity would not benefit from repeating this study.

The following EPA comments reflect the information in the robust summary (the full study report may address these comments). Comments are only made on those summaries considered either inadequate or deficient (study considered valid, but more information needs to be put in the summary).

Acute toxicity. The isoprene robust summary is considered inadequate for the following reasons: (1) the age, number, and sex of animals used are not specified; (2) the test substance concentrations used are not specified; and (3) there appeared to be no post-exposure observation period or documentation of clinical observations.

Genetic Toxicity (assessment of mutations): The neohexene robust summary (Hazleton Laboratories, 1982) needs to provide the following: (1) incidence by dose of mutations observed; (2) whether any cytotoxicity was observed (and at what dose); (3) and the level of response with the positive control.

Genetic Toxicity (assessment of chromosomal effects): Twelve robust summaries were submitted for isoprene (4 *in vivo* and 2 *in vitro*); 2-methyl-2-butene (2 *in vivo*); isoamylene [ $>90\%$  2-methyl-2-butene](3 *in vivo*); 2-methyl-1-butene (2 *in vivo*); and neohexene (1 *in vitro*). Eight robust summaries were considered deficient for the following reasons:

Isoprene. (*In vivo* sister chromatid exchange study in mouse bone marrow, Tice et al., 1988). The following information needs to be provided in the summary: (1) the incidence of SCEs in treated animals by dose; (2) the incidence and dose(s) at which cytotoxicity, inhibition of cellular proliferation, and rate of erythropoiesis suppression occurred; and (3) mitotic indices measured.

Isoprene. (*In vivo* chromosomal aberration study in mouse bone marrow, Tice et al., 1988). Although the study conclusion is negative because there was no statistically significant increase in the frequency of chromosomal aberrations between control and treated animals, the noted increased incidence in treated animals, by dose, needs to be provided.

Isoprene. (*In vivo* mammalian erythrocyte micronucleus study in mice, Tice et al., 1988). The incidences of micronuclei by dose and the PCE/NCE ratios need to be provided.

Isoamylene. (*In vivo* mammalian erythrocyte micronucleus study in mice, two summaries: Exxon Biomedical, 1990, 1991); 2-methyl-2-butene (*In vivo* mammalian erythrocyte micronucleus study in mice (Exxon Biomedical, 1991) and in rats (Exxon Biomedical, 1991)); and 2-methyl-1-butene (*In vivo* mammalian erythrocyte micronucleus study in mice, Exxon Biomedical, 1991). The PCE/NCE ratios need to be provided.

Repeat Dose Toxicity. There were five robust summaries describing repeat dose inhalation studies with isoprene (all inhalation studies with test durations ranging from two weeks to two years). EPA notes the following: (1) the incidence data for the non-neoplastic effects in male mice (26-week study) and in rats and mice (the two two-year studies) need to be provided in the robust summaries (similar incidence data are not necessary for the two-week study because the same dose levels were used in the longer duration tests and some specific incidence data are presented for the same study under the reproductive toxicity endpoint); and (2) in the robust summary for the two-year carcinogenicity study in rats (NTP 1997), because the first paragraph under Conclusions presents an interpretation different from that of the study authors, the submitter should also report the study authors' interpretation.

Developmental Toxicity. The isoprene robust summary needs to provide the incidence data by dose for all effects observed.

### **Ecotoxicity Studies**

No robust summaries were provided for these endpoints.

### **Followup Activity**

EPA requests that the Sponsor advise the Agency within 90 days of any modifications to its submission.