

**EPA Comments on Chemical RTK Challenge Submission:
3,5-Di-*tert*-butyl-4-hydroxyhydrocinnamic acid octadecyl ester**

SUMMARY OF EPA COMMENTS

The sponsor, Ciba Specialty Chemicals Corp., submitted to EPA Robust Summaries and a Test Plan that were received July 10, 2000, and submitted a test plan to the HPV Tracking System Web site (www.hpvchallenge.com), for 3,5-Di-*tert*-butyl-4-hydroxyhydrocinnamic acid octadecyl ester (CAS # 2082-79-3). EPA posted the submission on the ChemRTK website on July 20, 2000.

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

1. The submission does not meet minimal standards for data adequacy. There were many inadequacies in the health and ecological effects study summaries, which must be revised to be acceptable for the Challenge Program. EPA has provided specific comments on how to enhance the robust summaries. Sponsors should refer to the Challenge Program guidance.

EPA accepts the submission conditionally, believing that the issue is poor documentation but that enough information may be inferred to make tentative judgements. Eventual full acceptance of the submission is contingent upon the receipt within 90 days of substantially improved robust summaries and other information that can meet the standard set out in EPA's guidance documents.

2. Physicochemical and Environmental Fate Data. The sponsor supplied calculated data without citing available experimental data. Similarly, the sponsor used only estimated data as inputs into the fugacity model. EPA prefers measured data when available. To estimate transport and distribution, the sponsor used the EPIWIN Level III model which provides estimated values as default inputs. EPA recommends using the EQC level III model from the Canadian Environmental Modeling Centre at Trent University.

3. Health Effects: Most of the robust summaries are inadequate because not enough information is presented to allow for an independent assessment of the data. However, EPA's tentative scientific judgment is that no further testing is needed for the purposes of the U.S. HPV Challenge Program, provided that the sponsor supplies adequate documentation as discussed under Item 1 above.

4. Ecological effects: Although there were many inadequacies in the study summaries, EPA suggests that an analysis based on this chemical's physicochemical properties, including extremely low water solubility, may support the sponsor's conclusion that no further testing is necessary. EPA will take into account adequate documentation of such an analysis supplied by the sponsor in determining final acceptance of the test plan.

EPA is requesting that the Sponsor advise the Agency within 60 days of any modifications to its submission.

EPA COMMENTS ON THE 3,5-DI-*tert*-BUTYL-4-HYDROXYHYDROCINNAMIC ACID OCTADECYL ESTER CHALLENGE SUBMISSION

General

The submission does not meet minimal standards. There were many inadequacies in the health and ecological effects study summaries, which must be revised to allow final acceptance as an HPV Challenge submission. EPA has provided specific comments on how to enhance the robust summaries to the standard established in EPA's HPV Challenge Program Guidance (<http://www.epa.gov/opptintr/chemrtk/guidocs.htm>).

The test plan on the industry HPV Tracking System Web site and the test plan summary table submitted with the robust summaries to EPA were substantially different. EPA contacted the sponsor and learned that the sponsor was unable to make desired corrections to the Tracking System submission.

Test Plan

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

EPA believes that no additional test data are needed to satisfy the needs of the HPV Challenge Program.

Fate (photodegradation, stability in water, biodegradation, and transport/distribution).

EPA believes that no additional test data are needed to satisfy the needs of the HPV Challenge Program.

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

EPA's tentative judgement is that no additional test data are needed to satisfy the needs of the HPV Challenge Program pending receipt of adequate robust summaries.

Ecological Effects.

EPA's tentative judgement is that no additional test data are necessary to satisfy the needs of the HPV Challenge Program pending receipt of adequate robust summaries. An adequately documented analysis, such as a quantitative structure-activity relationship (QSAR) analysis, based on this chemical's physicochemical properties may provide additional support to the sponsor's conclusion that further aquatic testing is unnecessary.

SPECIFIC COMMENTS ON ROBUST SUMMARIES

Chemistry

All the physicochemical property data (estimated using EPIWIN) are acceptable, except for melting point, which is much higher than the measured value.

The sponsor supplied melting and boiling points, vapor pressure, logP, and water solubility values estimated with the EPI program (EPIWIN). The EPIWIN estimated melting point (240 °C) is far higher than reported values of 50-52 °C (Aldrich Catalog 2000-2001, p. 1242), 49-54 °C (Kirk Othmer, 3rd Ed., Vol. 2, p. 88), and 50-55 °C (MSDS from Ciba Specialty Chemical Corp.), which confirms the known large error tendency in calculating this endpoint. For chemicals with a molecular weight greater than about 200 or with more than 15 carbons, EPIWIN almost always predicts a melting point much higher than observed.

Entering a melting point value of 51 °C into the EPI program results in lower vapor pressure (3.978×10^{-11} mm Hg at 25 °C) and higher water solubility (3.978×10^{-8} mg/l at 25 °C) estimates. The Ciba MSDS reports a water solubility value of < 0.2 ppm (< 0.2 mg/l) at 20 °C. and vapor pressure as $\sim 2 \times 10^{-9}$ mmHg at 20 °C. Furthermore, although EPIWIN estimated a boiling point of 560.8 °C, the Ciba MSDS reports that the chemical decomposes at >350 °C.

As measured values are preferred as inputs to other estimation programs, sponsors should explain their use of estimated values when apparently measured values are available.

Fate

The biodegradation data (OECD 301B, Modified Sturm Test and OECD 302B, Modified Zahn-Wellens Test) are acceptable.

The use of EPIWIN to estimate properties and fate is acceptable for this chemical to the extent measured data are lacking. The EPIWIN program allows one to input measured values, thereby improving the accuracy of the values estimated for other properties.

In contrast to the EPIWIN estimated melting point of 240 °C, EPA finds that other sources list values in the range 49–55 °C. When 50 °C is input into EPIWIN the estimated values for water solubility and Henry's Law constant increase by factors of 7 and 20 respectively; the values of other properties were not affected.

EPA recommends using measured data as much as possible. The sponsor used the EPIWIN Level III model, which provides estimated values as default inputs. In order to estimate environmental fate endpoints, however, EPA recommends using the EQC level III model from the Canadian Environmental Modeling Centre at Trent University. This model can be found at the following Web address: <http://www.trentu.ca/academic/aminss/envmodel/>.

Health Effects

EPA evaluated 11 health endpoint robust summaries and found one (acute toxicity via inhalation) of them to be adequate for the purposes of the U.S. HPV Challenge Program. The inadequate summaries lack information that is necessary to evaluate the basic adequacy of the cited study. The two unpublished teratogenicity studies and the two-generation reproduction study are currently in EPA files and the robust summary information missing and listed below is available from the full study reports. EPA's tentative scientific judgment is that new studies are not necessary for the purposes of the U.S. HPV Challenge Program, pending receipt of adequate robust summaries.

The following EPA comments reflect the information in the robust summaries (the full study reports may address these comments):

Acute Toxicity. Three separate robust summaries were submitted (oral, dermal and inhalation routes of exposure). In the dermal and oral acute toxicity studies, because a vehicle was used a control should have been run. It is likely, however, that there are no vehicle effects because no mortality was observed in either study. In addition, the following important information should be added to the dermal robust summary (1) the observation period following dosing; and (2) a description of how the test material was applied (i.e., shaved back, occluded or not occluded, etc.). EPA believes that these three robust summaries collectively meet the U.S. HPV Challenge Program needs for the acute toxicity endpoint.

Genotoxicity (dominant lethal assay, mice). (1) No positive or negative controls were identified. (2) There is no indication of male:female mating ratio. (3) No supporting data are presented for the statement that no evidence of dominant lethality was observed.

Genotoxicity (somatic mutation assay, Chinese hamsters). (1) The number of animals/dose group is low and one cannot determine from the summary whether chromosomal aberrations or nuclear anomalies were scored. (2) There is no indication of the number of cells scored for either anomalies or aberrations. (3) No data are presented to verify the claim that there is no difference between treated and control cells in numbers of anomalies.

Genotoxicity (somatic mutation assay, Chinese hamsters). (1) There is no indication of the methodology used to prepare slides for scoring of aberrations nor of the method used to score the slides, i.e. were slides coded? (2) No data were submitted to support the claim that the chemical is nonmutagenic.

Genotoxicity (Ames test). (1) No rationale is given for dose selection; (2) the background revertant colony counts are not reported; (3) there is no indication whether positive controls were used; (4) there is no indication of the solvent or vehicle chemical used; (5) there is no indication of incubation time or temperature or method of counting, e.g. by hand or electronic colony counter; and (6) no data are presented to support the claim that there is no increase in reverse mutation with or without S9 fraction.

Repeat dose toxicity (oral via gavage, rats). This study was reported as GLP and adverse effects were reported (increased blood urea nitrogen in high dose females; increased relative liver weights in high and mid-dose females; a significant dose response in increased liver weight in males; and minimal centrilobular hepatocytic hypertrophy in high dose males). However, incidence by dose (values for each effect) are not provided and this is necessary to evaluate the significance of these findings. The age of the test animals was not given.

Reproductive toxicity (oral via the diet, rats). In this GLP study, the summary does not present the incidence by dose for all the effects described. A summary table, for example, would help greatly.

Developmental Toxicity. Two studies were submitted and summarized, one with rats and one with mice. Information missing from both summaries includes: (1) age of animals at study initiation; (2) number of animals per dose; (3) mating procedures (male/female ratios/cage, length of cohabitation, proof of pregnancy); and (4) data sufficient to show the effect of treatment upon other parameters such as number of successful matings, rate of implantation and resorption, litter weight, number of litters/treatment group, etc. Information missing from the rat teratology robust summary includes: (1) identity of vehicle; (2) adequate description of the maternal effects noted (the incidence of reduced food

intake by dose and whether or not body weight or body weight gain was affected); and (3) adequate description of the fetal effects observed (incidence by dose for growth retardation and increase in number of unossified phalangeal nuclei of the hind limb). The sponsor states in the robust summary for the rat study that the mouse findings were corroborative. From the information presented in the summary, it appears that the results were different and suggestive of a species difference in response.

Ecotoxicity Studies

The comments below reflect the information presented in the robust summaries; information in the full study report may address some of the issues identified.

Acute Aquatic Toxicity. Robust summaries were submitted for studies on fish (2), daphnia, and green algae. Many critical experimental details were omitted from the robust summaries, which effectively rendered them inadequate. EPA's tentative scientific judgment is that new studies are not necessary for the purposes of the U.S. HPV Challenge Program, pending receipt of adequate robust summaries and adequate analysis of potential effects.

Fish. Information missing from two submitted fish acute robust summaries includes: number of replicates/test, water chemistry, solvent/vehicle used, temperature, pH, test substance purity, dissolved oxygen, TOC, and hardness. Test concentrations were above the predicted water solubility limit and no information was supplied as to whether the test concentrations were measured or nominal. Because this chemical has low water solubility, the information provided does not allow a conclusion to be made as to how much of the test was conducted according to the Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, June 2000, available at <http://www.oecd.org/ehs/test/monos.htm>). The vehicle used was above the recommended concentration of #100 mg/L.

Aquatic plants. Information missing from the submitted algal inhibition test robust summary includes: total hardness, pH, TOC, exposure vessel type, size, lighting, temperature, and dissolved oxygen.

Aquatic invertebrates. Information missing from the submitted acute daphnid test robust summary includes: a description of the dilution water to include source, test substance purity, temperature, dissolved oxygen, pH, hardness, alkalinity, total organic carbon, test method (flow-through, static, static renewal), measured or nominal concentration, vehicle used, and number of animals per concentration tested. The vehicle concentration was almost 800 times higher than that allowed by OECD test guidelines. The chemical was tested above the calculated water solubility limit, and test duration was only 24 hours instead of the recommended 48 hours.

Followup Activity

EPA requests that the Sponsor submit adequate robust summaries and other modifications to its submission within 90 days.