

201-15504

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

**1620 I Street, N.W.  
Suite 925  
Washington D.C. 20006  
Tel. (202)-293-5800 Fax (202)-463-8998**

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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

August 16, 2004

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 the Chemical Category "Ionone Derivatives". The Terpene Consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for terpenoid substances under the Chemical Right-to-Know Program. Since 1999, the twenty-one (21) 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 for each of the sponsored chemicals, and conducted additional testing.

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 the Chemical Category, "Ionone Derivatives". The revised test plan and robust summaries contain the results of additional toxicity studies and additional physical properties information that addresses the questions and comments made by the EPA in its letter dated 8/15/2002. 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. New data includes:

- 1) Acute toxicity study in aquatic invertebrates [Ward, 2003a]
- 2) Acute toxicity study in aquatic plants [Ward, 2003b]
- 3) Calculated data on environmental fate using the EPIWIN Level III model [MacKay *et al.*, 1996]
- 4) Additional data for calculated melting point and photodegradation endpoints EPIWIN Level III model [MacKay *et al.*, 1996]
- 5) A comprehensive evaluation of the developmental toxicity studies for two ionone derivatives [Willhite *et al.*, 1986]

Based on this additional data, we conclude that the current test plan and robust summaries for this chemical category are now complete.

Based on this additional information, the Terpene Consortium concludes that the experimental and model data for physiochemical properties, environmental fate, ecotoxicity, and human health endpoints are consistent for the members of this chemical category. The database of information on category members permits one to reliably predict endpoint values for other untested members of the category. Therefore, these data support the inclusion of the two listed substances in the chemical category and would allow for other structurally related ionone derivatives to be included in the chemical category.

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 these substances. 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. The two ionone derivatives that constitute the members of this chemical category have been reviewed along with a group of 19 other ionone 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).

In 1998, ionone derivatives [WHO Food Additive Series: 42, 1999; see Revised Test Plan] were recognized as safe for use in food.

The substances in this category are also recognized as “Generally Recognized as Safe” (GRAS) for their 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 each of these substances in food are published in the Food Chemical Codex [FFC, 1996; see Revised Test Plan].

Based on the long history of use of these substances 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 these substances to the food supply, there is no compelling evidence that these substances 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

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

#### Category Definition

The submitter has proposed a category covering methylionone (mixed isomers, CAS No. 1335-46-2) and alpha-iso-methylionone (CAS No. 127-51-5). Ionone, the parent terpene, occurs in nature as three isomers (alpha, beta, and gamma) that differ in the position of one double bond. Methylionone has the same double bond isomers as ionone and a methyl group at the terminal position of the butanone side chain. There are contradictory descriptions of this mixture in the test plan. It is identified as both methyl- and isomethylionone isomers in the "identity of substances" section (page 1) but the "structural classification" section (page 6) states that the mixture contains only the three isomers of methylionone. EPA assumes the latter statement to be correct but recommends the ambiguity be addressed.

**Methylionone (mixture of isomers) is a mixture of alpha, beta, and gamma isomers in which the double bond is located at 2- (alpha), 1- (beta) and exocyclic 2-methylene (gamma) positions on the the cyclohexane ring.**

In addition, *α-iso-methylionone* is a mixture of isomers including alpha, beta, and gamma isomers of both *iso-methylionone* (methyl group substituted at the 2-position of the 1-buten-3-one side chain) and *n-methylionone* (methyl group substituted at the terminal 4 position of the 1-buten-3-one):

**The identity of all isomers have been included on Page 1 and throughout the rest of the test plan where appropriate.**

Iso-methylionone has the same double bond isomers as ionone and a methyl group at the 2-position of the butanone side chain; Only the alpha isomer is a category member.

**The name was assigned to the principal isomer (see above), although, in reality, the product of commerce contains both *iso-* and *n-methylionone* isomers:**

*alpha-iso-Methylionone* (55-65%)

*beta-iso-Methylionone* (4-7%)

*alpha-n-Methylionone* (22-32%)

*beta-n-Methylionone* (1-5%)

*gamma-n-Methylionone* (1-5%)

The structure presented for *alpha-ionone* in the test plan is identical with that presented for *beta-ionone* and all its metabolites in Figure 1. The error needs correction.

**According to CAS, the structure listed on page 1 for  $\beta$ -ionone is actually  $\alpha$ -ionone and vice versa. This has been corrected. Figure 1 is correct. The structures in Sections 1 and 2.2 have been switched to make the names correspond to the correct structures.**

## Category Justification

The submitter bases the category on similar chemical composition and metabolism. Chemically, the only structural difference between alpha-iso-methylionone and the methylionone mixture is that alpha-iso-methylionone contains an additional methyl group at the 2-position of alpha-ionone while methylionone contains an additional methyl group at the 4-position of alpha-ionone. Orally administered ionones are absorbed and metabolized in mammals by allylic hydroxylation of the ring followed by oxidation of the hydroxyl group to 3-oxo derivatives. Reduction of the ketone function to the corresponding secondary alcohol also occurs. Combinations of these detoxication reactions result in the formation of multiple polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acids. The metabolism of ionones is expected to be similar in humans. This is supported by human metabolism studies of retinoids and carotenoids that possess ionone fragments.

Although the submitter did not summarize the existing data on the category members and the parent ionones, the data are reasonably concordant which strengthens the justification for the proposed category. Based on the close structural similarity and expected similar metabolism, EPA agrees that the existing testing can be reasonably extrapolated to the category members for health. EPA believes that more testing is needed to determine if the same holds true for ecological effects.

As discussed below (see Test Plan comments), the submitter has not clearly explained how the data on the parent terpene ionone and other isomers are being used to support the proposed category.

A related concern is that the test plan refers to "pseudoionones" without providing structural information other than that they are open-chain synthetic precursors to ionones. This is significant because in the robust summaries data on pseudoionones are presented as analog data for category members, but without justification for an analogy between the two rather different structures. Similarly, data on "delta-methylionone" appear in the robust summaries, but the structure and reason for its inclusion are lacking.

**The structure for delta-methylionone contains the ring double bond at the 3-position of the cyclohexane ring. This structure is structurally related to the other three *n*-methylionone isomers and data related to this substance is considered relevant to the ionone derivatives.**

**Although pseudoionone is the open chain version of ionone (see Test Plan), it is an  $\alpha,\beta$ -unsaturated methyl ketone (the chemically and biochemically most reactive part of the molecule) of the same molecular weight and similar calculated bp, water solubility, log Kow, and aquatic toxicity as the three methylionone isomers as well as  $\alpha$ -iso-methylionone. Therefore, it could be expected to exhibit similar biological activities. The developmental toxicity data support such a conclusion. Data related to this substance is also considered relevant to the hazard assessment for the ionone derivatives. The discussion of the structural relationship between the members of the category and non-category members is discussed in the revised test plan.**

## Test Plan

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

EPA agrees with the submitter that no further testing is necessary for boiling point, vapor pressure and log Kow.

*Melting point.* Although the chemical is a liquid at room temperature, the melting point should

be determined according to OECD Guideline 102. If the melting point is greater than 0 C, then it should be reported.

The calculated melting points indicate that both substances are solids at room temperature. That they are liquid indicates they are mixtures. This is stated for methylionone and  $\alpha$ -iso-methylionone. If so, the melting points of both substances would be broad and not be useful either in characterization or in the hazard evaluation.

**NOTE:** If the CAS no. 1335-46-2 is entered into the EPI suite, the wrong structure is retrieved. All calculated values throughout for methyl ionone are incorrect. Given the small molecular differences between all the ionone derivatives discussed in the test plan, it is concluded that the calculated melting point for any correct ionone structure represents the melting point of any of these isomers. The calculated melting point of the *alpha*- and *beta*-n-methylionone isomers has been included in the test plan and robust summaries.

*Water solubility.* Insufficient information was presented in the water solubility robust summary for the methyl ionone mixture; the measured value did not agree well with an estimated value or with a measured value for alpha-iso-methylionone.

The measured solubility for  $\alpha$ -iso-methylionone is 16 mg/L by OECD methodology. Based on the fact that the only difference in structure between the iso-methyl derivatives and the n-methyl derivatives is the position of a methyl group in a C<sub>13</sub> ketone, the reliable figure for water solubility would be expected for the n-methyl isomers. A measured solubility value was reported for one of the n-methylionone isomers. Also, the water solubility of *gamma*-methylionone was reported to be of 90 mg/L at a temperature of 25 °C. Based on the solubility of the two members of this chemical category at 20 °C and 25 °C, both mixtures are expected to be soluble in the range from approximately 15 to 25 mg/L. The calculated values for  $\alpha$ -,  $\beta$ - and  $\gamma$ -ionone are 10.3, 8.0 and 8.83 mg/L, respectively, in good agreement with the measured value for  $\alpha$ -iso-methylionone. This agreement gives confidence in the algorithm used for calculation. Given the consistency of measured and calculated values, no additional studies should be performed.

**NOTE:** If either the CAS no. 127-24-3 ( $\alpha$ ) or 14901-07-6 ( $\beta$ ) is entered into EPI, the same structure is retrieved, and that is for  $\beta$ -ionone. To do the calculations for  $\alpha$ -ionone, the smiles notation must be entered.

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

Adequate data are available for the biodegradation endpoint.

*Photodegradation.* The submitter supplied a calculated value for alpha-iso-methylionone and proposes to extrapolate it to methylionone. While the results of a calculation for methylionone are expected to be similar, EPA prefers that the calculation be provided rather than extrapolating from a calculated value.

**The calculated values for each of the *alpha* and *beta* isomers have been included in the robust summaries and test plan.**

*Stability in water.* On page 12 of the test plan the submitter states that hydrolysis is not possible for any of the members of the category. However, in the test plan table on page 21 a calculated value is indicated for alpha-iso-methylionone and a NA (not applicable) for methylionone. The robust summary shows calculated t<sub>1/2</sub> values from 9 to 169 hours. The submitter needs to address this inconsistency.

**The test plan states that ketones cannot hydrolyze. This is not chemically possible. However, the calculated figures quoted from the model are for  $\alpha$ -iso-methylionone and**

**the reason for the large difference is the 10-fold higher wind velocity and 20 fold higher current velocity for the river (9 hr) compared to the lake (169 hr). The corresponding half-lives for  $\alpha$ -,  $\beta$ - and  $\gamma$ -ionone are 5.9 and 181; 6.1 and 183; and 6.7 and 190 hr, respectively, in good agreement.**

*Fugacity.* The submitter 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 a level III analysis, which is more rigorous. The EQC and EPIWIN Level III models are acceptable.

**The EPIWIN Level III fugacity calculations have been performed and included in the revised robust summaries and test plan.**

Health Effects (*acute toxicity, repeat dose toxicity, genetic toxicity, and reproductive/developmental toxicity*).

Test data are available for these endpoints from studies using ionone, methylionone, alpha-iso-methylionone, and other related isomers. However, the submitter needs to clarify how the data on the non-category members are being used. For instance, in the test plan table on page 22, the submitter indicates that the reproductive/developmental endpoints will be addressed by the "category approach" i.e., read across from other test data. But in the test plan text and table this is not articulated. For the endpoints in question, data on non-category members are merely summarized. It is left to the reader to make the proper connections. The submitter needs to revise the test plan table to indicate where the non-category member analog data will be used or revise the category to include the ionones.

**Based on a lengthy discussion of the absorption, distribution, metabolism, and excretion of ionone derivatives, it is apparent that the toxicologic potential of any of the ionone, *n*-methylionone and *iso*-methylionone derivatives is similar. The toxicity data support this conclusion. However, the test plan has been revised to reflect how the data non-category members are being used for each endpoint ( see background Section 2.2).**

EPA considers the developmental toxicity data inadequate and recommends that the submitter conduct a developmental toxicity screening test (OECD TG 421) on one of the category members.

**See comment below.**

*Acute Toxicity.* The submitter provided summaries on several isomers; however, none is complete and lack critical information. Considering the weight-of-evidence, EPA considers this endpoint addressed for the purposes of the HPV Challenge Program. However, the submitter needs to provide the missing information in robust summaries.

**Whatever data was presented in the original article has been included in the robust summaries**

*Repeated-dose Toxicity.* Available data on alpha-iso-methylionone are not adequate for the purposes of HPV Challenge Program because the studies were conducted using only one dose level that was also a NOAEL.

**There are, however, data from repeated dose studies on structurally related ionone derivatives (alpha- and beta-ionone) and there is no reason to suggest that  $\alpha$ -iso-methylionone should be significantly more toxic than either *alpha*- or *beta*-ionone. The doses in this study were chosen to be several orders of magnitude higher than**

**exposures from flavour or fragrance use and therefore should serve, along with the data on analogues, as sufficient for the hazard assessment. Unless evidence exists that suggests this material is significantly more toxic than its analogues or that a significantly higher exposure may occur, further testing is not justified.**

*Reproductive toxicity.* The two generation reproductive toxicity study on methyl ionone (mixture of isomers) was not conducted according to the OECD guidelines and alone is not adequate to address the reproductive toxicity endpoint because only one dose level was tested that was also the NOAEL. However, examination of the sex organs in the available repeated-dose studies did not reveal any effects and taken together address this endpoint.

*Developmental Toxicity.* The developmental toxicity study is inadequate because pregnant females were dosed only once on the 8th day of pregnancy. There was no evidence of maternal or fetal toxicity at the highest dose tested. This endpoint has not been adequately addressed for the purposes of HPV Challenge Program.

**This study was an extensive SAR study that was developed to address retinoid-type teratogenicity reported in humans. The study (Willhite, 1985) is one of numerous studies designed to evaluate the well-recognized Vitamin A- induced teratogenic syndrome in humans leading to characteristic malformations and elevated levels of spontaneous abortions (Benke, 1984; Fernhoff and Iamer, 1984; Rosa, 1984; and Willhite et al., 1986).**

**It was determined that the malformation syndrome observed in humans could be reproduced in fetal hamsters by treating the dam with a high dose of retinoid on Day 8 of gestation (Willhite and Shealy, 1984; Willhite et al., 1984a, 1984b). This protocol evolved from the normal treatment regimen, because it induced malformations in retinoid-treated hamsters at a higher rate than did the normal 6 to 15 daily treatment at slightly lower dose levels. Using this single dose protocol, a series of studies (Willhite and Shealy, 1984; Willhite et al., 1984; Willhite and Balogh-Nair, 1984) were undertaken to investigate the effect of structural changes of Vitamin A on teratogenic activity. Two of the substances chosen were beta-ionone, a retinoid-degradation product containing a 4-carbon side chain and pseudoionone, a ring opened analog containing the same functional groups and molecular formula as ionone. The results of studies on numerous compounds indicate that teratogenic potential is associated with specific structural features in the Vitamin A. The presence of the following structural requirements:**

- 1) a retinoid beta-cyclogeranylidene ring,**
- 2) a polyene chain of at least five carbons,**
- 3) a polar hydrophilic function group located on the polyene chain terminus, and**
- 4) a trans stereochemistry in the polyene chain giving rise to a curved plane in the chain.**

**Structural requirements 2, 3, and 4 were not met by beta-ionone while structural requirements 1-4 were not met by pseudoionone. Neither substance showed any evidence of teratogenicity in hamsters at dose levels up to 960 or 460 mg/kg, respectively. Given these results and well-documented protocol capable of identifying teratogenic potential in this group of substances, an additional developmental study using an OECD 421 or 414 protocol is not warranted at this time. In addition, ionone derivatives are readily metabolized via reduction of the ketone function and allylic oxidation of exocyclic methyl substituents to yield in both cases, polar excretable metabolites. Given the metabolic fate of this class of substances and the comprehensive developmental testing for carotenoid teratogenicity, there is no foundation to perform additional testing for this endpoint.**

Ecological Effects (*fish, invertebrates, and algae*). The submitter proposes to perform acute daphnia and algae testing on alpha-iso-methylionone and extrapolate the results to methylionone. However, there is little information supporting the SAR model for chemicals of this type. EPA believes that methylionone may be the most toxic member owing to its less-substituted vinyl ketone moiety, and should be tested instead of alpha-iso-methylionone for the proposed endpoints. If the results of the daphnid acute test show an increase in toxicity beyond what the SAR model predicts, then an acute fish test on methylionone should be conducted.

**Methyl ionone (mixture of isomers) has been substituted for iso-methylionone. The mixture of the isomers has been tested at the limit of their solubility. The 48-hour median effective concentration (EC50) in Daphnid magna was determined to be 2.65 mg/L. The 48-hour LC50 was 3.11mg/L while the 48-hour NOEC was reported to be 1.14 mg/L [Ward, 2003a]. Using an OECD 201 Guideline, the acute toxic potential of n-methylionone (mixture of isomers) was investigated in green algae (*Selenastrum capricornutum*). The 72-hour EC50 value for *Selenastrum capricornutum* was reported to be 7.47 mg/L using the average specific growth rate, 3.23 mg/L, calculated based on the number of cells/mL, and 2.89 based on average specific growth rate. The NOEC was determined to be 0.404 mg/L [Ward, 2003b].**

The 48-hour fish test on *beta*-ionone is considered inadequate owing to the shorter than 96-hour required test duration.

**It is true that the length is shorter than recommended but the facts that there were no deaths at 48 hrs at a concentration of 5 mg/L and *alpha*-isomethylionone showed an LC50 of 10.9 mg/L, supports the conclusion that the LC50 value of n-methylionone (mixture of isomers is approximately 10 mg/L. This concentration approaches the limit of solubility of this substance at 17 °C.**

The submitter also needs to consider performing a daphnid chronic test given that the measured log Kow for alpha-iso-methylionone of 4.6 suggests a concern for chronic toxicity. Generally, EPA would recommend the chronic testing instead of the acute tests, but in this case, because toxicity was seen in the fish acute studies using alpha-iso-methylionone, the acute endpoints need to be addressed. All testing should be done using measured concentrations to account for potential chemical losses over the duration of the test.

**Again, the problem is in testing at the limits of water solubility, especially with a mixture. This can be done, but before agreeing, there should be some evidence that predicted environmental concentrations justify such an expenditure of effort and animals.**

### Specific Comments on the Robust Summaries

General comment

Throughout the robust summaries the title substance is one of the two category members but often is not the substance tested. To avoid confusion it is preferable to title each summary with the name of the substance tested and then to identify it as an analog of a specific chemical.

**This has been done where appropriate.**

Health Effects

*Acute Toxicity.* Most summaries lack the following information: dose levels (when more than

one dose used), method description, animals sex, observation period, clinical signs, mortality per sex and per dose level.

**The original reports were checked and details included if available. However, in most cases these details were not presented.**

*Genetic Toxicity.* The missing information in the Ames test is the number of dose levels, number of replicates, quantitative data, and explanation for not testing at the required concentrations and absence of cytotoxicity.

**The original reports were checked and details included if available. However, in most cases these details were not presented.**

Ecotoxicity

*Fish.* A missing data element in the 96-hour study is water hardness.

**These data were included in the robust summary.**