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Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
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HEADQUARTERS
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Re: HPV test plan for diallyldimethylammonium chloride (DADMAC)

Dear Administrator Leavitt:

The following comments on the HPV test plan for DADMAC (CAS no. 7398-69-8), prepared by the DADMAC HPV Committee, 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.

DADMAC is an ionic quaternary ammonium monomer which is used in the manufacture of water-soluble cationic polymers for use as coagulants. The DADMAC HPV Committee is planning to conduct an acute fish toxicity test. On the assumption that this will be OECD test no. 203, it can be expected to kill at least 120 fish.

Our central objection to the proposed test is that an acute fish toxicity test has already been carried out, as stated in the test plan (p. 4), and the 72-hour LC_{50} of DADMAC was found to be 56 mg/L. The DADMAC HPV Committee provides no explanation as to why this test was unsatisfactory but, rather, states that it was "valid" and "comparable with guidelines/standards" (IUCLID data set, p. 14). There is therefore absolutely no justification for the DADMAC HPV Committee to conduct an additional fish test.

It would appear that the DADMAC HPV Committee wishes to carry out an additional fish test because the existing study was conducted with *Lepomis macrochirus*, which is highly intolerant of pollution (Lerczak and Sparks 1995), whereas the proposed test is to be with *Pimephales promelas*, which is one of the most pollution-tolerant North American freshwater fish species (Rice and Michael). It should go without saying that this approach is entirely unacceptable, from both a scientific and environmental standpoint, and that the toxicity obtained with *Lepomis macrochirus* should be the definitive value for regulatory, HPV, and other purposes.¹

¹ As a general note, the ecologic significance of fish testing should be taken into consideration. Ecotoxicity and mammalian toxicity tests have different purposes: mammalian tests are often considered 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 proposed fish test therefore aims to show whether exposure to DADMAC will result in large-scale fish death. However, because water pollution kills the food on which fish subsist, such as invertebrates and algae, it can deplete fish populations even with no direct fish toxicity. The toxicity of DADMAC to invertebrates and algae is uncertain, as shown by the inclusion in the test plan of tests on these organisms (p. 4). It is premature to carry out fish tests while other types of aquatic toxicity are uncertain.

I can be reached at 757-622-7382, ext. 8001, or via e-mail at JessicaS@peta.org.

Sincerely,

Jessica Sandler
Federal Agency Liaison

References

Friccius, T., Schulte, C., Ensenbach, U., Seel, P. and Nagel, R., "Der Embryotest mit dem Zebraabräbling: Eine neue Möglichkeit zur Prüfung und Bewertung der Toxizität von Abwasserproben", *Vom Wasser* 84:407-418, 1995.

Larsen, J., Schultz, T.W., Rasmussen, L., Hoofman, R. and Pauli, W., "Progress in an ecotoxicological standard protocol with protozoa: results from a pilot ring test with *Tetrahymena pyriformis*", *Chemosphere* 35:1023-1041, 1997.

Lerczak, T.V. and Sparks, R.E., "Fish populations in the Illinois River", <http://biology.usgs.gov/s+t/noframe/m2137.htm>. On *Our Living Resources* website, National Biological Service, US Dept. of the Interior, 1995.

Nagel, R., *Umweltchemikalien und Fische: Beiträge zu einer Bewertung*, Johannes Gutenberg-Universität, Mainz, 1998.

Nagel, R., "DarT: the embryo test with the zebrafish *Danio rerio*: A general model in ecotoxicology and toxicology", *ALTEX* 19 (Suppl. 1): 38-48, 2002.

Rice, D. and Michael, M., "Fish of Plumbrook Station", <http://osat-ext.grc.nasa.gov/emo/pub/PSMS/Volume%20I/D/D%20Fish-PBS.pdf>. On website of NASA Glenn Research Center at Lewis Field, Environmental Management Office.

Schulte, C. and Nagel, R., "Testing acute toxicity in the embryo in zebrafish, *Brachydanio rerio*, as an alternative to the acute fish test: preliminary results", *ATLA* 22:12-19, 1994.

Schulte, C., Bachmann, J., Flidner, A., Meinelt, T. and Nagel, R., "Testing acute toxicity in the

In addition, several *in vitro* test methods are now available. One example is the DarT Test (Nagel 2002; Schulte and Nagel 1994; Nagel 1998), which involves the use of fertilized fish eggs as a surrogate for living fish. This test has been confirmed to be reliable and relevant by the German Environmental Protection Agency (Schulte *et al.* 1996), and has therefore been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius *et al.* 1995). A second example is the TETRATOX assay, based on the protozoan *Tetrahymena pyriformis* (Schultz 1997; Sinks and Schultz 2001). The TETRATOX protocol has been standardized (Larsen *et al.* 1997), and the German Environmental Protection Agency is currently funding research with the goal of establishing an OECD test guideline. The DADMAC HPV Committee can feel free to ask us for more information about either of these tests.

embryo of zebrafish (*Brachydanio rerio*): An alternative to the fish acute toxicity test”, *Proceedings of the 2nd World Congress on Alternatives and Animal Use in the Life Sciences*, Netherlands, Utrecht, 1996.

Schultz, T.W. “TETRATOX: *Tetrahymena pyriformis* population growth impairment endpoint: A surrogate for fish lethality”, *Toxicological Methods* 7:289-309, 1997.

Sinks, G.D. and Schultz, T.W., “Correlation of *Tetrahymena* and *Pimephales* toxicity: Evaluation of 100 additional compounds”, *Environmental Toxicology and Chemistry* 20:917-921, 2001.