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To NCIC HPV@EPA
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Subject Fw: Submission of Responses to EPA Comments and Revised Test Plan and Robust Summaries for Estragole

----- Forwarded by JuanB Perez/DC/USEPA/US on 09/14/2005 07:59 AM -----

"Adams, Tim " <tadams@therobertsgroup.net>
09/13/2005 02:03 PM

To NCIC OPPT@EPA, Rtk Chem@EPA
cc skrixer@chemintox.com
Subject Submission of Responses to EPA Comments and Revised Test Plan and Robust Summaries for Estragole

Dear Administrator:

On behalf of the member companies of the Flavor and Fragrance High Production Volume Consortia, the Terpene Consortium is pleased to submit a letter responding to the EPA comments on the chemical category "Estragole". We also wish to submit a revised test plan and revised robust summaries for this substance. In addition to new human health data, the Terpene Consortium has sponsored additional studies on ecotoxicity and environmental fate endpoints. These documents represent the final submission by the Terpene Consortium for this substance. The cooperation of our Consortium with EPA has lead to the accumulation of relevant hazard data and provided many benefits both to the industry and the public. Our Consortium values the experience.

Respectfully,

Timothy B. Adams, Ph.D.

Technical Contact Person for FFHPVC

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2005 Revised Test Plan for Estragole.pdf 2005 Robust Summaries II for Estragole.pdf

Responses to EPA Comments for Estragole.pdf

201-16029

The Flavor and Fragrance High Production Volume Consortia (FFHPVC)

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2005 SEP 15 AM 11:09

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

July 25, 2005

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 "Estragole". 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 "Estragole".

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 "Estragole". The revised test plan and robust summaries contain additional data on existing studies and the results of additional ecotoxicity testing and environmental fate data. Newly published and unpublished reports on repeat dose, reproductive, and developmental endpoints have been added to the human health section. These data are related to the questions and comments made by the EPA in its letter dated 3/4/2003. 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 the chemical, estragole, in 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 estragole. A summary of the key hazard data has been included in this letter and also in the revised test plan (see Section 1.1.1. of test plan).

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. Estragole is 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 estragole in food are published in the Food Chemical Codex [FFC, 1996; see Revised Test Plan].

Based on the long history of both as naturally occurring component of food and as a substance intentionally added to food, the hazard assessments performed by the US FDA, 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.

Best regards,

Timothy B. Adams, Ph.D., Technical Contact Person for FFHPVC

Summary of Key Hazard Data for Estragole

ENDPOINT	SUBSTANCE/SURROGATE/ CHEMICAL CATEGORY[1]	VALUE/RANGE[2]	REFERENCE
Physical Properties			
Vapor pressure	Estragole (4-methoxyallylbenzene)	0.041 mm Hg (21°C) 1.0 mm Hg (52.6 °C)	Daubert, 1989 Stull, 1974
Partition Coefficient	Estragole (4-methoxyallylbenzene)	3.47	MacKay, 2000
Environmental Fate			
Biodegradation[3]	4-Hydroxy-3-methoxyallylbenzene)	+ (OECD 301B)	Quest, 1994
Ecotoxicity			
Fish	3,4-Dimethoxyallyl benzene(methyl eugenol)	96-hr LC50=8.1 mg/L (bluegill) 96-hr LC50=6.0 mg/L (trout)	Beroza, 1975
Aquatic Invertebrates	Estragole (4-methoxyallylbenzene)	48-hr EC50=8.7 mg/L (Daphnia magna) 48-hr LC50=6.0 mg/L NOEC=4.73 mg/L	Ward, 2003
Aquatic Plant	Estragole (4-methoxyallylbenzene)	72-hr EC50= 1.35 mg/L using the number of cells/mL. The 72-hr NOEC=0.118 mg/L	Boeri, 2003,
Human Health			
Repeat Dose[4] (route)	Estragole (4-methoxyallylbenzene)	NOEL=50 mg/kg bw/d (rat, dietary) NOAEL=75 mg/kg bw/d (mouse, gavage) LOAEL=150 mg/kg bw/d (mouse, gavage) LOAEL=37.5 mg/kg bw/d (rat, gavage)	Jones, 2003 NTP, 2005
Reproduction (route)	3,4-Dimethylene oxyallylbenzene (safrole)	NOAEL(maternal toxicity)=5 mg/kg bw/d (mouse, gavage) LOAEL(maternal toxicity)=50 mg/kg bw/d (mouse, gavage) NOAEL(fetal toxicity)=5 mg/kg bw/d (mouse, gavage) LOAEL(fetal toxicity)=50 mg/kg bw/d (mouse, gavage)	Moro, 1985
	3,4-Dimethoxyallyl benzene (methyl eugenol)	NOAEL for effects to reproductive organs (epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries)= 300 mg/kg bw/d (m,f rats)	NTP, 2002

[1] Surrogate is a structurally related substance that may include a metabolic product or precursor of the named substance. Range of values may be reported for substance, surrogate or chemical category.
[2] Experimental value or values for a substance or group of substances in the chemical category
[3] not biodegradable, (-); readily biodegradable, (+); ready and ultimately biodegradable, (++)
[4] Value is the NOAEL or NOEL(route, duration)

	3,4-Dimethyleneoxyallylbenzene	LOAEL(maternal toxicity)=50 mg/kg bw/d (mouse, gavage) NOAEL(teratogic effects)=200mg/kg bw/d (mouse, gavage) LOAEL(fetal toxicity)=50 mg/kg bw/d (mouse, gavage)	Moro, 1985
Developmental (route)	Nutmeg oil (contains 80% terpene hydrocarbons –see developmental effects for monoterpene and bicyclic terpene hydrocarbons;20% of allylalkoxybenzene derivatives	NOEAL(teratogenic effects)= 52 mg/kg bw/d as allylalkoxybenzene derivatives) (rat, f, gavage)	Morgeiridge, 1973c
[illegible]			
In vitro	Estragole (4-methoxyallylbenzene)	+ UDS + Rec	Howes, 1990 Chan, 1992 Muller, 1994 Zani, 1991
In vivo	Estragole (4-methoxyallylbenzene)	+ DNA adducts P^{32}-post labeling + DNA adducts P^{32}-post labeling	Randerath, 1984 Phillips, 1981

[5] (-), no significant genotoxic potential; (=/-), equivocal evidence; (+), positive evidence of genotoxicity. AMS, Ames assay; MLA, Mouse Lymphoma assay; ABS, chromosomal aberration assay; UDS, Unscheduled DNA Synthesis; MN, Micronucleus test, SCE, Sister Chromatid Exchange assay, SLA, Sex-linked Lethal assay.

EPA Comments on Chemical RTK HPV Challenge Submission:
Estragole

Summary of EPA Comments

The sponsor, the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC), submitted a test plan and robust summaries to EPA for estragole (p-allylanisole, CAS No. 140-67-0) dated October 21, 2002. EPA posted the submission on the ChemRTK HPV Challenge Web site on November 4, 2002.

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

1. **Analog Justification.** In some sections of the test plan, the analogs selected and justifications provided were insufficient to support the use of surrogate data to satisfy an endpoint. For health effects, methyl eugenol is a reasonable analog whereas *trans*-anethole does not appear to be an appropriate analog.

Response: We agree with EPA that allylalkoxybenzene derivatives such as methyl eugenol, safrole, and asarone are appropriate surrogates for estragole. We also agree that propenylalkoxybenzene derivatives such as analogs are not relevant to the human health endpoints. We have added relevant data for methyl eugenol and safrole for these endpoints,

2. **Physicochemical Properties.** The data provided by the submitter for boiling point, vapor pressure, water solubility, and partition coefficient are adequate for the purposes of the HPV Challenge Program. The submitter needs to provide measured melting point data for this chemical.

Response: Relevant melting point data has been added to the robust summaries and test plan.

3. **Environmental Fate.** The data provided for photodegradation and fugacity are adequate for the purposes of the HPV Challenge Program. EPA agrees that biodegradation testing should be conducted for this chemical. The submitter needs to address some deficiencies in the robust summaries.

Response: Available data relevant to environmental fate have been added to the robust summaries

4. **Health Effects.** Adequate data are available for the acute toxicity endpoint for the purposes of the HPV Challenge Program. EPA reserves judgment on the adequacy of the genetic toxicity and repeated-dose toxicity endpoints pending submission of additional critical information. EPA believes that *trans*-anethole is not an appropriate analog for estragole given its different metabolic profile and thus does not support the reproduction and developmental toxicity endpoints for estragole. EPA recommends that the submitter provide data from an appropriate analog or conduct a combined reproduction/developmental toxicity screening test on estragole.

Response: The data for human health endpoints based on anethole studies have been removed from the robust summaries. Repeat dose data on estragole from a recent 90-day study (NTP, 2005) and a 28 day dietary study and 28 day gavage study with methyl eugenol

have been included in the repeat dose endpoint. Given that safrole is an allylalkoxybenzene derivative, data on this substance has been added to the reproductive and developmental endpoints. Also, data on the reproductive organs from the 2-year bioassay on methyl eugenol has been added to the reproductive endpoint. Finally, reproductive and developmental data on nutmeg oil are considered relevant to estragole. Nutmeg oil is primarily composed on terpene hydrocarbons (monoterpenes and bicyclic monoterpenes) and allylalkoxybenzene derivatives. The reproductive and developmental toxicity of hydrocarbons (see test plans for monoterpene hydrocarbons and bicyclic terpene hydrocarbons) indicate a very low order of toxicity. Therefore any significant reproductive effects can be safely assumed to be associated with administration of the group of allylalkoxybenzene derivatives that accounts for up to 20% of the essential oil.

5. **Ecological Effects**. EPA agrees with the test plan for these endpoints. However, the submitter needs to address deficiencies in the robust summaries.

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

EPA Comments on the Estragole Challenge Submission

Test Plan

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

The data provided by the submitter for boiling point, vapor pressure, water solubility, and partition coefficient are adequate for the purposes of the HPV Challenge Program.

Melting point. The submitter provided calculated data for this endpoint from the EPIWIN program. EPIWIN has sometimes provided melting point data that do not match experimental data, and therefore should not be used as the only source of melting point information. Furthermore, the use of estimated values introduces uncertainties that then become magnified in modeling applications. The submitter needs to provide measured melting point information following OECD Guideline 102, or provide data from a reliable literature source.

Response: Although we agree that model data is not desirable in the presence of experimental data, it should not be the responsibility of the submitter to provide data to improve the results of modeled physiochemical data. The objective of any study is to provide appropriate data for hazard assessment. Because estragole is a liquid at ambient temperature and is handled as a liquid, melting point data is not relevant information for completion of the hazard assessment for this substance. However, we have empirical data on a number of structural relatives (3,4-dimethoxyallylbenzene, 3,4-dimethoxypropenylbenzene, 4-methoxypropenylbenzene) in which the effect of adding a methoxy group on melting point has been evaluated. Given this semi-empirical approach the melting point of estragole is estimated to be approximately 1 degree C. This estimate is consistent with the prediction of the model.

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

The data provided by the submitter for photodegradation are adequate for the purposes of the HPV Challenge Program.

Stability in water. While the test plan correctly states that estragole cannot hydrolyze, the submitter also needs to provide a brief explanation in robust summary format.

Response; A robust summary has been included on stability in water.

Biodegradation. EPA agrees with the submitter's recommendation that biodegradation testing should be conducted for this chemical. The submitter needs to provide ready biodegradation data following OECD Guideline 301.

Response; A robust summary has been included for the eugenol and anethole. Both substances are readily biodegradable. Based on these data and the fact that there is no indication that this naturally occurring substance is persistent in the environment, it is safe to assume estragole is also readily biodegradable

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

Acute Toxicity. The acute oral toxicity data are adequate for the purposes of the HPV Challenge Program on a weight-of-evidence basis.

Genetic Toxicity. EPA reserves judgment on the adequacy of gene mutation and chromosomal aberration endpoints pending submission of the additional critical information for the robust summaries.

Response: Based on the fact that genotoxicity studies on estragole and 1'-hydroxyestragole (To, 1982; Swanson, 1979: Howes, 1990;Chan, 1992; Muller, 1994; Zani, 1991) show evidence of a genotoxic potential in selected bacterial assays. Also, there is evidence of unscheduled DNA synthesis assays and DNA interaction in 32P-post-labelling experiments (Muller, 1994; Randerrath, 1984; Phillips, 1981). Therefore, it can be concluded that estragole exhibits a significant potential for genotoxicity under the conditions used in these experiments.

Repeated-Dose Toxicity. EPA reserves judgment on this endpoint for the following reasons:

(1) All the submitted studies assessed carcinogenicity as an endpoint. Except for the methyl eugenol NTP cancer bioassay range-finding study, these studies were not complete enough to satisfy the repeated-dose endpoint requirements.

Response: Robust summaries for the recently concluded 90-day repeat dose studies for estragole in mice and rats have been prepared. Like the methyl eugenol studies, gavage dose levels were set such that all dose levels were toxic in rats. A NOAEL could be determined in mice. A 28-day study on methyl eugenol administered in the diet is more relevant given that exposure to methyl eugenol, estragole, and safrole is principally through the diet. In rats, methyl eugenol produced no toxic effects at dietary levels of 50 mg/kg bw per day. Biochemical studies (Ellis, 2005) on livers and forestomach of these rats showed evidence of DNA adduct formation in the forestomach at the 50 mg/kg bw level using P^{32}-post-lableing as a detection method. The NOAEL is approximately 5 mg/kg bw per day

(2) The 14-week repeated-dose studies performed as range-finding studies for the methyl eugenol cancer studies were not presented or summarized. The submitter needs to provide summaries for the 14-week methyl eugenol studies. This information may be important because of the testicular effects observed in mice (NTP, 2000).

Response: Robust Summaries of both studies have been prepared for the 14-week methyl eugenol study. More relevant 90-day gavage studies with estragole have also been prepared. Also a robust summary for a 28-dietary study with methyl eugenol has been preparted. These data are compared below:

The 90-day gavage study on estragole performed by the National Toxicology Program in mice revealed a NOAEL of 75 mg/kg bw/day in mice. There was no evidence of alterations to any organ or tissue, including the testes, in mice up to a dose level of 300 mg/kg bw/day. In the 14 week methyl eugenol gavage study (robust summary included), male mice experienced changes in testes weight at gavage dose levels (100 and 300 mg/kg bw per day but not at 1000 mg/kg bw per day) greater that those associated with other toxic effects in male mice (liver effects at 30 mg/kg bw per day). The NTP study on estragole shows no such testes effects at any dose level in the 90-day study. Therefore, the NOAEL for toxic effects is 75 mg/kg bw per day in mice. Based on the 90-day study, toxicity to reproductive organs associated with administration of estragole is 300 mg/kg bw per day.

In rats, the 90-day NTP study using estragole revealed histopathological changes of the liver at 37.5 and 75 mg/kg bw per day including bile duct hyperplasia, oval cell hyperplasia, hepatocyte hypertrophy, periportal inflammation all of which were described as being minimal effects. Therefore, the NOAEL for rats is less than 37.5 mg/kg/day. The 14-week study with methyl eugenol showed increased relative liver weights (14.08 g) in male rats at 30 mg/kg bw per day when compared to the vehicle controls (12.87 g) but not with respect to untreated controls (13.56 g). A significant increase in testis weight was observed in male rats receiving 1,000 mg/kg/d. Clearly, effects to reproductive organs occur at toxic dose levels. Therefore, a NOAEL for estragole in rats is concluded to be less than 37.5 mg/kg bw per day. However, these data were taken from gavage studies. In a dietary study, the principal route of human exposure, rats administered methyl eugenol in the diet exhibited no evidence of toxicity at dose levels up to 50 mg/kg bw per day.

(3) NTP recently completed a 90-day study with estragole in rats and mice. According to the NTP website (http://ntp-server.niehs.nih.gov/), a 90-day study with estragole was started in September of 2001. At this time (March, 2003), there is no indication what the results were or when a draft report will be available. EPA believes this information to be critical as it addresses the toxicity of the sponsored chemical. The submitter needs to check on the status of this study. *Reproduction and Developmental Toxicity*. The submitter has used *trans*-anethole data to address the reproductive and developmental toxicity endpoints. Although *trans*-anethole is structurally similar to estragole, its metabolic pathway and thus its toxicity may be significantly different at higher dose levels. Submitted Information for estragole and additional information in a separate HPV submission for anethole (posted on the ChemRTK Web site December 4, 2002) describe O-demethylation as the predominant pathway at lower intake levels for both substances, but at higher levels (greater than 10 mg/kg bw), estragole (and methyl eugenol) utilize a 1'-hydroxylation pathway producing reactive intermediates that have been associated with toxicity. However, in the case of *trans*-anethole, when the O-demethylation pathway is saturated, omega-oxidation occurs producing end products similar to those seen with estragole but without the formation of the reactive intermediate. Also, both estragole and *trans*-anethole can form epoxides; however, the epoxidation pathway, as presented, is a minor metabolic route. Since the formation of a reactive intermediate is an important difference in the metabolism of estragole, *trans*-anethole is not an appropriate surrogate for estragole, especially at higher doses.

Response: Based on the differences in biochemical fate, we agree that anethole is not an adequate surrogate for estragole and other allylalkoxybenzene derivatives. Robust summaries for anethole in the human health section were originally intended only for comparison with estragole. Human health data on anethole has been removed from the robust summaries and test plan. Only data on allylalkoxybenzene derivatives (estragole, methyl eugenol, safrole) has been included. The recent 90-day data presented for estragole in mice and rats (NTP, 2005), data for the 14-week NTP studies for methyl eugenol in mice and rats (NTP, 2000), data for a mixture of allylalkoxybenzene derivatives (Morgareidge, 1973, and the more completely translated data from the 1985 developmental study for safrole (Moro, 1985) now provide the basis for evaluating the reproductive and developmental endpoints for estragole.

Other studies submitted for the reproductive and developmental toxicity endpoints used a mixture referred to as oil of nutmeg or FDA 71-28, defined as a mixture of 10-20% p-allylalkoxybenzene derivatives (myristicin, elemicin, safrole and methyl eugenol) and 80 to 90% bicyclic terpene hydrocarbons. Although methyl eugenol is a reasonable surrogate for estragole, the amount present in this mixture is very small and suggests that the mixture itself may not be a good analog for estragole. No comparison of the metabolic pathways for the other p-allylalkoxybenzene derivatives–or the bicyclic terpene–components of the mixture has been supplied and therefore the information provided does not address the reproductive/developmental toxicity endpoint for estragole.

Response: The reviewer may not recognize the common names in the mixture above, but safrole, myristicin, methyl eugenol, etc. are all allylalkoxybenzene derivatives that participate in the same 1'-hydroxylation pathway as does estragole. Therefore, the mixture of allylalkoxybenzene derivatives does provide an adequate surrogate fro estragole. In addition, the essential oil is principally composed of two structural chemical groups, monoterpene hydrocarbons and allylalkoxybenzene derivatives. Monoterpene hydrocarbons that account for approximately 80% of the oil have been tested for developmental toxicity (see test plan for Monoterpene Hydrocarbons). They exhibit no potential for developmental toxicity at dose levels exceeding those exerting general toxicity or even carcinogenic effects. Therefore, monoterpene hydrocarbons are relatively inert compared to allylalkoxybenzene derivatives. Allylalkoxybenzene derivatives are known to be toxic at dose levels as low as 30 mg/kg bw/day (NTP, 2000, 2005; More, 1985; Long, 1963). The Moro gavage study with safrole (a surrogate for stragole) showed no teratogenic effects at dose levels (200 mg/kg bw/day) 4 time those causing maternal and fetal toxicity. Gavage administration in a mixture diluted by the presence of hydrocarbons better duplicated dietary conditions. Under these conditions, a dose level equivalent to 52 mg/kg bw per day in rats produced no toxic or developmental effects (Morgareidge, 1973). Based on the observations that developmental effects occur at dose exceeding general toxicity, the hazard assessment for the developmental endpoint should be based on the the above data and the fact that developmental toxicity occurs at dose levels exceeding either maternal or fetal toxicity.

EPA recommends that the submitter provide data from a more appropriate analog or conduct a combined reproduction/developmental toxicity screening test (such as OECD 421) on estragole.

Response: Studies on estragole and more appropriate surrogates (safrole and methyl eugenol) have been included.

Ecological Effects (*fish, invertebrates, and algae*).

The test plan for these endpoints is adequate for the purposes of the HPV Challenge Program. The submitter needs to provide missing study details in the robust summaries.

Response: Details have been added to existing robust summaries were available. Data on recently performed studies for aquatic invertebrates and aquatic plants have been added.

Specific Comments on the Robust Summaries

Generic comments

The submitter should consult EPA guidance documents for the preparation of robust summaries (http://www.epa.gov/chemrtk/guidocs.htm).

Summaries should list the substance purity or explicitly state if that information was not reported.

In the robust summaries for analogs, it would be preferable to use the analog name as the

Substance Name, with analog status indicated in parentheses, and show the CAS number of the analog. For example:

Substance Name Safrole (analog for estragole)
CAS No. 94-59-7
For test mixtures, each robust summary needs to include all available compositional information. For example, test material FDA 21-78 was only completely defined on page 29 of the test plan; none of the robust summaries mentioned that it contained 80-90% bicyclic terpenes (not identified as estragole analogs).

Response: These changes were made to the robust summaries where appropriate.

In some cases, the submitter did not use the term NOAEL/NOEL correctly. If the lowest dose was a LOAEL, the study did not have a NOAEL since these terms refer to 'observed', i.e., tested levels; in this situation, the correct NOAEL/NOEL field is 'undetermined' or 'none.'

A positive control is required for genotoxicity assays. Negative genotoxicity data are not valid if the summaries do not report the positive controls.

Response: These changes were made to the robust summaries where appropriate.

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

Transport and distribution (fugacity). The data provided by the submitter for transport and distribution (fugacity) are adequate for the purposes of the HPV Challenge Program. However, the submitter needs to incorporate in the robust summary the actual values of the input parameters used in the model.

Response: Input values were added to the robust summary.

Health Effects

Acute Toxicity. The Jenner et al. (1964) study summaries should provide the doses used and the length of the observation period, and whether there were any gross necropsy findings.

Response: Available data (observation period) was included in the robust summary.

Repeated-Dose Toxicity. 12-month cancer study with estragole. A robust summary for a 12-month carcinogenesis assay (Miller et al., 1983) in mice exposed to estragole or its metabolite, 1-hydroxyestragole, in the diet provided some information on systemic toxicity (noncancer effects), but was incomplete. Omissions included the purity of the test material, the group sizes, the sizes of body weight gain reductions, time-weighted averages for the doses (modified during the test), and information about organ weights or histopathology of organs besides the liver.

Response: The Miller study (Miller et al., 1983) was a structure-activity study on the carcinogenic potential of allylalkoxybenzene derivatives to the liver. Given the limited scope of the study protocol (*e.g.*, tissues analyzed and body weight changes during the study), studies on estragole and methyl eugenol performed by the National Toxicology Program and Jones (Jones, 2005) provide a more consistent and comprehensive database of information upon which to assess the repeat dose hazard of estragole.

Genetic Toxicity. Ames assays. All the summaries of mutation assays in Salmonella typhimurium need the following: information on positive controls (except for To et al., 1982), the cytotoxic concentration, the number of replicates, and the statistical methods or the criteria for determining levels of significance.

Response: Additional data for positive controls, number of duplicates, cytotoxicic concentrations, etc. were included in the robust summaries where available.

In vitro chromosomal aberration study. A robust summary for a negative chromosomal aberration assay in cultured rat V79 cells omitted information on the use of positive controls, the concentrations administered, the criteria for positive results, and the numbers of cells examined.

Response: These data were added to the robust summary if available.

Ecological Effects

Fish. The robust summary submitted for a study with *trans*-anethole did not indicate the number of fish tested per concentration, control use and response data, and the statistical methods used.

Response: Additional available data for both acute fish studies were added to the robust summaries.

Invertebrates. The two robust summaries submitted for studies with estragon oil (70-88% estragole) and *trans*-anethole did not indicate one or more of the following study details: the number of organisms tested per concentration, control response data, signs of toxicity/mortality data, statistical methods used, and/or the test system used (i.e., static vs. renewal or flow-through).

Response: Given that an OECD Guideline study was recently performed using estragole, the study on anethole was deleted. Additional requested information for the estragon oil study has been included in the robust summary.

Algae. The robust summary submitted for a study with *trans*-anethole reported an 96-hour IC_{50} value rather than a 72-hour LC_{50} or NOEC value. Missing study details included control response data and water chemistry measurements.

Response: Given that an OECD Guideline study was recently performed using estragole, the study on anethole was deleted.

References

Smith et al. (2002). Safety assessment of allylalkoxybenzene derivatives used as flavouring substances–methyl eugenol and estragole. Food Chem. Toxicol., July, vol. 40(7): 851-70. (This report is listed as an unpublished report in the robust summary.)

National Toxicology Program (NTP), 2000. Toxicology and carcinogenesis studies of methyl eugenol (CAS No. 93-15-12) in F344/n rats and B6C3F1 mice (gavage studies). DRAFT NTP-TR-491; NIH Publication No. 98-3950.