

December 31, 2003

Timothy Adams, Ph.D.
Technical Contact
The Flavor and Fragrance High Production Volume Consortia
The Cyclohexyl Derivatives Consortia
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Dear Dr. Adams:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for Cyclohexanol Derivatives Category posted on the ChemRTK HPV Challenge Program Web site on August 28, 2003. I commend The Flavor and Fragrance High Production Volume Consortia, The Cyclohexyl Derivatives 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 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 Consortia advise the Agency, within 60 days of this posting on the Web site, of any modifications to its submission. Please send any electronic revisions or comments to the following e-mail addresses: oppt.ncic@epa.gov and chem.rtk@epa.gov.

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: W. Penberthy
M. E. Weber

EPA Comments on Chemical RTK HPV Challenge Submission: Alkyl-substituted Cyclohexanol Derivatives Category

Summary of EPA Comments

The sponsors, Cyclohexyl Derivatives Consortium and the Flavor and Fragrance High Production Volume Cyclohexyl Derivatives Consortium, submitted a test plan and robust summaries to EPA for the alkyl-substituted cyclohexanol derivatives category dated August 21, 2003. EPA posted the submission on the ChemRTK HPV Challenge Website on August 28, 2003. The category consists of two sponsored compounds: 4-*tert*-butylcyclohexanol (CAS No. 98-52-2) and 4-*tert*-butylcyclohexyl acetate (CAS No. 32210-23-4). The submission also includes data on analogs.

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

1. Category Justification. EPA believes that 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate may be reasonably grouped into a single category based on structural similarity; however, some health effects data on various analogs do not fully support the category. Most of the data are developed either on 2-isopropyl-5-methylcyclohexanol or a mixture of seven analogs. Based on the significant difference in toxicity and potency of 4-*tert*-butylcyclohexanol, 2-isopropyl-5-methylcyclohexanol does not appear to be an adequate analog for 4-*tert*-butylcyclohexanol.
2. Physicochemical Properties. The submitter needs to provide measured values for boiling point, vapor pressure and water solubility for 4-*tert*-butylcyclohexanol and for 4-*tert*-butylcyclohexyl acetate.
3. Environmental Fate. The data for photodegradation, stability in water, and biodegradation are adequate for the purposes of the HPV Challenge Program. The submitter may need to run the fugacity model again if measured physicochemical data are developed.
4. Health Effects. The submitted data for 4-*tert*-butylcyclohexanol are adequate for acute, repeated-dose, and genetic toxicity endpoints for the purposes of the HPV Challenge Program. The submitted data for reproductive and developmental toxicity on the analog 2-isopropyl-5-methylcyclohexanol and the seven analog mixture do not adequately address these endpoints. A combined reproductive/developmental toxicity screening test is needed. The submitter needs to address deficiencies in the robust summaries.
5. Ecological Effects. The submitted data are adequate for the daphnia and green algae toxicity endpoints for the purposes of the HPV Challenge Program. EPA reserves judgement on the adequacy of the fish toxicity data pending submission of critical missing data elements in the robust summary. If the information is not available, the submitter needs to perform an acute fish toxicity test.

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

EPA Comments on the Alkyl-substituted Cyclohexanol Derivatives Challenge Submission

Category Definition

The submitter proposed a category consisting of 4-*tert*-butylcyclohexanol (CAS No. 98-52-2) and its ester, 4-*tert*-butylcyclohexyl acetate (CAS No. 32210-23-4). Both substances exist in *cis* and *trans* forms. The alcohol serves as a synthetic precursor of the acetate and variation in the ratio of the *cis* and *trans* isomers does not significantly alter the physical properties. The test plan also includes supporting data on the following seven alkyl-substituted cyclohexanol and cyclohexanone compounds:

- (1) 2-isopropyl-5-methylcyclohexanol (CAS No. 1490-04-6);
- (2) 2-*tert*-butylcyclohexanone (CAS No. 1728-46-7);
- (3) 3-*tert*-butylcyclohexanone;
- (4) 4-*tert*-butylcyclohexanone (CAS No. 98-53-3);
- (5) 2-methylcyclohexanone (CAS No. 583-60-8);
- (6) 3-methylcyclohexanone (CAS No. 591-24-2); and
- (7) 4-methylcyclohexanone (CAS No. 589-92-4).

The submitter should have included CAS registry numbers for the supporting compounds in the test plan; EPA located CAS registry numbers for 6 of the 7 compounds.

Category Justification

The submitter supports grouping the category members based on a common use (soap perfumes) and common metabolic pathways in mammalian species. The submitter provided experimental data or estimated values for all physicochemical, environmental fate, and ecotoxicity endpoints but does not use these data to support the category. To support the common metabolism, the submitter reported that the ester undergoes rapid hydrolysis to the alcohol in *in vitro* and *in vivo* studies. In addition, the submitter provides further evidence to show that after administration of either the ester or alcohol in animal models, elimination of these two chemicals occurs through common pathways and intermediates, including the formation of glucuronic acid conjugates of cyclohexanol. The sponsor also described study data to demonstrate common metabolic and elimination pathways for related compounds such as 4-*tert*-butylcyclohexanone, isomers of *tert*-butylcyclohexanol, and 2-isopropyl-5-methylcyclohexanol, which are used as analogs for compounds in the category. In the case of 4-*tert*-butylcyclohexanone, the submitter states that the ketone and 4-*tert*-butylcyclohexanol are interconvertible *in vivo*. Therefore, based on common chemical structures (an alkylated cyclohexanol) and metabolic pathways, the submitter expects to find similar toxicological properties for the sponsored and analog compounds.

Analog Justification

EPA believes that 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate may be reasonably grouped into a single category based on structural similarity; however, some health effects data on various analogs do not fully support the category based on the following:

1. *The isomer, 2-isopropyl-5-methylcyclohexanol shows different toxicity than 4-tert-butylcyclohexanol.* The 28-day repeated-dose toxicity data for 4-*tert*-butylcyclohexanol show significant toxic effects in rats different than those for the analog, 2-isopropyl-5-methylcyclohexanol. These effects include severe neurotoxic signs such as convulsions, ataxia, fasciculation, aggressiveness, hunched posture, hypoactivity, etc. In addition, relative weights of epididymis were increased at all dose levels. These effects were not seen in the other 28-day repeated-dose toxicity study conducted on the mixture of seven components or in the 90-day repeated-dose toxicity study with 2-isopropyl-5-methylcyclohexanol. There is also a significant difference in potency of these two chemicals; 2-isopropyl-5-methylcyclohexanol being less potent (NOAELs of 750 and 1125 mg/kg/day for rats and mice, respectively) than 4-*tert*-butylcyclohexanol (NOAEL of 50 mg/kg/day or less based on the changes in epididymis weights). Therefore, it is questionable whether 2-isopropyl-5-methylcyclohexanol is an appropriate analog for 4-*tert*-butylcyclohexanol and to extrapolate less toxic analog data to the more toxic sponsored chemicals.
2. *Reproductive and developmental toxicity endpoints.* Data provided for reproductive toxicity with the mixture of seven analogs was conducted using only dosed females. The two dominant lethal assays in males (acute and subacute doses) with 2-isopropyl-5-methylcyclohexanol may not be appropriate for

reasons outlined above. In all four developmental toxicity assays with 2-isopropyl-5-methylcyclohexanol, no maternal toxicity was evident at the highest tested doses which were significantly lower than the guideline recommended dose of 1000 mg/kg/day.

Overall, 2-isopropyl-5-methylcyclohexanol does not appear to be an adequate analog for 4-*tert*-butylcyclohexanol. In addition, most of the data are developed on either a mixture of seven analogs or on 2-isopropyl-5-methylcyclohexanol and do not support the SIDS endpoints for the sponsored chemicals. EPA recommends that the submitter conduct a combined reproductive/developmental toxicity screening test (OECD TG 421) on 4-*tert*-butylcyclohexanol to address these endpoints.

Test Plan

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

Boiling point. It is unclear whether some of the submitted data are measured or calculated. For the purposes of the HPV Challenge Program, boiling point values under 300 °C need to be measured unless precluded by experimental obstacles.

*Vapor pressure for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate.* It is unclear whether the vapor pressure values of less than 0.1 hPa for 4-*tert*-butylcyclohexanol and 0.01 hPa for 4-*tert*-butylcyclohexyl acetate from an unpublished report are measured or calculated. The HPV Challenge Program specifies testing for substances with calculated vapor pressures above a cut-off value of 1×10^{-5} Pa (7.5×10^{-8} mm Hg) and if the submitted data are calculated, measured data following OECD TG 104 are needed. In addition, for 4-*tert*-butylcyclohexanol, there is a discrepancy between the value reported in the test plan (less than 0.1 kPa at 0.75 mm Hg) and that provided in the robust summary (0.1 hPa).

*Water solubility for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate.* It is unclear whether values of less than 100 mg/L at 20 °C for 4-*tert*-butylcyclohexanol and ca. 90 mg/L at 20 °C for 4-*tert*-butylcyclohexyl acetate from an unpublished report are measured or calculated. The HPV Challenge Program specifies testing for substances with calculated water solubilities greater than 1 µg/L. If these values are calculated, the submitter needs to provide measured water solubility data for both of these chemicals following OECD TG 105.

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

The data provided by the submitter for photodegradation, stability in water, and biodegradation are adequate for the purposes of the HPV Challenge Program.

Fugacity. The submitter may need to run the fugacity model again if measured physicochemical data are developed as discussed above under Physicochemical Properties.

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

The submitted data for 4-*tert*-butylcyclohexanol are adequate for acute, repeated-dose, and genetic toxicity endpoints for the purposes of the HPV Challenge Program.

Reproductive/Developmental Toxicity. The submitted data are for the analog 2-isopropyl-5-methylcyclohexanol or for the analog mixture and EPA does not believe that these are appropriate analogs for the sponsored substances (see analog justification). Consequently, EPA recommends that the submitter conduct a combined reproductive/developmental toxicity screening test (OECD TG 421) on 4-*tert*-butylcyclohexanol to address these endpoints.

Ecological Effects (fish, invertebrates, and algae).

The submitted data are adequate for the daphnia and algae toxicity endpoints for the purposes of the HPV Challenge Program. EPA reserves judgement on the adequacy of the 4-*tert*-butylcyclohexyl acetate *Cyprinus carpio* study pending submission of critical missing data elements in the robust summary. If the specified information is not available, the submitter needs to perform a fish toxicity test according to the OECD guideline to adequately address this endpoint. (The two studies of 4-*tert*-butylcyclohexanol, submitted on the Golden orfe are inadequate to address the fish toxicity endpoint.)

Specific Comments on the Robust Summaries

Health Effects

None of the summaries listed the purity of the test material and none of the summaries for studies on analogs provided the CAS registry number of the analog.

Acute Toxicity. Robust summaries for acute oral toxicity studies (one for 4-*tert*-butylcyclohexanol and three for 4-*tert*-butylcyclohexyl acetate) in rats were missing the following information: doses administered, length of the observation period, gavage vehicle (if used), sex and strain of rat, group size, results for mortality by sex and dose, and method for calculating the LD₅₀. The summary for the study by Opdyke (1976) mis-stated the LD₅₀ in the Value field as *less than 5 but greater than 500 mg/kg*; this should probably read *less than 5 g/kg but greater than 500 mg/kg*.

Genetic Toxicity. Robust summaries were missing the following information: all of the tested concentrations (a range was given), and the criteria for a positive result. The Remarks fields included a statement on the number of metaphases analyzed, which is irrelevant to prokaryotic systems and should be deleted.

Ecological Effects

Fish. Information missing from the robust summary of the studies of 4-*tert*-butylcyclohexyl acetate in *Cyprinus carpio* includes test substance purity, control use/response, water hardness, test temperature, organism specifications, mortality at each concentration, and statistical methods used.

Invertebrates. The submitter needs to identify a key study for this endpoint and provide the following missing information in the robust summaries: the number of daphnids, concentrations tested, control use/response, effects (percentage immobilization) at each concentration, temperature, and statistical methods used. In addition, in the summaries of the studies on 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate, it was not clear whether the pH and the dissolved oxygen ranges were for the entire test duration, and whether the dissolved oxygen values represented at least 60% of air saturation at test temperature.

Algae. Information missing from one or more of the summaries includes water quality characteristics (pH and test temperature), light intensity and quality, details about cell density/inhibition of cell growth at all concentrations tested, control use/response, statistical methods used, and whether or not the EC₅₀ values were based on nominal or measured concentrations. The submitter needs to provide this missing information.

Followup Activity

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