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PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

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Marianne L. Horinko, Acting Administrator
U.S. Environmental Protection Agency
Ariel Rios Bldg. (1101A)
1200 Pennsylvania Ave., NW
Washington, DC 20460

Re: Comments on the HPV test plan for formaldehyde, reaction product with tetrapropenyl phenol, methylamine and sulphur

Dear Acting Administrator Horinko:

The following are comments on the HPV test plan for formaldehyde, reaction product with tetrapropenyl phenol, methylamine and sulphur (CAS no. 68855-34-5), submitted by the American Chemistry Council (ACC). These comments are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health and environmental protection organizations have a combined membership of more than 10 million Americans.

This compound could have been excluded from the HPV program since it could be considered a polymer due to its high molecular weight and repeating units within the structure. Even if it is not strictly considered a polymer, its macromolecular properties (>1000 daltons) make this compound non-bioavailable and of no toxicological consequence when mixed with other, lighter compounds in lubricating oils. The ACC reports the results of acute toxicity tests with this substance in which toxic effects were not seen in rats and rabbits at the limit dose. Nevertheless, the ACC is planning to kill at least an additional 795 animals in an acute fish toxicity test (OECD 203) and a combined repeat-dose, reproductive and developmental toxicity test (OECD 422).

The acute fish toxicity test is clearly inappropriate since the octanol/water partition coefficient is unknown and most likely high. The EPA has stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2, and it recommends that with such highly hydrophobic compounds a chronic *Daphnia* test be used instead of the acute fish toxicity test (EPA, *Federal Register* 2000, pp. 81679, 81695). The log K_{ow} value of 68855-34-5 is unknown and is to be measured as one of the planned tests (p. 7). However, 68855-34-5 likely has a high log K_{ow} and low water solubility due to its great molecular weight (between 967 and 1167 daltons), which is more than twice the molecular weight of many compounds with log K_{ow} values greater than 4.2. Two examples include 2,5-furandione,3-(dodecenyl)dihydro-, reaction products with propylene oxide with a molecular weight of 458.64 daltons and a log K_{ow} of 4.6, and the lubricating basestocks oils category with average molecular weights from 300 to 500 daltons and log K_{ow} values all greater than 4.9. It is therefore premature to carry out a fish test. If the log K_{ow} is greater than 4.2, all further analysis of this endpoint using fish is unnecessary. If it is less than 4.2, appropriate structure activity relationships, exposure evaluation, and known chemistry data render the need for any fish toxicity testing unnecessary.

We note that the ACC also appears to have ignored the use of ECOSAR and the fact that running ECOSAR is a first step that should be conducted prior to making any decisions regarding the possible need for acute fish testing. After the ACC has determined the physical and chemical parameters of the substance and run ECOSAR, if ECOSAR yields ambiguous results and the ACC wishes to further investigate the acute fish toxicity of 68855-34-5, we urge it to use one or more of the several available *in vitro* and *in silico* methods (see Appendix I).

However, it should be further noted that 68855-34-5 exposure is associated with physical fouling. The sole commercial use of 68855-34-5 is as an additive to lubricants for use in railroad engines. The concentration of 68855-34-5 in these lubricants is only 1-3% so, although there is the possibility of marine and freshwater pollution with 68855-34-5, this will invariably be associated with pollution with a far larger volume of lubricant. Water pollution with lubricants results in the physical fouling of aquatic organisms, such as gill-coating in the case of fish, and lubricants also often form a surface sheen which can lead to oxygen deficiency. It is therefore highly probable that the physical fouling with which 68855-34-5 is associated is more severe than its toxicity. It therefore appears that determining the fish toxicity of 68855-34-5 is purely an academic exercise.

With respect to OECD 422, the ACC may well be correct in stating that no non-acute mammalian toxicity data are available. However, as in the case of many lubricant additives, including the compounds in this category, the high molecular weight, low solubility, and the fact that they are diluted in a relatively non-toxic oil base in most exposure scenarios limits the toxicity and bioavailability of these compounds and renders the toxicity analysis of them essentially moot. In the screening and prioritization process of the HPV program, it strikes us that the analysis of the toxicity of this category and similar compounds is quite easily identified without conducting further unreliable animal tests. Conducting additional tests for compounds such as these merely serves the check-the-box mentality which EPA purports to disavow.

Furthermore, the EPA has stated that "Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships" (Wayland 1999 and *Federal Register* 2000), and we are therefore concerned that no attempt to categorize 68855-34-5 with similar compounds appears to have been made. In a brief data search we found that numerous studies have been carried out on the toxicity of tetrapropenyl phenol, the principal compound from which 68855-34-5 is prepared. We therefore recommend that the ACC use the information obtained in these studies, together with the current state of knowledge about the relationship between polyphenolic structural chemistry and toxicity, to identify the compounds that can be expected to be of similar toxicity to 68855-34-5. In particular, we suggest that categorization with other calcium phenates and alkylated phenol sulfides is one avenue that should be explored. Numerous toxicity studies of these types of compound have been carried out, and several academic and governmental reports have been published (Faust 1984, Hewstone 1994). Lastly, the rodent embryonic stem cell test (Appendix II) can be used to supplement this information, thus sparing another 675 animals.

We note that the ACC plans to carry out an *in vitro* chromosomal aberration test (OECD 473; p. 14). This assay is most commonly carried out using Chinese hamster ovary cells. However, human lymphocytes can be used equally readily, and we hope that the ACC will use this option.

Thank you for your attention to these comments and we look forward to the ACC's response. I can be reached at 757-622-7382, ext 1304 or by email at JessicaS@PETA.org.

Sincerely,

Jessica Sandler
Federal Agency Liaison

Appendix I: Alternatives to the acute fish toxicity test

With respect to *in silico* methods, several quantitative structure activity relationship (QSAR) programs for estimating toxicity to fish and other aquatic organisms are available. The EPA itself encourages the use of one established QSAR: ECOSAR (EPA 2002).

With respect to *in vitro* methods, TETRATOX, an assay based on the *protozoan Tetrahymena pyriformis* (Larsen 1997), is the most appropriate. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997). The extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After almost two years, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for action rather than words.

The recently validated *Dar*T test is another prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, the *Dar*T test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for

immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

Appendix II: Alternatives to the *in vivo* developmental toxicity test

An *in vitro* embryotoxicity test method, the rodent embryonic stem cell test, has been validated by the European Centre for the Validation of Alternative Methods, and the Centre's Scientific Advisory Committee has concluded that this test is ready to be considered for regulatory purposes (Genschow 2002). Although we have written to the EPA repeatedly, for more than a year, about its inclusion in the HPV Program, we have received no detailed reply. This test is available commercially in the U.S. and we urge the ACC to correspond directly with the EPA about the feasibility of incorporating the rodent embryonic stem cell test. At the very least, the ACC should consider carrying out this test before undertaking an *in vivo* test, as it is relatively inexpensive, at about $10,000 per compound, including both range-finding and definitive tests. If the result is clearly positive, 68855-34-5 should be treated as a developmental toxicant, and no further testing should then be carried out within the HPV program, which is intended to be a screening-level program.

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