June 21, 2001

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

Dear Dr. Adams:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Terpenoid Tertiary Alcohols and Related Esters, posted on the ChemRTK Web Site on February 14, 2001. I commend The Terpene Consortium of The Flavor and Fragrance High Production Volume Consortia for its 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 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.

As noted in the attached Comments, the proposed category was not adequately justified. The inclusion of certain proposed members needs better explanation. The general discussion of the category justification for health endpoints and concomitant recommendations are unacceptable for a variety of reasons. The test plan for aquatic toxicity provided even less explanation for defining these chemicals as a category. The Consortium needs to explain more fully why the category holds together for all chemicals in the test plan for the different endpoint groups, and how testing or using SAR predictions may satisfy the ecological toxicity needs of all members of the category for the purposes of the Challenge program. In addition, the robust summaries for ecological effects in general provided insufficient information to determine data adequacy or adequately interpret the studies.

The Consortium also needs to reconsider its analysis of the potential biodegradation and testing needs of 2-pinanol hydroperoxide and the bicyclic terpenoids, and to better explain how available data on related substances can be used to address the biodegradation potential of "-terpineol.

The Consortium estimated the transport and distribution of these chemicals using a Level I Model. EPA recommends using the EQC Level III model; see Specific Comments on the Robust Summaries. For those chemicals having experimental biodegradability data available, the sponsor should run the Level III model using the half-lives derived from the test results.

Ten of these substances (not identified in the Test Plan) are on FDA's GRAS list. 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 HPV Challenge Program. Nonetheless, while this line of reasoning could have been used to support the recommendations not to test the substances in this category, the information was only provided as background; few examples, and no actual data, were cited.

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 Consortium 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 this 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 tsca-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

Attachment

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

EPA Comments on Chemical RTK HPV Challenge Submission: Terpenoid Tertiary Alcohols and Related Esters

SUMMARY OF EPA COMMENTS

The sponsor, the Terpene Consortium (one of the Flavor and Fragrance High Production Volume Consortia, or FFHPVC), submitted a Test Plan and Robust Summaries to EPA dated January 26, 2001, for the Terpenoid Tertiary Alcohols and Related Esters. EPA posted the submission on the ChemRTK Web site on February 14, 2001. The proposed information-gathering plan is for thirteen substances (see Category Definition, below) considered by the sponsor to constitute a category.

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

1. Category justification. The proposed category was not adequately justified for the different endpoint groups. The inclusion of certain proposed members needs better explanation.

2. Physicochemical and Environmental Fate Data. The submitter needs to provide measured data for a number of physicochemical endpoints. For environmental fate, the submitter needs to reconsider its analysis of the potential biodegradation and testing needs of 2-pinanol hydroperoxide and the bicyclic terpenoids, and to better explain how available data on related substances can be used to address the biodegradation potential of "-terpineol.

The submitter estimated the transport and distribution of these chemicals using a Level I Model. EPA recommends using the EQC Level III model; see Specific Comments on the Robust Summaries. For those chemicals having experimental biodegradability data available, the sponsor should run the Level III model using the half-lives derived from the test results.

3. Health Endpoints. Adequate data exist for some category members for some SIDS endpoints, but the general discussion of the category justification for health endpoints, and the plan not to conduct any health tests, are not supported for a variety of reasons detailed below.

4. Ecotoxicity. The test plan for aquatic toxicity provided limited explanation for defining these chemicals as a category, since several in the group are structurally different (acetate esters, a peroxide, mixtures) from the others. The sponsor needs to explain more fully why the category holds together for all chemicals in the test plan, and how testing or using SAR predictions may satisfy the ecological toxicity testing needs of all members of the category for the purposes of the Challenge program. In addition, the robust summaries in general provided insufficient information to determine data adequacy and adequately interpret the studies.

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

EPA COMMENTS ON THE TERTIARY TERPENOID ALCOHOL AND RELATED ESTERS CATEGORY

Category Definition

The category Terpenoid Tertiary Alcohols and Related Esters contains 13 terpenoid substances that are used extensively in flavors and fragrances. All but one of the substances is either a tertiary alcohol or an ester; the exception is 2-pinanol hydroperoxide. Seven of the substances are discrete alcohols: linalool (CAS # 78-70-6), tetrahydrolinalool (CAS # 78-69-3), myrcenol (CAS # 543-39-5), dihydromyrcenol (CAS # 18479-58-8), "-terpineol (CAS # 98-55-5), *cis*-2-pinanol (CAS # 4948-28-1), and *trans*-2-pinanol (CAS # 4948-29-2), two substances are acetate esters: linalyl acetate (CAS #115-95-7) and "-terpineol acetate (CAS # 80-26-2), one substance is a mixture of two chemicals: 2-pinanol (CAS # 473-54-1), a mixture of *cis*- and *trans*-2-pinanol), and two substances are complex mixtures: pine oil (CAS # 8002-09-3) and "pinanol thermal rearrangement products" (CAS # 125252-49-5). EPA commends the Consortium for providing information on the mixture components and the estimated per cent range of each component.

The identity of the proposed category members is clear. However, the inclusion of 2-pinanol hydroperoxide (28324-52-9) is questionable, given its unique chemical properties. This is discussed further below.

Category Justification

The sponsor considers the proposed category justified because "...of [the members'] close structural relationships, [*and*] their similar physico-chemical properties, including the ready conversion of linalool to " -terpineol. They are also considered together by virtue of the fact that they participate in the same pathways of metabolic detoxication and have similar toxicologic potential" (Section 2.3.3, Test Plan).

EPA's comments on each of these criteria follow.

Close structural relationships

Considering all 13 substances, the proposed category does not appear to cohere on the basis of structural considerations. The inclusion of 2-pinanol hydroperoxide, acetate esters and several mixtures (pine oil and the thermal rearrangement products of pinanol) requires better justification.

Of the seven alcohols in the proposed category, one is noncyclic and saturated (tetrahydrolinalool), three are noncyclic and unsaturated (linalool, myrcenol, and dihydromyrcenol), two are cyclic and saturated (*cis*-2-pinanol and *trans*-2-pinanol, and one is cyclic and unsaturated (" -terpineol). Furthermore, the unsaturated compounds include both mono- and diolefins. These different structure types may have different physiological (see discussion of metabolism below) and environmental properties (cyclic chemicals generally do not biodegrade as readily as acyclic chemicals, possibly making their persistence in the environment vary over a wide range).

Similar Physicochemical Properties

This criterion was stated in the submission but not discussed.

Metabolic Pathways/Ready Conversions

The sponsor provides adequate information on the metabolism/elimination of the esters, linalool and alpha-terpineol, and the bicyclic tertiary alcohols, but does not make a case for similarities or application to other category members (tetrahydrolinalool, the peroxide, and the mixtures). In addition, the data show how cyclicity and the level of unsaturation likely play a role in the differing elimination of linalool and " -terpineol; linalool appears to be metabolized predominantly by glucuronic acid formation whereas oxidation predominates for " -terpineol.

The submission also postulates the "ready conversion" of some group members to others under a variety of conditions. However, while some of the chemicals may be converted to others in the gut (linalool and " -terpineol), tetrahydrolinalool, for example, will not. It is further stated that some members will form epoxide intermediates (minor pathway suggested for alpha-terpineol) without addressing this possibility for others.

As a result of the incomplete description of the metabolic pathways (only seven of 13 category members discussed), and the failure to bring all category members into the discussion, the submission provides little support for the conclusion that all members of this group have a common metabolic fate in mammalian systems.

Similar Toxicologic Potential

The sponsor suggests that mammalian toxicities of the substances in this category are likely to be similar. However, the majority of data supporting this conclusion are from acute and genetic toxicity testing. The lack of a complete toxicity profile on even one member of this group, and the limited data on the other members, make it difficult to conclude that these substances should be grouped together because of similarities in

toxicologic properties, and that no additional testing is required because of the demonstrated similarity of the group. Details are discussed below under Test Plan: Health Effects–Repeat Dose Toxicity.

Industrial/Biogenic Production

A flow diagram depicting the manufacture of the various substances would be very helpful. Details were provided only for five of the 13 category members: the 2-pinanol hydroperoxide is reduced to make *cis*- and *trans*-2-pinanol which in turn are used to make linalool, which is "readily converted" to " -terpineol.

Again, reporting of this information is useful for the category members discussed, but there was no attempt to show how all category members fit into a category on the basis of industrial/biogenic production.

Other Considerations

The thrust of the category justification was health effects-related. Adequate attention must be given to justifying the category approach for other endpoint areas.

Test Plan

Overall, the Test Plan could have benefitted from better organization and better support for many of the conclusions. It is difficult to understand the reasoning behind many of the conclusions in the Test Plan.

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

According to the Test Plan Table (page 32) most endpoints are or will be calculated. EPA strongly recommends that the submitter provide measured data for these endpoints.

Melting Point

The submitter's approach to this endpoint is acceptable.

Boiling Point

The submitter states that no further testing is necessary for this endpoint. Measured data were not submitted for 2-pinanol hydroperoxide, which the submitter notes is thermally unstable. The submitter needs to supply whatever information is accessible on the decomposition point for the chemical. In addition, measured data were not submitted for pinanol thermal rearrangement products; a measured value for the acetate of plinol, the major mixture component, was provided, but none for plinol itself. The submitter needs to provide measured data for this chemical or explain why the acetate is an adequate surrogate for the mixture.

Vapor Pressure

The submitter states in the text that no further testing is necessary for this endpoint. Most of the data submitted, however are based on estimations. Therefore, EPA recommends that the submitter provide measured data for these chemicals.

Water Solubility

The submitter states in the text that no further testing is necessary for this endpoint. Most of the data submitted, however are based on estimations. Therefore, EPA recommends that the submitter provide measured data for these chemicals.

Environmental Fate (Photodegradation, Stability in Water, Biodegradation, Fugacity)

Biodegradation

The submitter mentions that 2-pinanol hydroperoxide will decompose to 2-pinanol, and that it will biodegrade in the environment, but does not explain how the decomposition occurs except to cite a report on the substance's decomposition under severe, environmentally irrelevant laboratory conditions. This information does not support the submitter's conclusion that the hydroperoxide will biodegrade in the environment. In addition, since peroxides are toxic to microbes, they are generally considered to inhibit biodegradation. EPA recommends that the submitter re-evaluate its analysis of the potential biodegradation of this chemical and its testing recommendation.

The submitter's conclusion that bicyclic substances in this category, 2-pinanol cis-2-pinanol and trans-2-pinanol should be biodegradable in the environment is based on their structural similarity with non-bicyclic substances in this class that have been shown to be readily or ultimately biodegradable. Without study data, it is unclear whether the bicyclic substances will biodegrade in a manner that is similar to that of acyclic and monocyclic substances in this category. While it is possible that the submitter's conclusion is valid, the conclusion can only be supported by test data on the bicyclic compounds of this class. EPA recommends that the submitter re-evaluate the biodegradation potential of the bicyclic chemicals and how they should be characterized for this endpoint.

The submitter mentions data that could be related to the biodegradation potential of "-terpineol, such as studies on "-terpineol acetate and pine oil (containing up to 60% "-terpineol), but does not explicitly address the issue.

Fugacity

The submitter indicates in its Test Plan that it estimated the transport and fugacity of these chemicals using the Level I Fugacity-based Environmental Equilibrium Partitioning Model. EPA recommends using the EQC Level III model; see Specific Comments on the Robust Summaries.

Because many of these chemicals have experimental biodegradability data available, in those cases the sponsor should run the Level III fugacity model using the half-lives derived from the test results.

Health Effects.

The sponsor presents a matrix for health endpoints on page 35 of the Test Plan, and does not plan to conduct any health testing for this category. No data are available for 2-pinanol hydroperoxide. This is important because there was no discussion in the Test Plan about which category member might be used to "inform" the toxicity of the hydroperoxide given available information on alcohols and esters. As a general comment, EPA questions the order in which the substances are presented and how that will be used in a read-across (or read-down in this case) approach from cells with available data to cells for which no data exist (and for which no tests are planned). The specific comments presented below take into account the narrative presented above under Category Justification:

Acute Toxicity

Although EPA does not believe that the inclusion of all 13 substances as a category is adequately supported, it is likely that the range of acute toxicity in the available data from ten of 13 substances would include the three substances not tested.

Genetic Toxicity

EPA questions the column headings used for genotoxicity because the main issue is assessing possible mutations to the gene or the chromosome; not whether the tests were *in vitro* versus *in vivo*. If one re-organizes the data accordingly, the majority of the data are negative and cover seven of the 13 substances. Without establishing some sort of relationship (structural, metabolic, etc.) between the chemicals with data and those without, EPA fails to see the logic in the matrix array presented and how the genotoxicity data would be used in a category approach.

Repeat Dose Toxicity

The limited oral and dermal subchronic administration of the tested members of the proposed category are not considered acceptable for a category approach for the following reasons:

1. Two studies (linalool and linalyl acetate) are 5-day immunotoxicity screens that do not meet the SIDS endpoint for repeat dose toxicity.

2. The 67-week carcinogenicity study of linalyl acetate has limitations because it used only one dose and did not appear to assess any other endpoint except appearance of tumors.

3. Three studies were conducted with test substances that are not category members: a 12 week study with a mixture of linalool and citronellol; an 18 week study with linalyl isobutyrate; and another 12-week study with a test material that was a mixture of linalyl isobutryate and geranyl acetate. Importantly, the two 12-week studies were conducted to assess the effects of the test substances on food utilization.

4. One 20-week study and two 28-day studies (one range-finding and one definitive test) were performed with category members (alpha-terpineol acetate and pinanol thermal rearrangement products, respectively) and the study summaries appear adequate. However, these studies alone may not address all other category members in terms of systemic toxicity. For example, the pinanol thermal rearrangement products study reports a NOAEL of 15 mg/kg/day with effects (liver and kidney weight increases with associated pathological changes) at the higher doses of 150 and 1000 mg/kg/d. No such effects were observed in the 20-week study (up to 500 mg/kg/d estimated dose, according to the Test Plan) or in the three studies identified above at doses as high as 50 mg/kg/d of each substance in the linalool/citronellol study; 48 mg/kg/d (linalyl isobutryate); and 500 mg/kg/d (acetate mixture).

Reproductive Toxicity

The two reproductive toxicity studies in the submission are not considered acceptable for a category approach for the following reasons:

1. Neither protocol included an evaluation of male reproductive performance (only females were treated with the test substance). Although this may be considered acceptable if there is an adequate repeat dose study of at least 90 days' duration in which male reproductive organs were evaluated (see OECD SIDS guidance), given the repeat dose data supplied this appears not to be the case.

2. The "linalool" study in fact used coriander oil (only 73% linalool).

3. The "alpha-terpineol acetate" study in fact used cardamom oil (65% alpha-terpineol acetate).

Given the difficulties with the category justification, repeat dose studies, and the study protocols, it is not appropriate, from a category perspective, to use these studies as "anchors" in a reproductive toxicity category analysis for 13 substances.

Developmental Toxicity

The single study submitted for this endpoint did use a proposed category member, pine oil (though the study summary is deficient; see below). However, using only one study or testing one member of a group of 13 substances has several problems: (1) in this case, the positive outcome would be "applied" (read-across) to all category members; and (2) strictly speaking, even if there were considerable similarities in structure, metabolism, and toxicity, using only one "anchor" for a 13-member category is usually not appropriate.

Ecological Effects.

The test plan says little about how the chemicals in the category are tied together for ecological effects. For example, insufficient explanation is provided why two acetate esters, a hydroperoxide, and mixtures of substances should behave similarly toxicologically to the alcohols in the proposed category. The test plan also lacks a discussion of why SARs were used to predict ecological effects for some of the chemicals but not others. The robust summaries for measured and predicted ecological toxicity end points were insufficient to determine data adequacy for several reasons (see Robust Summary Comments section). If supplied, this information can help EPA to determine the adequacy of the test plan to satisfy the hazard screening needs of this proposed category.

The test plan cites an *in vitro* assay in Section 2.6 showing that the enzyme carboxylesterase in the gut of fish hydrolyzes acetate esters in minutes, and implies that the primary chemical exposure for fish is thus the hydrolyzed alcohols. EPA believes that exposure to the hydrolysis product is not the most dominant route of exposure for acetate esters. In fact, it is likely that ester exposure and toxicity will occur first via the gills and elicit intrinsic toxicity in fish which may overshadow secondary effects. In addition, it is not known if this enzyme is present in invertebrates, and is likely not present in algae. Furthermore, the test plan states that under environmental conditions the ester hydrolysis half-life is over 23 days, reducing the likelihood of exposure to hydrolysis products. Therefore, all base set testing of the esters is necessary to characterize the potential hazard to aquatic organisms.

The sponsor needs to explain its selection of representative test substances for these effects and why they are sufficient to characterize the thirteen substances, particularly when 2-pinanol thermal rearrangement product components, 2-pinanol hydroperoxide, and one of the acetate esters appear to be structural outliers in the proposed category. For the alcohols, the sponsor may consider basing test substance selection on the most and the least hydrophobic members.

The sponsor should explain why none of the thirteen substances is recommended for testing in all three species (fish, daphnid, algae). Although the sponsor recommended testing linalyl acetate for daphnid and algae on the basis of predicted SAR values, the test plan does not explain why fish testing is not planned as well.

Specific ecotoxicity testing proposals

Fish. The submitter recommends testing linalool only, using OECD Guideline 203. Linalool has the lowest ECOSAR-estimated LC_{50} and is an excellent candidate for testing. However, no testing has been performed on either 2-pinanol thermal rearrangement product components or 2-pinanol hydroperoxide. Since both of these represent potential outliers (complex mixture and hydroperoxide), the sponsor needs to explain the rationale for including these chemicals as category members.

Aquatic Invertebrates. The submitter recommends testing for linalyl acetate only, on the basis of the low estimated EC_{50}; however, ECOSAR also predicts a low estimated EC_{50} for " -terpinyl acetate, and no estimates were provided for seven of the 13 substances. Given the complexity and structural differences of this group, estimated EC_{50} values using estimated log P values should be obtained for all members of this category, or an explanation provided as to why not. The sponsor needs to explain why other chemicals and species are not considered for testing.

Aquatic Plants. The submitter suggested that linalyl acetate and 2-pinanol be tested using OECD guideline 201; however, given the paucity of information and the types of substances in the category, it would be better to

provide experimental data for pine oil and 2-pinanol thermal rearrangement products as well as the suggested substances. In addition, ECOSAR values should be developed for all the members of the category.

Specific Comments on the Robust Summaries

Environmental Fate (Photodegradation, Stability in Water, Biodegradation, Fugacity)

Fugacity

In all the fugacity estimations the submitter used the Level I Fugacity-based Environmental Equilibrium Partitioning Model. EPA recommends using the EQC Level III model, which is more realistic and useful for estimating a chemical's fate in the environment. In order to develop the Level III fugacity model, EPA recommends using the EQC Level III model from the Canadian Environment Modeling Centre at Trent University, which allows full control of data inputs. This model can be found at the following web address: http://www.trentu.ca/academic/aminss/envmodel

Health Effects

In general, the robust summaries presented the information necessary to understand the study design and results. However, the robust summaries would benefit from additional information as discussed below. Inclusion of this information in the robust summaries would facilitate interpretation of the testing methodology used to evaluate the endpoints. In addition, it would be useful to organize the robust summaries by endpoint (*e.g.*, group all acute oral studies together).

Repeat Dose Toxicity

Co-carcinogenicity study with linalyl acetate. This robust summary is inadequate for the repeat dose toxicity endpoint for the following reasons: (1) only one dose was used; and (2) it does not appear to assess any other endpoint except appearance of tumors.

The 12-week studies with (a) a mixture of linalool and citronellol and (b) linalyl isobutryate and geranyl acetate. These robust summaries are inadequate because there is no documentation of the relevance of the study design to systemic toxicity. The studies were conducted primarily to assess the effects of the test substances on food utilization and the primary endpoints assessed were urinary output of sugar and albumin.

Reproductive Toxicity

The two (coriander oil and cardamom oil) studies presented used unusual protocols (not specifically identified) and contain useful information on developmental toxicity. However, both robust summaries are considered deficient because they did not provide the incidence by dose for the effects (maternal and developmental) observed.

In addition, the developmental toxicity information should be presented in that section of the Test Plan and in separate developmental toxicity robust summaries.

Developmental Toxicity

The two study summaries submitted include a dose-range finding study and the definitive study with pine oil. The definitive study is more important for this endpoint because all appropriate parameters were assessed as opposed to an abbreviated protocol as was the (apparent) case for the dose-range finding study. Therefore, the comment that the robust summary is deficient because it did not provide the incidence by dose for the effects observed is confined to the definitive study summary.

Environmental Effects/Ecotoxicity Studies

Robust summaries were submitted for studies on fish, daphnia, and green algae. Many critical experimental details were omitted from most of the robust summaries, which effectively rendered many of them inadequate.

A number of ECOSAR estimations were included in the robust summaries. These also provided little detail on the data inputs; in some cases, inappropriate log Ps were used. The sponsor used measured log P values to predict toxicity instead of calculated log P values that the model used to develop the SAR regression equations. The use of SAR in this manner may give varying results, as illustrated by comparing the predicted and measured aquatic toxicity values submitted for certain chemicals. The sponsor needs to justify using measured log P as an input into the ECOSAR model. For linalool, the sponsor used an ECOSAR class based on allyl alcohols to predict a fish toxicity value of 0.6 mg/l. While the prediction would generally be appropriate for primary and secondary alcohols, it should not be extended to tertiary alcohols such as linalool. Therefore, the more appropriate predicted fish toxicity value for this chemical, which is based on the neutral organic (baseline toxicity) class, is 13.0 mg/L.

Some of the tests were performed on plinyl acetate as a surrogate for pinanol thermal rearrangement products. While pinanol thermal rearrangement products contain 47-50% plinol, other components include *trans*-2-pinanol (24%), linalool (7-9%), *cis*-"-terpineol (1-2%), *p*-menthan-1-ol (1-2%), "-fenchol (<4%), and others at less than 1% each (e.g., simple aliphatic alcohols, geraniol, myrcene, limonene, and "-pinene). ECOSAR estimates were provided for some of the components, but not for plinol, the major component.

Fish. Experimental and predicted 96-hour fish toxicity data were provided in the robust summaries. Many critical experimental details were missing from the summaries of measured aquatic data, including: pH; water hardness; DO; stability of the chemical; TOC; type and amount of solvent used; and temperature.

Aquatic Invertebrates. Both experimental and estimated data were presented in the robust summaries for the tertiary terpenoid alcohols. Many critical experimental details were missing from the measured aquatic data submitted to included: pH; water hardness; DO; stability of the chemical; TOC; type and amount of solvent used; and temperature.

Aquatic Plants. Both experimental and estimated data were presented in the robust summaries for the terpenoid tertiary alcohols and esters. Critical experimental details missing from the measured aquatic data summaries included: pH; water hardness; DO; stability of the chemical; TOC; type and amount of solvent used; and temperature.

Followup Activity

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