April 1, 2010

Thomas M. Gray, M.S. D.A.B.T.
Technical Manager
Regulatory and Scientific Affairs Department
American Petroleum Institute
1220 L. St. N.W.
Washington, DC 20005-4070

Dear Mr. Gray:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for Reclaimed Substances: Disulfides, Diethyl and Diphenyl, Naphtha Sweetening (aka Disulfide Oil) posted on the ChemRTK HPV Challenge Program Web site on March 10, 2009. I commend the American Petroleum Institute 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 American Petroleum Institute 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 me at 202-564-8617. 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,

Mark W. Townsend, Chief
HPV Chemicals Branch

Enclosure

cc: O. Hernandez
R. Lee
J. Willis

EPA Comments on Chemical RTK HPV Challenge Submission:
Disulfide Oil

SUMMARY OF EPA COMMENTS

The sponsor, the American Petroleum Institute, submitted a test plan and robust summaries to EPA for the complex mixture identified as Disulfides, Diethyl and Diphenyl, Naphtha Sweetening (Disulfide Oil, CAS No. 68955-96-4) dated December 19, 2008. EPA posted the submission on the ChemRTK HPV Challenge website on March 10, 2009.

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

1. Testing Approach and Analog Justification. The use of dimethyl disulfide data to characterize the sponsored substance is reasonable for ecological effects and biodegradation. Additional support is needed for the approach to health effects.

2. Physical Chemical Properties. Available data appear adequate for these endpoints for the purposes of the HPV Challenge Program, but the submitter needs to provide robust summaries for some submitted data.

3. Environmental Fate. Available data appear adequate for these endpoints for the purposes of the HPV Challenge Program, but the submitter needs to provide robust summaries for some submitted data.

4. Health Effects. EPA reserves judgment on the data for these endpoints pending receipt of additional information. The submitter needs to supply additional data and additional robust summaries as described in more detail below.

5. Ecological Effects. Submitted data are adequate for invertebrate and algal toxicity for the purposes of the HPV Challenge program. Available data for dimethyl disulfide are tentatively adequate for fish, pending submission of adequate robust summary data by the sponsor of that chemical. The submitter needs to incorporate additional available data into the test plan.

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

EPA COMMENTS ON THE DISULFIDE OIL CHALLENGE SUBMISSION

Testing Approach and Analog Justification

Disulfide oil (DSO) is a complex mixture comprising 44 individual constituents quantitatively identified by the submitter (Test Plan, Appendix I, page 41). Ten of the constituents are short-chain (C1 to C3) dialkyl disulfides that make up about 87% of the mixture (Test Plan, page 10, Table 2). Four of these 10 dialkyl disulfides are present at concentrations of greater than 10% (methyl ethyl disulfide [18.2%]; methyl isopropyl disulfide [14.4%]; dimethyl disulfide [12%]; and diethyl disulfide [11.2%]). The focus of the test plan is on the dialkyl disulfides. The submitter proposes to use data for dimethyl disulfide (12% of the total mixture) to characterize the SIDS endpoints for the mixture. Thirty of the 44 components listed in Appendix I contain sulfur; the remaining 14 components make up <1% of the mixture. Thus, approximately 12% of the mixture represents “other” sulfur containing compounds.

Disulfides. The submitter’s approach proposes that the low molecular weight disulfides will have similar toxicological and other properties and that dimethyl disulfide presents the worst case for the toxicological endpoints. The test plan cites available data to support this view

The Test Plan presents a good discussion of the proposed rationale for the submitter’s approach involving a free-radical mechanism of toxicity. However, much of this discussion infers toxicological

behavior from chemistry studies, with few toxicity studies to confirm the proposed structure-toxicity relationships. Some of the stated conclusions are not convincingly supported, or are somewhat contradicted, by the cited data or publications. For example, the test plan states on p. 20 that ecotoxicity data confirm that "DMDS is the most toxic member of the disulfide series in DSO." However, the fish toxicity data cited do not show a clear trend vs. structure, and the values in Daphnia given for DMDS and the diethyl derivative cannot be considered different, especially given the differing test durations.

For mammalian toxicity, the submitter's claim, based on a generalization in the cited 1989 review by Munday that longer chain lengths lead to reduced radical stabilization and lower oxidation rates and consequently lower expected health effects toxicity, is not justified beyond the comparison of methyl and ethyl groups. In fact, in the structure-toxicity discussion on health effects, there is much reliance on the Munday review, but that author states clearly that "(t)he comparative toxicity of the saturated aliphatic thiols and disulphides cannot…be judged on the present evidence and further studies, using a single species of animal, are required."

However, the overstatements and inconsistencies in the submission are not fatal to the general argument for disulfides. For example, the submitter's assertion that secondary and tertiary alkyl groups will decrease the toxicity compared to DMDS and unbranched dialkyl disulfides is reasonable. The available evidence supports a more modest conclusion that, for both ecotoxicity and health effects, DMDS is likely to be at least as toxic as the other disulfides and is an acceptable source of toxicity data for the disulfide components of the sponsored mixture for screening purposes.

Trisulfides. The test plan does not address potential trisulfide toxicity. Although trisulfides make up a small percentage of the mixture (four components totaling approximately 3% of DSO (data from Appendix 1 of the Test Plan)), available data show that trisulfides are more biologically potent than disulfides. Munday et al. (2003) (cited in the Test Plan) present *in vitro* and *in vivo* rat data for dipropyl disulfide and dipropyl trisulfide. The paper evaluates redox cycling, hemolytic activity and phase 2 enzyme induction. In all experiments, the trisulfide was more active or more potent than the disulfide. In terms of *in vitro* activity in rat erythrocytes, the trisulfide inhibited catalase (68% vs. 0% for the disulfide); depleted GSH (73% vs. 17% for the disulfide); increased methemoglobin activity (46% vs. 3% for the disulfide); and increased sulfhemoglobin formation (4% vs. 0%). Studies with rats (five days of dosing) resulted in significant differences from controls for the trisulfide (but not the disulfide) for the following hemolytic parameters: decreases in packed cell volume and hemoglobin levels and increases in Heinz bodies; increased relative spleen weights; and increases in erythropoietic activity in the spleen and liver (all data from Tables 3-6 in Munday et al., 2003 as cited in the Test Plan). These data suggest that the trisulfides are more potent hemolytic agents than the disulfides and could contribute to overall DSO toxicity even at low relative concentrations. The submitter needs to revise the test plan to adequately address this potential health effects concern. As one approach, EPA recommends that the submitter provide data from one or more *in vitro* studies as described in Munday et al. (2003) comparing the response of the DSO mixture to the responses of the individual sulfides examined in the Munday paper, to support the contention that the trisulfides do not contribute to overall toxicity.

Test Plan

Physical Chemical Properties (melting point, boiling point, vapor pressure, partition coefficient, and water solubility)

The submitted data appear adequate for these endpoints for the purposes of the HPV Challenge Program. The submitter provided some data for the mixture and summarized measured and modeled data for the individual disulfides. EPA recommends that the submitter report the range of values for individual endpoints rather than (or in addition to) the individual values for representative chemicals. EPA considers this approach more reasonable for a complex mixture and has begun to apply it in evaluations of HPV data (see the following example):

Rosin and Rosin Salts:
http://www.epa.gov/chemrtk/hpvis/rbp/Cat_Rosin%20and%20rosin%20salts_Web_SuppDocs_Sept2008.pdf

Some data were reported for constituent disulfides in addition to DMDS. However, no robust summaries were provided for these substances. The submitter needs to provide the data for DSO and the various supporting chemicals discussed in the test plan in a DSO robust summary format for each endpoint.

The introduction to this section of the test plan asserts that "…approximately 64% of DSO is composed of five dialkyl disulfides with an alkyl carbon number of C4 or less. Consequently, the chemical and physical properties associated with these disulfides will exert a disproportionate impact on the properties of the substance." However, in the section on vapor pressure the submitter points out the "relatively high volatility of the *non-disulfide* [emphasis added] chemicals in DSO and their disproportionate contribution to the overall volatility of the substance." The conflicting sentences need to be modified to eliminate the inconsistency. For these endpoints, "disproportionate effects" of lower-concentration constituents are by no means unusual.

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

Existing data appear adequate for all four endpoints for the purposes of the HPV Challenge Program. The submitter needs to provide a DSO robust summary for each endpoint that discusses the test plan data for DSO and the various supporting chemicals. For the stability in water endpoint, the summary needs to describe the likelihood of hydrolysis in terms of whether water-sensitive functional groups are present.

Biodegradation. The submitter provided adequate OECD 301 D ready biodegradability test data previously submitted to EPA for dimethyl disulfide. EPA agrees that it is reasonable to use these data to characterize the other disulfides in the mixture, and the mixture as a whole, for this endpoint.

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

As noted above, EPA believes the use of DMDS is a reasonable representation of possible human health effects for the disulfide portion of the DSO mixture. However, available data on the trisulfide portion (mentioned above) raise a concern about the possible role of this more biologically potent portion of the DSO mixture. Thus, EPA reserves judgment on the health effects part of the submission pending receipt of the following:

(1) A revised test plan that adequately addresses potential health effects concerns for the trisulfide component of the mixture. One possible approach is for the submitter to provide one or more *in vitro* studies as described under the Trisulfides subsection of the Testing Approach and Analog Justification section above.

(2) Missing robust summaries as specified below.

Ecological Effects (fish, invertebrates, and algae)

Submitted data are adequate for invertebrate and algal toxicity for the purposes of the HPV Challenge program. The submitter needs to provide robust summary data for fish, unless such data are provided in accordance with the test plan for DMDS (http://www.epa.gov/chemrtk/pubs/summaries/dimthdsl/c16161tc.htm) (the submitter's statement that DMDS *chronic* fish toxicity testing will be performed in conjunction with the previously submitted test plan for DMDS is incorrect and should be corrected to reference *acute* toxicity). The submitter of the DMDS test plan has informed EPA that it has completed testing for ecological effects and will provide robust summaries by June 30, 2010. That submitter has also provided EPA with the endpoint values. EPA judges the fish and other new values provided as tentatively adequate pending receipt of adequate robust

summaries, because they were performed according to standard OECD guidelines and the results are consistent with other reported values. In addition to the 96-hour rainbow trout LC50 value of 0.97 that already appears in Table 6, the DSO submitter needs to incorporate these values into the revised DSO test plan: Daphnid 48-hr LC50 = 1.82 and algal 72-hr EC50 = 14.3 (growth rate), 11 (biomass) (all values cited as Dr. U. NOACK Laboratorien, 2007). EPA will post the corresponding summaries at the Web link cited earlier in this paragraph when they become available.

General and Specific Comments on the Robust Summaries

General

The Test Plan refers to robust summaries on several endpoints using DSO constituents. The majority of these are with DMDS and the summaries are presented in Appendices III and IV. However, the robust summaries for all other data are missing:

- Physical chemical properties and environmental fate (discussed above).
- Health: acute toxicity data with DSO (Furedi-Machacek, 1991a-c and Drummond, 1991) and DPDS (cited as an MSDS from Chevron Phillips Chemical Co., 2005)); irritation and sensitization data with DSO (Furedi-Machacek, 1991d-f); repeated-dose toxicity data with DPDS (Posternak et al, 1969); and genetic toxicity data (Ames test) with DPDS (Tsai et al., 1996). In addition, a robust summary for the 90-day inhalation study with DMDS reported by Kim et al. (2006) is not included in either Appendix.

Agency files also contain the following TSCA Section 8(e) studies that should be included in the dataset for DSO.

- A Minimal Toxicological Study With Diethyl Disulfide in Rats (Fiche #: OTS0544426; Doc#: 88-920005643; Old#: 8EHQ-0792-6997)
- Acute Inhalation Toxicity Study with Diethyl Disulfide in Rats (Fiche #: OTS0544443; Doc#: 88-920005660S; Old#: 8EHQ-0792-7014S)
- A 10-Day Repeated Inhalation Toxicity Study with Diethyl Disulfide in Rats (Fiche #: OTS0540990;Doc#: 88-920005016S; Old#: 8EHQ-0892-6370S)

Finally, the submitter should be aware of the comments that the Agency posted to Arkema regarding some deficiencies in the robust summaries for DMDS (http://www.epa.gov/chemrtk/pubs/summaries/dimthdsl/c16161tc.htm)

Specific Comments: Health Effects

Reproductive/Developmental Toxicity. There needs to be a discussion in the robust summary section for reproductive toxicity about the evaluation of reproductive organs in the 90-day inhalation study with DMDS.

Followup Activity

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