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Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
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PETA

PEOPLE FOR THE ETHICAL
TREATMENT OF ANIMALS

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Re: Comments on the HPV test plan for benzene phosphorous dichloride (BPD)
and benzene phosphinic acid (BPA)

Dear Administrator Leavitt:

The following comments on the BPD/BPA Coalition's test plan for BPD (CAS 644-97-3) and BPA (CAS 1779-48-2) 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 ten million Americans.

We commend the BPD/BPA Coalition for its decision not to carry out additional mammalian studies on BPD or BPA. This decision was based primarily on the fact that the primary toxicity of BPA is its caustic action in the gastrointestinal tract, due to its highly acidic properties, and testing dilute solutions would not be relevant to the toxicity of BPA in industrial use (test plan, pp. 5-6). The BPD/BPA Coalition's decision represents an appropriate application of the following ruling from the EPA:

In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested. (Wayland, 1999)

We are grateful that the BPD/BPA Coalition took animal welfare into consideration in reaching this decision.

At the same time, we disagree with the BPD/BPA Coalition's proposal to carry out an acute toxicity test on fish, which will kill at least 40 animals per test conducted. We are pleased that the Coalition has used ECOSAR as a first step, and has predicted BPD to be slightly toxic to fish (summaries, p. 9). However, we suggest that the Coalition, continuing its avoidance of animal experiments, use *in vitro* methods for its assessment of fish toxicity.

The recently validated *DarT* Test (Nagel 2002) is a prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte and Nagel (1994) and Nagel (1998). Briefly, the *DarT* test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish; because the eggs will not hatch during the test period, the *DarT* is classified as a non-animal test. The exposure period is 48-hours, and assessed endpoints include

coagulation, development of blastula, gastrulation, termination of gastrulation, development of somites, movements, extension of the tail, development of eyes, heartbeat, circulation, heart rate, pigmentation, and edema. Endpoints comparable to lethality *in vivo* include failure to complete gastrulation after 12-hours, no somites after 16-hours, no heartbeat after 48-hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of the effects of test substances. The reliability and relevance of the *DarT* test have recently been confirmed through an international, multi-laboratory validation study coordinated and financed by the German Environmental Protection Agency; and predictions of acute toxicity from the *DarT* test were highly concordant with *in vivo* reference data (Schulte *et al.* 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 *et al.* 1995), and has since been nominated for development into an OECD Test Guideline. It is clearly suitable for immediate use as a replacement to the use of fish in SIDS screening studies.

Another promising *in vitro* assay is TETRATOX. In this assay, the protozoan *Tetrahymena pyriformis* is used as a biomarker for acute lethality in fish (Schultz 1997). The biochemistry and physiology of *T. pyriformis* have been thoroughly investigated since the 1950s and this assay has been used, in various forms, for aquatic toxicity testing since the 1970s (Sinks & Schultz 2001). In this test, a range-finding study followed by three replicate definitive tests is performed for each test substance. Each treatment replicate consists of a minimum of five different concentrations per substance tested; thus, at least 30 data points comprise each analysis. The current, standardised protocol is for a 40-hour static test, which provides for multigenerational exposure. Range-finding tests are also included to allow an accurate approximation of both the highest concentration with no observed effect on population growth and the lowest concentration with total inhibition of cell replication. Output measures from the TETRATOX assay are the 50 percent inhibitory growth concentration (IGC50, mmol/L) and the 95 percent fiducial interval. The current TETRATOX database includes more than 2,000 industrial organic chemicals, including over 800 aliphatic chemicals, 900 aromatic chemicals, 400 neutral narcotics, and 400 direct-acting electrophiles, among others (Schultz, personal communication). The TETRATOX protocol has now been standardised and has undergone a preliminary ring test (Larsen *et al.* 1997). The German EPA is currently funding a second, more elaborate ring test, with the goal of establishing an OECD Test Guideline. In the interim, data generated by TETRATOX demonstrate a consistently high degree of concordance to data from *in vivo* acute studies in fish, which supports the use of this assay as a prospective replacement for toxicity studies in fish (Seward *et al.* 2001).

In addition, the ecologic significance of fish tests should be taken into consideration. Ecotoxicity and mammalian toxicity tests have different purposes: mammalian tests are assumed to be useful for predicting toxicity in individual humans, whereas fish tests are not intended to predict toxicity in individual fish, but to predict economic loss to commercial and “sport” fisheries, and ecologic damage. The fish test therefore aims to show whether exposure to BPA or BPD will result in large-scale fish death. However, because water pollution kills the food on which fish subsist, it can deplete fish populations even with no direct fish toxicity. Carp and catfish are herbivorous, eating mostly algae, whereas most other familiar North American freshwater fish species are carnivorous, eating worms, small crustaceans, smaller fish, insect larvae, etc. The toxicity of BPA/BPD towards these types of organism is unknown, as shown by the inclusion in

the test plan of tests on aquatic invertebrates and algae. Fish tests should not be carried out while other types of aquatic toxicity are uncertain.

Finally, the BPD/BPA Coalition proposes to conduct an *in vitro* chromosomal aberration test on BPA and, conditionally, on BPD. The Coalition does not specify the cells it intends to use, and we therefore urge it to use either human lymphocytes or Chinese hamster ovary cells from an established and immortal cell line.

Please feel free to contact me at 757-622-7382, ext. 8001, or via e-mail at JessicaS@peta.org.

Sincerely,

Jessica Sandler
Federal Agency Liaison

References □

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