

**The Flavor and Fragrance High Production Volume Consortia  
(FFHPVC)**

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Administrator  
U.S. Environmental Protection Agency  
Ariel Rios Building  
Room 3000, #1101-A  
1200 Pennsylvania Avenue N.W.  
Washington, D.C. 20460

September 27, 2006

Dear Administrator:

On behalf of the Flavor and Fragrance High Production Volume Consortia, I wish to thank the Environmental Protection Agency (EPA) for their comments on the test plan and robust summaries on "Monoterpene Hydrocarbons". The Terpene Consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for chemical substances under the Chemical Right-to-Know Program. Since 1999, the companies that are current members of the Terpene Consortium have supported the collection and review of available test data, development of test plans and robust summaries, and conducted additional testing for "Aromatic Terpene Hydrocarbons".

Based on our initial recommendations for testing and the peer-reviewed comments of the EPA, the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC) is pleased to submit the following revised test plan and robust summaries for "Monoterpene Hydrocarbons". The revised test plan and robust summaries contain additional data on existing studies and the results of additional toxicity and environmental fate studies that are related to the questions and comments made by the EPA in its letter dated 06/11/2002. This letter contains responses to the specific comments made by the EPA. These responses taken together with the inclusion of new study data and other information constitute the key changes to the original test plan and robust summaries.

Based on these additional data, the Terpene Consortium concludes that the current test plan and robust summaries for this category is now complete. The experimental and model data for physiochemical

properties, environmental fate, ecotoxicity, and human health endpoints are consistent and provide a comprehensive basis upon which to evaluate the hazard potential of monoterpene hydrocarbons. A summary of the key hazard data has been included in this letter and also in the revised test plan for Monoterpene Hydrocarbons.

In an EPA letter dated 19 October 2001 concerning HPV-sponsored chemicals that are recognized as GRAS by the Food and Drug Administration, it was pointed out that:

“ It may well be, on the basis of experience gained over years of use, that most of the substances have little compelling evidence suggesting that testing is needed in the context of the HPV Challenge Program. Nonetheless, while this line of reasoning could have been used to support the recommendation not to test the substances in this category, the information was only provided as background; few examples, and no actual data, were cited.”

Without prior guidance from EPA, the Terpene Consortium felt responsible to report endpoint data for this substance. Most of these data have already been provided to the US Food and Drug Administration and the World Health Organization during their evaluation of these substances as food additives. Human health hazard data on monoterpene hydrocarbons have been reviewed by the World Health Organization/Food and Agriculture Organization Joint Expert Committee for the Evaluation of Food Additives (WHO/FAO JECFA) for use as flavoring substances in food. As part of its responsibility, JECFA maintains an ongoing program of review of the safety of food additives (WHO Technical Series Nos. 38, 40, 42, 44, 46, 48, 50, 52, 54). In 2004, the group of monoterpene hydrocarbons [WHO Food Additive Series: 52, 2004; see Revised Test Plan] were recognized as safe for use in food.

The group of monoterpene hydrocarbons in this chemical category is also recognized as “Generally Recognized as Safe” (GRAS) for its intended use in food by the United States Food and Drug Administration under the Code of Federal Regulations (CFR 172.515). Under supervision of the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, specifications for the commercial use of monoterpene hydrocarbons in food are published in the Food Chemical Codex [FFC, 1996; see Revised Test Plan].

Based on the long history of monoterpene hydrocarbons both as naturally occurring components of food and as substances intentionally added to food, the hazard assessments performed by the US FDA and WHO/FAO JECFA, and the current regulatory status for the addition of this substance to the food supply, there is no compelling evidence that this substance should be further tested for physiochemical properties and human health endpoints in the EPA Chemical “Right to Know” Program. We do, however, maintain that data on the environmental fate and ecotoxicity are relevant to the HPV Challenge program. In this context, we have sponsored ecotoxicity studies to provide a robust database on ecotoxicity endpoints. We consider that the test plan and robust summaries for this category are final and have no plans to provide additional data. The EPA comprehensive comments provided the necessary guidance to complete the test plan for this category. The collaboration between the Terpene Consortium and the

Environmental Protection Agency in the Chemical "Right to Know" Program has produced a hazard database that will be useful to the public for decades to come. Thank you for the opportunity to participate in such a program.

If you have any questions or comments concerning the contents of this letter, please feel free to contact me at any time (202-331-2325) or [tadams@therobertsgroup.net](mailto:tadams@therobertsgroup.net).

Best regards,

Timothy B. Adams, Ph.D.

Technical Contact Person for FFHPVC

## Summary of Key Hazard Data for Monoterpene Hydrocarbons

Endpoint	Substance/Surrogate <sup>1</sup>	Value/Range <sup>2</sup>	Reference
<b>Physical Properties</b>			
<b>Partition Coefficient</b>	Terpinolene	5.3 (OECD 117)	Givaudan Roure Inc. ,1996a
<b>Partition Coefficient</b>	Terpenes & terpenoids, sweet orange oil	5.3 (OECD 117)	Givaudan Roure Inc. ,1996a
<b>Environmental Fate</b>			
<b>Biodegradation</b>	Terpinolene	28d/62.1%/(OECD 301B)	Birch R., 1996
<b>Biodegradation</b>	Terpinolene	28d/80%/(OECD 302C)	Rudio J., 1998
<b>Biodegradation</b>	Terpinolene	28d/51%/(OECD 301F)	Rudio J. ,1997
<b>Ecotoxicity</b>			
<b>Fish</b>	<i>d</i> -Limonene	96-hr/LC50=0.702 mg/L	Broderius et al., 1990
<b>Fish</b>	<i>d</i> -Limonene	96-hr/LC50=0.720 mg/L	Broderius et al., 1990
<b>Fish</b>	Terpinolene	96-hr/LC50=1.210 mg/L	Broderius et al., 1990
<b>Aquatic Invertebrates</b>	<i>d</i> -Limonene	48-hour LC50 = 0.577 mg/L	Broderius et al., 1990
<b>Aquatic Invertebrates</b>	<i>d</i> -Limonene	48-hour LC50 = 0.924 mg/L	Broderius et al., 1990
<b>Aquatic Invertebrates</b>	Terpinolene	48-hour LC50 = 2.55 mg/L	Broderius et al., 1990
<b>Aquatic Plants</b>	<i>d</i> -Limonene	No significant inhibition	Broderius et al., 1990
<b>Aquatic Plants</b>	Terpinolene	No significant inhibition	Broderius et al., 1990
<b>Human Health</b>			
<b>Repeat Dose (route)</b>	<i>d</i> -Limonene (oral-gavage)	103 wks LOAEL: 75 mg/kg bw/d (male); 600 mg/kg bw/d (female)	NTP, 1990

<sup>1</sup> Surrogate is a structurally related substance include a metabolic product or precursor of the named substance

<sup>2</sup> Experimental value or values for a substance or group of substances in the chemical category

		103 wks NOAEL=300 mg/kg bw/d (female rat); undetermined (male rat)	
<b>Repeat Dose (route)</b>	<i>d</i> -Limonene (oral-gavage)	103 wks LOAEL: Undetermined for males; 1000 mg/kg bw/d for female 103 wks NOAEL=500 mg/kg bw/d	NTP, 1990
<b>Repeat Dose (route)</b>	<i>d</i> -Limonene (oral-gavage)	16 d LOEL=3300 mg/kg bw/d 16 d NOEL=1650 mg/kg	NTP, 1990
<b>Repeat Dose (route)</b>	<i>d</i> -Limonene (oral-gavage)	13 wk LOEL=600 mg/kg bw/d 13 wk NOEL=300 mg/kg	NTP, 1990
<b>Repeat Dose (route)</b>	<i>d</i> -Limonene (oral-gavage)	13 wk LOEL=500 mg/kg bw/d 13 wk NOEL=1000 mg/kg	NTP, 1990
<b>Repeat Dose (route)</b>	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck) (oral-gavage)	28 d LOEL = 240 mg/kg bw/d 28 d NOEL = Not determined	Serota, 1990
<b>Repeat Dose (route)</b>	<i>beta</i> -Myrcene (oral-gavage)	13-week LOEL = 500 mg/kg bw/d 13-wk NOEL = Not determined	NTP draft, 2003
<b>Repeat Dose (route)</b>	<i>beta</i> -Myrcene (oral-gavage)	13-week LOEL= 500 mg/kg bw/d (female) 13-wk NOEL = 250 mg/kg bw/d 13-week LOEL = 1000 mg/kg bw/d (male) 13-wk NOEL= 500 mg/kg bw/d (male)	NTP draft, 2003
<b>Reproductive</b>	<i>beta</i> -Myrcene (oral-gavage)	86-112 d NOEL= 300 mg/kg bw/d LOEL=500 mg/kg bw/d	Paumgarten et al., 1998
<b>e) Developmental(rout</b>	<i>beta</i> -Myrcene (oral-gavage)	128 d NOEL (rat): 250 mg/kg bw/d 128 d LOEL(rat): 500 mg/kg bw/d (oral-	Delgado et al., 1993b

		gavage)	
<b><i>in vitro</i> Genotoxicity<sup>3</sup></b>	d-Limonene; beta-Myrcene; Sweet Orange Oil	-(AMS); - /+(MLA); -, (ABS); - (SCE);	Heck et al., 1989; Florin et al., 1980; Muller, 1993; Haworth et al., 1983; Anderson et al., 1990; Myhr et al., 1990; Kauderer et al, 1991; Roscheisen et al, 1991; Crebelli et al., 1990; Kuroda et al., 1989.
<b><i>in vivo</i> Genotoxicity</b>	d-Limonene beta-Myrcene	(-) mouse embryo (-/+ ) ABS  (-) MN	Fahrig, 1984; Zamith et al., 1993.  NTP, 2003.

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<sup>3</sup> (-), no significant evidence; (+/-), equivocal evidence; (+), positive evidence of genotoxicity

## Responses to EPA Comments on Monoterpene Hydrocarbons

Excerpted comments from EPA concerning the test plan and robust summaries for terpene hydrocarbons with suggested response/actions in bold type.

### Category Justification

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.

**It is agreed that Terpenes and terpenoids, turpentine-oil residue, CAS 68938-00-1, consists primarily of polymers of terpenes and terpenoids. These polymers should meet the exemption criteria and therefore not be included in the program. The substance, therefore, can be considered as a polymer with some monomer content and therefore be exempt or it can be considered a mixture of terpenes and terpenoids with a high (exempt) polymer content. If considered as the latter, it fits well within this group but if the former, should be exempt from the program.**

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.

**The mixture, terpenes and terpenoids, turpentine oil, limonene fraction, distillation residue, CAS 68334-40-7, contains significant quantities of oxygenated derivatives (15-20% tertiary monoterpene alcohols (mainly linalool and *alpha*-terpineol) 5-12% unspecified oxygenated terpenes). However, these components are essentially similar to those that would be formed by P-450 oxidation of limonene and other monoterpene hydrocarbons *in vivo*. As stated in section 2.5, “The principal metabolic pathway involves side chain oxidation to yield monocyclic terpene alcohols and carboxylic acids. These metabolites are mainly conjugated with glucuronic acid and excreted in the urine, or to a lesser extent in the feces.” Similar oxidation**

**products would be expected to form in the process of biodegradation in the environment. Thus, it is appropriate to consider this mixture in this group of terpene hydrocarbons.**

## 2. Physicochemical Properties and Environmental Fate.

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 robust summaries were revised to address the confusion concerning testing decisions. Robust summaries on mixtures were revised, particularly noting that most of the mass of naturally occurring mixtures is contributed by monoterpene hydrocarbons .**

The submitter's approach to melting point, boiling point, partition coefficient and water solubility for -limonene, *dl*-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.

**Searches of the common compilations of physical properties did not find additional data. Melting points below 25 °C are not particularly reliable for substance identification and are not used in most models for environmental effects. Additional efforts do not seem justified given that the manufacture, transport, and potential exposure occurs when these substances are present in the liquid or vapor state.**

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.

**The Table 3.5 has been appropriately revised.**

*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 measured vapor pressure of 2.01 mm Hg at 25 °C for myrcene from this source has been added to the robust summary.**

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.

**This has been corrected.**

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 degrees 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.

**Terpenes and terpenoids, turpentine oil, limonene fraction, distillation residue, CAS 68334-40-7, is a complex mixture. Therefore, any measurement of melting (freezing) point or boiling point will result in a wide range for physical property values that would not be useful for either substance characterization or for evaluation. Since water solubilities of its components would vary, it is not clear what an attempt to measure this property would produce that would be helpful in evaluation nor even what water solubility means in the context of complex mixtures. It is true that the oxygenated components would lower the partition coefficient but it is not known how this could be calculated for this complex mixture. Listing the higher range that encompasses the hydrocarbon terpene components is a conservative approach to evaluation.**

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

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.

**As pointed out above, the oxygenated components of this complex mixture reflect the intermediates that would be expected upon biodegradation. The presence of these components would increase the rate of biodegradation. Comparing this complex mixture to the hydrocarbon terpenes in this group for prediction of biodegradation, therefore, represents a conservative approach to evaluation.**

*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.

**EQC Level III fugacity calculations for limonene, terpinolene, myrcene and dihydromyrcene have been added to the test plan.**

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

**The calculated data for photodegradation and Level III fugacity for dihydromyrcene have been added to the robust summaries.**

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

**A table presenting a summary of the data has been included in the test plan and the requested detail in the robust summaries for mammalian toxicity has been included.**

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.

**The relative high boiling points for this group of substances (158-186 °C) preclude substantial evaporation under the conditions and short time periods of the tests. Nevertheless, the reports can be checked for evidence of cytotoxicity. The two *in vivo* tests, where volatility is not an issue, were also negative.**

**NOTE: In checking the boiling points, an error was discovered. The listing of a bp for myrcene of 44 °C at 760 mm Hg should say 44 °C at 10 mm Hg.**

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.

**While we agree that additional chronic ecotoxicity data would be appropriate for most substances showing log Kow values of 4.88-5.3, these substances are naturally occurring monoterpenes that are ubiquitous in the environment. The results of any chronic test would need to be evaluated in the context of the ubiquitous nature of these substances. In addition, the relatively high EC50 and LC50 values for monoterpene hydrocarbons in acute studies does not warrant further chronic testing. All animals including aquatic species, have evolved in the presence of these substances. Daphnid are known to contain the cytochrome P-450 enzymes necessary to metabolize these substances.**

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

**This has been corrected.**

#### Health Effects

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

**The original reports were checked and test substance purity was not given where indicated.**

**Purity has been added to robust summaries**

*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 haematology details, and statistical methods and analyses.

**The original reports were checked but these details are often not presented. Requested statistics for the studies performed by the National Toxicology program have been added.**

*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.

**The requested data has been added where available.**

*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).

**The original reports were checked but these details not present in many of the published articles.**

*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.

**These data were added where available.**

#### Ecological Effects

*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.

**The original reports were checked but these details were not often presented. Input data for ECOSAR calculations have been added to the robust summaries.**

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

**This has been corrected.**