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December 16, 2002

Christine Todd Whitman, Administrator  
U.S. Environmental Protection Agency  
Ariel Rios Building  
Room 3000, #1101-A  
1200 Pennsylvania Ave., N.W.  
Washington, DC 20460

Subject: Comments on the HPV Test Plan for the Mixed Xylenol Category

Dear Administrator Whitman:

The following comments on the Merisol USA LLC High Production Volume (HPV) Challenge test plan for the chemical class known as mixed xylenol isomers are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

Merisol submitted its test plan on July 29, 2002. The mixed xyleneol category is comprised of six xyleneol isomers, as follows:

Chemical	CAS Number
2,3-xyleneol	526750
2,4-xyleneol	105679
2,5-xyleneol	95874
2,6-xyleneol	576261
3,4-xyleneol	95658
3,5-xyleneol	108689

Since the mixed xylenols are generally moved into commerce as starting materials for further chemical processing, there is little consumer exposure. However, due to the large production volume of these chemicals, they are subject to the HPV program.

Overall, the test plan for mixed xylenols proposes limiting the amount of new animal testing by grouping the various isomers of xylenols into one testing category. While we agree with this approach, which results in fewer animals being used in the SIDS battery, we are

concerned that the proposed testing includes tests that are unjustified by any reasonable standard as noted below:

1. Acute oral toxicity study (OECD No. 425)
2. Combined repeat dose/reproductive/developmental study (OECD No. 422)
3. Mammalian erythrocyte micronucleus test (OECD No. 474)

These tests are clearly unnecessary. If this test plan is conducted in its present form, up to 810 animals will be killed. The inclusion of an acute fish toxicity study (OECD No. 203) would kill an additional 40 animals. Our objections are summarized later in these comments.

Merisol bases the chemical category of the xylenols on their chemical similarity, i.e., they are all di-methyl phenols substituted with 2 methyl groups in various positions on the phenolic ring, sharing the same molecular weight, or, in the case of the mixture, average molecular weight and the physical-chemical properties of the isomers are similar. In addition, as stated in the Merisol test plan, methyl phenols, known as cresols, “have demonstrated that the methyl phenol isomers are remarkably equivalent in toxicity and that binary and tertiary mixtures of cresol isomers do not produce toxic interactions among the isomers, i.e., mixtures of cresol isomers do not exhibit more than additive toxicity.” A similar pattern would be expected for the mixed phenols based on structural similarity among this group of isomers. We agree with this assessment.

In Table 4 of the Merisol test plan, the toxicity data on the various xylene isomers are summarized. It is clear that the data are already available on the following:

1. acute oral toxicity (five of six isomers have LD<sub>50</sub>'s ranging from 296 mg/kg/day in rats for 2,6-xylene to 2300 mg/kg in rats for 2,4-xylene);
2. repeat dose toxicity (two of six with lowest NOEL = 0.6 mg/kg/day in rats after 6 month oral exposure to 2,6-xylene);
3. negative Ames assays for five of six isomers;
4. negative *in-vivo* chromosomal aberration assay for one of six isomers (2,6-xylene) and
5. developmental toxicity for one of six isomers (rat maternal NOEL = 60 mg/kg/day and developmental NOEL = 180 mg/kg/day in rats with 2,6-xylene).

Although Merisol states in its plan that the available toxicity data, when viewed as a whole, strongly support the toxicological category for mixed xylenols, they nonetheless propose new testing using animals, specifically, acute oral, repeat-dose with reproductive/developmental screen, and mammalian erythrocyte tests, as well as acute oral toxicity in fish. These tests would be conducted using a mixture of equal parts for the six isomers. Our objections to these additional tests may be summarized as follows:

1. Merisol does not follow some of the main tenets laid out in the *Federal Register* notice in December 2000 regarding the development of test plans. It is not appropriate to create a testing category, reject the use of toxicity data on individual components to bridge between isomers, and conduct new animal tests on an arbitrary mixture of the individual components. The test plan appears to deliberately ignore information available on 2,4- and 2,6-xylenol that can reduce the use of animals in SIDS tests. The data available from the 3- and 8-month oral gavage studies, taken together, are sufficient to address subchronic toxicity of individual components and the mixture. The use of additional animals in a new repeat dose study is both unnecessary and violates the principles stated in the *Federal Register*. Perhaps Merisol is unaware of the guidance the EPA has provided to manufacturers in the development of test plans and the goals of minimizing the use of animals in the HPV program.
2. Moreover, a new LD<sub>50</sub> test on the mixture, when there are individual LD<sub>50</sub>'s on five of the six isomers, is absolutely unwarranted. Any new information gathered from this test will not enhance the understanding of the acute oral toxicity of the mixture, nor is it needed for the individual isomers, as they have already been tested. Merisol has stated in its own test plan that mixtures of cresols do not produce toxic interactions among the isomers and a similar pattern would be expected for the mixed xylenols. Thus, if Merisol insists on conducting an acute oral toxicity test for mixed xylenols mixture, we urge it to use *in-vitro* cytotoxicity assays. This approach was incorporated into the HPV program as a result of the National Toxicology Program- and National Institute of Environmental Health Sciences-sponsored *Workshop on International on In-Vitro Methods*, held on October 17-20, 2000. This workshop reviewed the validation status of available *in-vitro* methods for predicting acute oral toxicity (among other goals). As a result of this workshop, the EPA encouraged those participating in the HPV program to “consider using the recommended *in-vitro* tests...as a supplemental component in conducting any new *in-vivo* acute oral toxicity studies,...[and] to note the intention to use these protocols in the HPV Challenge test plans submitted to EPA...” The two *in-vitro* tests recommended are the neutral red uptake assays using the mouse fibroblast cell line BALB/c 3T3 and normal human keratinocytes. Guidance on these recommended *in-vitro* tests, protocols for their use, and a reporting template for results can be found on the ICCVAM Web site at <http://iccvam.niehs.nih.gov/docs/docs.htm#invitro>. Finally, although Merisol states that it “may” use alternative testing strategies, it should be noted that its proposal to use the traditional LD<sub>50</sub> is unacceptable under any circumstances as TG 401 is being deleted internationally.
3. The same principle applies to the mammalian micronucleus test proposed by Merisol in their test plan for the mixture of the six xylenols. With regard to the genotoxicity study with RBC's, there are negative Ames assays for five of the six isomers and a negative *in-vivo* chromosomal aberration assay for 2,6-xylenol. Additional testing using the RBC micronucleus test is not warranted on the mixture, although if Merisol wishes to conduct an *in-vitro* Ames bacterial

mutation assay or other *in-vitro* tests, at least no animals would be subjected to needless suffering to complete the study. Furthermore, in light of the negative results in separate Ames and *in-vivo* chromosomal aberration assays, no new *in-vivo* testing is warranted, especially in a screening level program. The December 2000 *Federal Register* notice states that genotoxicity testing should be conducted *in vitro* unless physical properties preclude use of such studies.

4. There are ample data available on acute fish toxicity, with EC<sub>50</sub>'s (ranging from 3 – 27 mg/L) available on five of six xylenols; additional testing with OECD No. 210 is not needed for the mixture.
5. Merisol makes note in its test plan (footnote 3) that one other company may come under the HPV program due to importation of 2,4-xylene in excess of one million pounds/year for 1999, 2000, and 2001. We encourage Merisol to coordinate any HPV work with others who may propose duplicative testing in animals. This approach is consistent with the EPA's stated goals of maximizing the use of existing data in order to limit additional animal testing. We have encouraged the EPA in past test plan comments to ensure inter-industry cooperation in the development of chemical categories and test plans, including comments on the American Petroleum Institute Petroleum Coke test plan, the Phosphite Producers HPV Consortium test plan on tris(nonylphenol)phosphite, and the General Electric test plan on p-cumylphenol. We are concerned that the EPA is not encouraging inter-company and inter-industry cooperation in the development of test plans and chemical categories, thus greatly increasing the number of animals killed in the HPV program.
6. We have identified other data, separate from the data summarized by Merisol in Table 4 and Appendix E, which apparently have not been included in the evaluation of the xylenols and may be useful in determining data gaps. An evaluation of all relevant information is required in the December 2000 *Federal Register*. The citations for this information are provided below, specifically:

Ten and 90-day toxicity studies of 2,4-dimethylphenol in Sprague-Dawley rats (Daniel et al., 1993; *Drug and Chemical Toxicology: An International Journal For Rapid Communication*, 16[4], 351-68).

Results of range-finding toxicological tests on 3,5-xylene (1987; EPA/OTS Doc. No. 86-870002229).

Health and environmental effects profile for dimethylphenols (Anon, 1988; *Govt Reports Announcement & Index [GRA&I, Issue 19]*).

Experimental data on assessing the toxicity of 2,6-dimethylphenol (Laronov, 1976; *GIG TR PROF ZABOL*; [4], 43-46).

Health effects assessment for dimethylphenols (EPA Working Group, 1987, EPA PG:30 p: EPA /600/8-88/031).

A thorough evaluation of all of the toxicity data as a whole would likely lend further support to what is known about the mixed xylenols and further obviate the need for additional animal testing under the HPV program. Such an evaluation is set forth as a principle in the *Federal Register*.

7. Finally, there is not a data vacuum surrounding mixed xylenols' reproductive and developmental toxicity, as a developmental toxicity study under GLP's has been conducted on 2,6-xylenol. Extensive testing has already been conducted on cresols, the toxicity database of which was used in part to justify the mixed xylenols category. The cresols are monomethyl phenols instead of dimethyl phenols. Thus, with everything that is known about the mixed xylenols and cresols, further testing of the xylenols for reproductive/developmental toxicity is unnecessary. An *in-vivo* study using up to 750 animals in stressful experiments is neither warranted nor justified. As an alternative to *in-vivo* testing, an *in-vitro* embryotoxicity test would be adequate to characterize any possible adverse reproductive effects of these materials. If, in fact, Merisol insists on further exploration of developmental endpoints, we urge it to consider the use of an *in-vitro* test for embryotoxicity (a critical endpoint in developmental toxicity) using the rodent Embryonic Stem Cell Test (EST) protocol that has been validated by the European Centre for the Validation of Alternative Methods (ECVAM). For additional information, please refer to E. Genschow et al., "The ECVAM international validation study on *in-vitro* embryotoxicity tests: results of the definitive phase and evaluation of prediction models" (*Alternatives to Laboratory Animals* 30:151-76, 2002). If a positive result is found, the substance should be treated as a developmental toxicant/teratogen, and no further testing should be conducted under the screening-level HPV program.

### **Summary:**

There are sufficient data on the individual xylene isomers, in conjunction with what is known from testing on cresols with regards to mixture interactions (or lack thereof), which renders any new animal tests with xylenols completely unnecessary. There are also additional data which have apparently not been considered in the development of the test plan. In spite of these facts, Merisol has proposed clearly unnecessary tests on animals and has failed to fully utilize the available toxicity data on xylenols to meet HPV SIDS requirements. Furthermore, conducting these new tests clearly violates Sections 1 and 8 of the animal protection agreement and the EPA's December 2000 *Federal Register* notice that states a) "In analyzing the adequacy of data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there are sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested" and b) "As with all chemicals, before generating new information, participants should further consider whether any additional information obtained would be useful or relevant." Conducting a new LD<sub>50</sub> study, a repeat dose/reproductive screening study, an *in-vivo* micronucleus test, and acute fish

toxicity tests on the mixture of xylenols violates the standard set forth in the *Federal Register*.

I look forward to a prompt and favorable response to our concerns. I may be reached at 202-686-2210, ext. 302, or via email at [csandusky@perm.org](mailto:csandusky@perm.org).

Sincerely,

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Senior Toxicologist