December 20, 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 Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Phenethyl alcohol submission posted on the ChemRTK HPV Challenge Program Web site on August 22, 2002. I commend the Aromatic Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC) 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 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 FFHPVC 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 Hefter, 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 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

Enclosure

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

**EPA Comments on Chemical RTK HPV Challenge Submission:**
**Phenethyl Alcohol**

## SUMMARY OF EPA COMMENTS

The sponsor, the Flavor and Fragrance High Production Volume Consortia, submitted a test plan and robust summaries to EPA on August 2, 2002, for phenethyl alcohol (CAS No. 60-12-8). EPA posted the submission on the ChemRTK HPV Challenge Web site on August 22, 2002.

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

1. Physicochemical Properties and Environmental Fate. All appropriate SIDS-level endpoints have been addressed for the purposes of the HPV Challenge Program.

2. Health Effects. The data for acute, repeated-dose, and developmental toxicity are adequate for the purposes of the HPV Challenge Program. The submitter needs to either expand the robust summaries of the genotoxicity studies or provide additional data for both endpoints (gene mutations and chromosomal aberrations). EPA reserves judgement on the reproductive toxicity endpoint pending either additional information on the metabolism of this compound or the results of the 90-day histopathological examination of reproductive organs.

3. Ecological Effects. All appropriate SIDS-level endpoints have been addressed for the purposes of the HPV Challenge Program. However, the submitter needs to provide more detailed data elements for fish, aquatic invertebrates, and algae in robust summaries.

EPA requests that the submitter advise the Agency within 60 days of any modifications to this submission.

## EPA COMMENTS ON THE PHENETHYL ALCOHOL CHALLENGE SUBMISSION

### Test Plan

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

All appropriate SIDS-level endpoints have been addressed for the purposes of the HPV Challenge Program.

*Water Solubility.* The calculated water solubility value needs to be corrected to read 32,720 mg/L.

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

*Stability in water*. The submitter needs to state clearly in the test plan as well as the robust summary the rationale for not testing the hydrolysis of phenethyl alcohol. Phenethyl alcohol does not have a functional group that is susceptible to hydrolysis and so hydrolysis is not expected to occur in the environment.

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

The data for acute, repeated-dose, and developmental toxicity are adequate for the purposes of the HPV Challenge Program. The submitted data for genotoxicity may be inadequate (questions are raised below for clarification). EPA reserves judgement on the reproductive toxicity endpoint pending receipt of additional information on the metabolism of this compound or the results of the 90-day histopathological examination of reproductive organs.

*Genotoxicity (gene mutations)*. Four robust summaries were submitted, but none of those assays were considered adequate. These summaries were for a reverse mutation assay in bacteria (Ames test) using phenethyl alcohol, an unscheduled DNA synthesis assay using phenylacetic acid in primary rat hepatocytes, and two sex-linked recessive lethal mutation assays in *Drosophila melanogaster* (using phenyacetic acid ester, isoeugenol phenylacetate and 2-methyl phenacetaldehyde).

In the Ames test, the test concentration of 366 µg/plate is only a small fraction of the 5000 µg/plate specified in OECD Guideline 471 as the limit concentration in the absence of precipitate and cytotoxicity; the robust summary made no mention of a precipitate or cytotoxicity.

In the tests conducted in *Drosophila*, there was no evidence of cytotoxicity at the maximum concentrations used. Also, isoeugenol phenylacetate may not be an appropriate surrogate because the chemical structure is too different from the chemicals in the phenethyl alcohol metabolic series. In particular, isoeugenol phenylacetate may not hydrolyze rapidly and therefore, the toxicological properties of this chemical's metabolites may differ from the metabolites of phenethyl alcohol.

Additional information in the Ames test or the *Drosophila* test using 2-methyl phenacetaldehyde needs to be provided to indicate that the cytoxicity issue has been addressed. Although well-conducted mutation assays in *Drosophila* using appropriate chemicals are also acceptable to meet this endpoint, the bacterial reverse mutation test in *Salmonella typhimurium* is preferred.

*Genotoxicity (chromosomal aberrations)*. Three robust summaries were submitted, but none of those assays were considered adequate. There was one *in vitro* sister chromatid exchange assay of phenethyl alcohol (in human lymphocytes) and there were two mouse micronucleus tests using metabolites or surrogate chemicals.

The *in vitro* study had the following flaws: (1) no information on whether sister chromatid exchanges (SCEs) were the only effects measured (and no other chromosomal aberration as per OECD guideline studies); and (2) no metabolic activation in the human cultures.

Both micronucleus assays also had several shortcomings. The doses are too low to provide an adequate test of the potential to induce chromosomal aberrations. However, there was no discussion in the robust summary of whether doses were reduced because of cytoxicity. Furthermore, in the test on 2-methyl phenacetaldehyde, samples of bone marrow were taken at only one time (instead of 3 different times) during the proper sampling interval. The other test was conducted using the phenylacetic acid ester isoeugenol phenylacetate, which is not an appropriate surrogate. The submitter needs to provide appropriate information from the micronucleus study using 2-methyl phenacetaldehyde or supply data from another study to satisfy the chromosomal aberrations endpoint for the purposes of the HPV Challenge program.

*Reproductive toxicity*. A single study (a reproductive/developmental toxicity screening study in the rat) was submitted using the metabolite phenylacetic acid. EPA agrees with the submitter based on the data provided that phenethyl alcohol will be at least partly metabolized to phenylacetic acid. However, in the single metabolism study in humans, only 26 percent of a 4,000 mg oral dose of phenylethyl alcohol was excreted in the urine after 24 hours as the glutamine conjugate of phenylacetic acid. Although a higher percentage of the acid may have been excreted if a lower dose was used in this study, it is difficult to draw

definitive conclusions about the metabolism of this chemical from the available data. Also, animal data indicate variable rates of acid excretion.

The submitter is encouraged to further address this endpoint by submitting additional metabolic information, as well as results of the histopathological examination of the reproductive organs from the 90-day study on phenethyl alcohol by Owston et al. (1981).

Ecological Effects (fish, invertebrate and algal toxicity)

The endpoints for fish, aquatic invertebrates, and algae have been adequately addressed for the purposes of the HPV Challenge Program. The submitter needs to provide additional information in the robust summaries.

**Specific Comments on Robust Summaries**

In general, the submitter should include test guideline information or methodology where possible.

Physicochemical Properties

*Water Solubility.* The calculated water solubility value needs to be corrected to read 32,720 mg/L.

Environmental Fate and Transport

The submitter needs to add the missing stability in water section to the robust summary.

*Fugacity*. The submitter needs to provide the values of the input parameters for the fugacity calculations.

Health Effects

*Acute toxicity*: For the three key studies: 1) for the 1982 dermal study, the submitter needs to provide information on the sex of the test animals and additional details on the test conditions (e.g., rat strain) and results (number of deaths at each dose); 2) for the 1982 oral study, the submitter needs to provide information on the purity of the test material, the age of the animals if different from the guidelines, and necropsy results; and 3) for the 1983 dermal study, the submitter needs to provide information on test substance purity, animal age, and necropsy data.

*Repeated-dose toxicity.* In the 90-day dermal study, the submitter needs to provide information on the number of animals per test group, the weight and age of the animals, analytical and statistical methods used, and more quantitative results if available.

*Genotoxicity (gene mutations).* Details missing or inadequate in the robust summary for the Ames test included: (1) use of negative, solvent, or positive controls, (2) use of only one replicate instead of the recommended 3 replicates, and (3) only one concentration tested (which was too low). In the robust summaries for both sex-linked recessive lethal *Drosophila melanogaster* tests, there is no indication that the tests were conducted up to a cytotoxic concentration.

*Genotoxicity (chromosomal aberrations).* The robust summaries for both micronucleus studies were considered inadequate because there was no indication that the highest dose was limited by toxicity and it was lower than the limit dose of 5000 mg/kg. Other deficiencies noted in the study on 2-methyl phenacetaldehyde included: (1) samples were taken at only one time (instead of 3 times) between 12 and

72 hrs; (2) the numbers of males and females per group were not specified; (3) there is no indication that a positive control was used; and (4) no information was reported on the ratio of polychromatic cells to normochromatic cells.

*Reproductive toxicity*. The submitter needs to provide a reproductive toxicity robust summary using data from the 90-day dermal study on phenethyl alcohol.

For Vollmuth et al. (1995), the robust summary and test plan both indicate that the NOAEL is the lowest dose of 250 mg/kg. However, the test plan states that there was a decrease in the mating index at the mid-dose, whereas the robust summary states that the decrease only occurred at the highest dose. The submitter needs to resolve this discrepancy. In the robust summary under "Parental data...", the value of 50 should be changed to 500. The submitter should provide more quantitative data for all reproductive effects.

*Developmental toxicity.* The critical study (Palmer et al., 1986) was conducted under GLP according to a modified OECD TG 414. In the robust summary there is a heading "Actual doses received..." followed by a dose level of 430 mg/kg. This heading is used to describe all doses received by the test animals. The submitter should provide additional quantitative data for all developmental effects.

An apparent error in the test plan regarding the Palmer et al. study should be corrected. Specifically, the last two sentences on p. 18 are conflicting. The first sentence states that 0.14 mL/kg was without effects (this matches the robust summary discussion) but the next sentence notes slight differences in effects between this dose and the controls. It appears that the second sentence actually refers to the 0.43 mL/kg dose level (which would agree with the previously stated results and the robust summary). If so, the value in the second sentence should be changed to 0.43 mL/kg.

In the description of the results for the Mankes et al. (1983) study, both the robust summary and the test plan state that birth weights were lower in all treated groups but that the weights were greater in the mid-dose group than the controls. The submitter needs to resolve this discrepancy. Also, the developmental LOAEL is incorrectly given as 4300 mg/kg; this should be 430 mg/kg.

Ecological Effects (fish, invertebrates, and algae).

The submitter needs to provide the following information: pH, dissolved oxygen, and water temperature; age of the testing organisms at test initiation; statistical analyses used; 95% confidence intervals; control mortality; composition of the algal medium used for this test; purity of the test substance; light intensity and quality; initial cell concentration; and growth rate of the control culture.

**Followup Activity**

EPA requests that the submitter advise the Agency within 60 days of any modification to its submission.