

October 30, 2002

Timothy Adams, Ph.D.  
Technical Contact  
The Flavor and Fragrance High Production  
Volume Consortia  
1620 I Street, N.W.  
Suite 925  
Washington, D.C. 20006

Dear Dr. Adams:

The Office of Pollution and Toxics is transmitting EPA's comments on the robust summaries and test plan for Monoterpene Hydrocarbons posted on the ChemRTK HPV Challenge Program Web site on June 14, 2002. I commend The Flavor and Fragrance High Production Volume Consortia for their commitment to the HPV Challenge Program.

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 Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed Comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that The Flavor and Fragrance High Production Volume Consortia advise the Agency, within 60 days of this posting on the Web site, of any modifications to its submission.

If you have any questions about this response, please contact Richard Heftner, Chief of the HPV Chemicals Branch, at 202-564-7649. Submit questions about the HPV Challenge Program through the "Contact Us" link on the HPV Challenge Program Web site pages 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,

/S/

Oscar Hernandez, Director  
Risk Assessment Division

Enclosure

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

**EPA Comments on Chemical RTK HPV Challenge Submission:  
Monoterpene Hydrocarbons**

**SUMMARY OF EPA COMMENTS**

The sponsor, the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia, submitted a test plan and robust summaries to EPA for monoterpene hydrocarbons dated May 21, 2002. EPA posted the submission on the ChemRTK HPV Challenge Web site on June 14, 2002. The category consists of five individual monoterpene hydrocarbons and seven mixtures with varying quantities of monoterpenes and other products.

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

1. Category Justification. The submitter's approach for combining these chemicals into one category is reasonable except for the mixtures with CAS Nos. 68938-00-1 and 68334-40-7. EPA believes that CAS No. 68938-00-1 should not be included in the category given its significantly low monoterpene content and the presence of high amounts of polymers. The mixture having CAS No. 68334-40-7 contains higher terpenes, alcoholic groups and unspecified oxygenated terpenes that make it inconsistent with the other category members. The submitter needs to provide further chemical identification, chemical composition, and the structures of mixture components not defined in the test plan and to explain the relevance of CAS No. 68334-40-7 to the rest of the category members, or consider a different approach.
2. Physicochemical Properties and Environmental Fate. The submitter needs to provide measured vapor pressure data for myrcene or dihydromyrcene. The submitter also needs to provide additional robust summaries and address discrepancies between summaries and the test plan tables.
3. Health Effects. EPA reserves judgement on data adequacy for the purposes of the HPV Challenge Program, pending submission of additional critical information in the robust summaries. The submitter also needs to explain whether adequate measures were taken during testing of volatile substances for genetic toxicity.
4. Ecological Effects. EPA agrees that all appropriate acute SIDS-level endpoints have been addressed; however, the submitter needs to provide required data elements missing from the robust summaries. Because several chemicals have calculated log Kow values in the range of 4.8 to 5.3, EPA recommends conducting chronic invertebrate toxicity testing on the most hydrophobic single chemical (CAS No. 2436-90-0).

EPA requests that the submitter advise the Agency within 90 days of any modifications to this submission.

**EPA COMMENTS ON THE MONOTERPENE HYDROCARBONS CATEGORY  
CHALLENGE SUBMISSION**

**Category Definition**

The submitter proposed a category covering five individual monoterpene hydrocarbons (both cyclic and acyclic) and seven mixtures. The category members are listed below. While most of the mixtures are composed primarily of monoterpenes, two of the mixtures, terpenes and terpenoids, turpentine oil, limonene fraction, distillation residue (CAS No. 68334-40-7) and terpene and terpenoids, turpentine-oil residue (CAS No. 68938-00-1), contain smaller percentages of monoterpenes compared to the other category members (from approximately 31-38% and less than 3.2%, respectively) with higher terpene hydrocarbons, oxygenated terpenes, and polymeric material. The category definition is adequate.

<u>Chemical Name</u>	<u>CAS No.</u>	<u>Comments</u>
1. <i>d</i> -Limonene	5989-27-5	Single enantiomer
2. <i>d</i> -Limonene	138-86-3	Racemic modification
3. Terpinolene	586-62-9	
4. Myrcene	123-35-3	
5. Dihydromyrcene	2436-90-0	
6. Hydrocarbons, terpene processing by-products	68956-56-9	22-34% Limonene 22-33% Terpinolene 5-10% Myrcene 18% Limonene isomers 10% Unspecified terpene hydrocarbons
7. Orange peel oil, sweet ( <i>Citrus sinensis</i> (L.) Osbeck)	8008-57-9	91-94% Limonene 2.0-2.1% <i>beta</i> -Myrcene
8. Terpenes and terpenoids, sweet orange oil	68647-72-3 Limonene	91-95% <i>d</i> -  1-3% <i>beta</i> -Myrcene 1-2% <i>alpha</i> -Pinene
9. Terpenes and terpenoids, turpentine oil, limonene fraction	65996-99-8	59-64% <i>d</i> -Limonene 14-18% <i>beta</i> -Phellandrene 4-11% <i>beta</i> -Pinene 5-10% Unspecified terpene hydrocarbons
10. Terpenes and terpenoids, limonene fraction	65996-98-7	96-98% <i>d</i> -Limonene 1-2% Myrcene 5-10% Unspecified terpene hydrocarbons
11. Terpenes and terpenoids, turpentine oil,		

limonene fraction, distillation residue	68334-40-7	30-35% <i>d</i> -Limonene 1-3% Terpinolene 30-35% Unspecified diterpene and sesquiterpene hydrocarbons 15-20% Tertiary monoterpene alcohols (mainly linalool and <i>alpha</i> -terpineol) 5-12% Unspecified oxygenated terpenes
12. Terpenes and terpenoids, turpentine-oil residue	68938-00-1	3.2% <i>d</i> -Limonene, <i>beta</i> -Phellandrene, <i>beta</i> -Myrcene 4.8% Pinene, camphene, <i>delta</i> -3-carene 82% Polymers 10% Nonvolatile terpenes

### **Category Justification**

The submitter states that the basis for grouping the members of this category is that these substances are monoterpene hydrocarbons or are mixtures composed primarily of monoterpene hydrocarbons. The submitter also states that monoterpene hydrocarbons are naturally occurring compounds that are released primarily from coniferous trees and are found in nearly all fruits and vegetables. Commercially, the monoterpene hydrocarbons are derived from natural sources including fruits and pine trees. Some monoterpene hydrocarbons are used as flavorings and as fragrances.

The five single chemical substances (both limonenes, terpinolene, myrcene and dihydromyrcene) in the category are monoterpene hydrocarbons, are structurally similar, have similar molecular weights and functional groups, and are expected to have similar physicochemical, environmental, and toxicological properties. Grouping these chemicals into the category is reasonable.

The submitter states that the basis for including the seven mixtures in this category is that they are composed primarily of two or more of the following monoterpene hydrocarbons: *d*-limonene, *dl*-limonene, terpinolene, and myrcene. The submitter also states that it is appropriate to evaluate the monoterpene hydrocarbon substances and their structural analogs in the same chemical category. Owing to their structural similarities, the submitter expects that these substances will have similar metabolic pathways.

Three mixtures, sweet orange peel oil; sweet orange oil terpenes and terpenoids; and terpenes and terpenoids, limonene fraction contain greater than 90% monoterpene hydrocarbons, mainly limonene and myrcene. These substances are expected to have physicochemical, environmental, and toxicological properties similar to the major components that constitute the mixture. The inclusion of these three mixtures in the category is reasonable.

Two mixtures, CAS No. 68956-56-9 and CAS No. 65996-99-8, are composed mostly (67–95%) of monoterpene hydrocarbons. The inclusion of these two mixtures in the category is reasonable. However, CAS No. 68956-56-9 contains 18% limonene isomers and 10% unspecified terpene hydrocarbons and CAS No. 65996-99-8 contains 5-10% unspecified terpene hydrocarbons. The submitter needs to clarify the unspecified substances that are present in these products as to whether these are mono-, sesqui-, di-, or higher terpenes so that a better understanding of the mixtures can be formed.

The last two mixtures, CAS No. 68334-40-7 and CAS No. 68938-00-1, are predominately composed of non-monoterpene hydrocarbon substances. CAS No. 68334-40-7 contains approximately 31-38% monoterpene hydrocarbons. The rest of the mixture is composed of 15-20% tertiary monoterpene alcohols (linalool and *alpha*-terpineol), 30-35% unspecified sesquiterpene and diterpene hydrocarbons and 5-12% unspecified oxygenated terpenes. The submitter does not provide information that supports the inclusion of this substance in the category or an explanation of why any of the anticipated properties are either similar to the other members or fit a predictable pattern. As no testing is planned for this mixture, the submitter needs to provide this information to determine whether its inclusion in the category is appropriate.

The mixture having CAS No. 68938-00-1 is composed of less than 3.2% of monoterpenes, *d*-limonene and *beta*-myrcene, with the remainder composed of mostly polymeric (82%) and nonvolatile terpenes (10%). This substance is not consistent with the other members of the category and EPA believes that it should not be included.

## **Test Plan**

### **General Comment**

As indicated under Category Justification, the submitter needs to explain the relevance of CAS No. 68334-40-7 to the rest of the category members. If the submitter cannot support the inclusion of this substance as a category member, then a different approach is needed for it.

### **Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient and water solubility).**

Many of the individual physicochemical property and fate discussions lack explicit conclusions and thus require the reviewer to consult the test plan tables to ferret out the conclusions. The submitter's rationales for its testing decisions, particularly on mixtures, are unclear. Summary tables of measured and calculated physicochemical property and fate data would also help significantly in understanding the data.

The submitter's approach to melting point, boiling point, partition coefficient and water solubility for *d*-limonene, *d**l*-limonene, terpinolene, myrcene, and dihydromyrcene is generally adequate for the purposes of the HPV Challenge Program. However, melting points estimated from models are not satisfactory, and the submitter should attempt to locate literature values for more of the substances.

In the test plan text, the submitter indicates that the vapor pressure, octanol/water partition coefficient, and water solubility are expected to fall within ranges of values estimated by the submitter. This suggests that, for the category members lacking these data, fields for these endpoints in Table 3.5 that are designated as "NA" (i.e., not applicable owing to substance properties) should instead have the designation "R" (satisfied using SAR) as is done for boiling points. The submitter needs to ensure that its use of symbols in the Tables are appropriate and consistent.

*Boiling Point.* As test plan Table 3.5 indicates that boiling point data are available for sweet orange peel oil, the submitter needs to provide a corresponding robust summary.

*Vapor Pressure.* The submitter provided measured vapor pressure data only for *d*-limonene and calculated data for four other chemicals. EPA believes that the data presented insufficiently represent the vapor pressure for this category. The submitter needs to provide measured vapor pressure data for the noncyclic myrcene or dihydromyrcene in order to permit a more reliable assessment of this endpoint. A value for myrcene in the National Library of Medicine Hazardous Substance Databank (HSDB) cites as the source Perry's Chemical Handbook (Perry, R.H.; Green, D. 1984. Perry's Chemical Handbook. Physical and

Chemical Data, New York, NY: McGraw-Hill, 6<sup>th</sup> ed.). Verification of this value may obviate the need for further testing of this endpoint.

The calculated vapor pressure value in the robust summaries (page 10) for sweet orange peel oil is not reflected correctly in test plan Table 3.5.

For CAS No. 68334-40-7, even if its inclusion in the category can be justified, the submitter needs to provide measured melting point, boiling (or decomposition) point, and water solubility data following OECD guidelines. With components that are significantly different from the rest of the category members, its physicochemical properties may not follow the same pattern as the others. The melting point determination for this mixture could be satisfied under OECD Guideline 102 by a preliminary test showing that the value will be < 0°C. In addition, the submitter needs to amend the partition coefficient discussion. An expected Log P range of 4.8-5.3 is not appropriate for this mixture because its oxygenated components such as linalool and terpineol have literature Log P values of about 3. The discussion and testing decisions should reflect this information.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity).

The submitter's approach to photodegradation and stability in water are adequate for the purposes of the HPV Challenge Program.

*Biodegradation.* Adequate data are available for the purposes of the HPV Challenge Program and no additional testing is necessary. However, the submitter needs to include in its test plan a technical discussion on application of the category read-across approach to the monoterpene hydrocarbon category members for which no biodegradation data are available.

For CAS No. 68334-40-7, if its inclusion in the category can be justified, the submitter needs to specifically address the identity and biodegradation of the key components of the mixture in their "read across" discussion. This discussion might obviate the need for testing; however, EPA would reserve judgement pending receipt of such a discussion.

*Fugacity.* The sponsor estimated the fugacity of these chemicals using a Level I EQC model. Although EPA had previously recommended the use of EQC Level I, this model is somewhat limited. EPA now recommends the use of EQC level III, which provides a more rigorous level of analysis. The submitter needs to incorporate in its robust summaries the values of the input parameters to the fugacity models.

The test plan table indicates that fugacity and photodegradation data are available for dihydromyrcene. The submitter needs to provide the corresponding robust summaries.

Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity).

For the submitted substances other than CAS Nos. 68334-40-7 and 68938-00-1, robust summaries were presented for all human health effects endpoints using the studies conducted on *d*-limonene, beta-myrcene, terpinolene, dihydromyrcene, or sweet orange peel oil. The evaluation of the mammalian toxicity data in the robust summaries was limited by a general lack of detailed descriptions of several parameters, including methods used and results obtained. For presentation purposes, a table summarizing the derived toxicity values (NOAELs, NOELs, LOAELs, LOELs etc.) for all the endpoints would have improved the readability and comprehension of the text in the test plan summary. The text in the test plan was also difficult to read because of the exclusion of the CAS No. of each substance (particularly the mixtures) from the body of the report.

The submitter needs to address a general issue about the validity of the *in vitro* genotoxicity studies. It is questionable whether the results are valid because *d*-limonene, beta-myrcene and terpinolene are volatile substances and the robust summaries did not indicate whether these studies were modified appropriately. In addition, none of the robust summaries indicated whether testing was done up to cytotoxic concentrations, which would preclude the need to control for volatility. If the submitter cannot demonstrate that one or more of the *in vitro* genotoxicity studies was conducted in a manner that accounted for the volatility of the test substances, then additional genotoxicity testing may be warranted.

*Acute Toxicity.* Adequate data may exist for acute oral toxicity on a weight-of-evidence basis, but the submitter needs to provide the missing study details to allow independent assessment of study adequacy.

*Repeated-Dose Toxicity.* Adequate data may exist for the repeated dose toxicity endpoint. The submitter needs to address several deficiencies in robust summaries.

*Genetic Toxicity – Gene Mutations.* Adequate data may exist for the gene mutation endpoint; however, the submitter needs to justify the adequacy of the available studies by providing conditions under which the tests were performed and needs to provide missing study details to allow independent assessment of study adequacy. If one or more of the robust summaries can be satisfactorily revised to substantiate data adequacy, the available studies may be sufficient to assess the gene mutation potential of the category.

*Genetic Toxicity – Chromosomal Aberrations.* The weight of evidence suggests that data may be adequate to address the chromosomal aberrations endpoint; however, as stated above, the submitter needs to provide missing study details so that their adequacy can be independently assessed.

*Reproductive Toxicity.* The data are adequate for the reproductive toxicity study done in male and female rats on myrcene. For the other studies, the submitter needs to provide several study details in the robust summaries.

*Developmental Toxicity.* The submitter needs to provide missing study details to ensure an adequate and independent evaluation of study data.

#### Ecological Effects (fish, invertebrates, and algae).

No additional acute ecotoxicity testing is necessary; however, the submitter needs to include all required study elements in all robust summaries for them to be adequate. EPA believes the log Kow range of 4.8 to 5.3 for this category (reported in section 3.1.4 of the test plan) suggests that chronic invertebrate toxicity testing is necessary. The chronic daphnia 21-day test should be considered for the most hydrophobic single chemical, CAS No. 2436-90-0. Given the volatility of these chemicals EPA recommends that the chronic tests be conducted using the no-head-space flow-through method and analytical monitoring.

### **Specific Comments on the Robust Summaries**

#### Physicochemical Properties

*Partition Coefficient.* In section 1.4 of the robust summaries, page 13, myrcene is under CAS No. 123-11-5. The correct CAS No. is 123-35-3.

#### Health Effects

*Acute Toxicity.* The robust summaries lacked adequate description of test substance purity.

*Repeated-Dose Toxicity.* The robust summaries did not indicate the following study details for one or more of the studies: mortality/signs of toxicity per concentration tested, body weight monitoring data, tissues examined, clinical chemistry and hematology details, and statistical methods and analyses.

*Genetic Toxicity – Gene Mutations.* The robust study summaries for the bacterial and mammalian tests did not provide sufficient detail to independently assess study adequacy. There was little evidence that the *in vitro* testing had been carried out up to cytotoxic concentrations. Other missing details included test substance purity, culture conditions, rationale for dose selection, number of replicates, control use/response data, statistical methods used, and whether or not the studies controlled appropriately for volatility.

*Genetic Toxicity – Chromosomal Aberrations.* Certain *in vitro* study summaries were missing details such as test substance purity, cultures per test concentration, characterization and use of positive or negative controls, culture conditions and statistical methods and analyses. The summary for the *in vivo* test was also missing information on test substance purity and the specific chromosomal aberration results by dose (an increase was implied, but was described as not statistically significant).

*Reproductive and Developmental Toxicity.* The submitter needs to provide the following missing information so that the adequacy of these studies can be independently evaluated: test substance purity, reproductive/developmental parameters examined (it appears that many tests were nonstandard), magnitude of observed changes, and statistical methods and analyses.

#### Ecological Effects

EPA recommends that the submitter use the guidance on how to enhance the robust summaries to the standard established in the EPA's HPV Challenge Program Guidance at <http://www.epa.gov/chemrtk/guidocs.htm>.

*Fish, Invertebrates, and Algae.* The submitter needs to provide the following required data elements lacking in the robust summaries: mortality, DO, pH, water temperature, replicate numbers, and water hardness. Additionally, the submitter needs to report the input values for the ECOSAR predictions for invertebrates and algae.

Some 48-hr. daphnia tests were erroneously reported as 96-hr. studies.

#### **Followup Activity**

EPA requests that the submitter advise the Agency within 90 days of any modifications to this submission