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Statement on EPA Estrogenic Screening Results for Nonylphenol and Nonylphenol Ethoxylates

July 14, 2010 – EPA recently released results from the first round of the Agency's toxicity testing on oil dispersant products, which showed that none of the eight dispersants tested, including those that contain nonylphenol ethoxylate (NPE) surfactants, displayed biologically significant endocrine disrupting activity. [1] [2]

EPA used *in vitro* reporter gene assays, which are rapid lab-bench tests conducted in specially developed cell lines. The assay results determine, at a screening level, whether test compounds are capable of binding with hormone receptors and, if so, how their binding potency relates to natural human hormones, such as the estrogen, 17β-estradiol.

In addition to screening the dispersant products, EPA also conducted limited testing directly on NPEs and two forms of nonylphenol (NP), branched and linear. Branched NP can be an intermediate formed during the biodegradation of commercial NPEs in the environment. Linear NP is laboratory chemical that is not used in commerce and is not a degradant found in the environment.

In the EPA screening tests the estrogenic activity for branched NP ranged from 250 to 80,000-fold less potent than 17β-estradiol. Results previously reported in the published literature found branched NP to be one thousand to one million-fold less potent than 17β-estradiol, depending on which assay was used. Short-chain NPE degradants (NPE1 and NPE2) have been found to be less estrogenic than NP, while longer chain NPE (NPEn≥4), which are most commonly found in commercial products, appear to have little to no estrogenic activity.[3] [4] [5] [6] [7] [8] [9] [10] These results are from a variety of *in vitro* tests that measure biomarkers of exposure to estrogens (*e.g.*, vitellogenin induction in fish liver cells) and *in vivo* tests, which measured estrogen mediated changes in living fish or rodents. EPA's screening results appear to be fairly consistent with these previously reported results.

Across all aquatic organisms, the weight-of-evidence shows that commercial NPEs (*e.g.*, NPE9) are much less toxic than NP. [11,12] In 2006, EPA established Water Quality Criteria (WQC) for NP, the most toxic of the NPE environmental degradants. [13] WQC are surface water concentrations that, when met, will protect aquatic life. EPA develops WQC based on aquatic toxicity studies that measure adverse effects, such as growth, survival, and reproduction. [14] These types of effects can have broader impacts on species, such as population reductions.

EPA's WQC document for NP states:

> *"Whole organism endpoints such as reproductive and growth effects are used to derive aquatic life ambient water quality criteria for nonylphenol. To the extent that such endpoints reflect the integration of molecular, biochemical and tissue-level effects at the whole organism level, the nonylphenol criteria address the estrogenicity of nonylphenol."* [15]

It is important to remember that the results of an *in vitro* assay are not directly relevant to exposures and effects in living organisms; bathing cells in a chemical solution is not the same as exposing living fish or other aquatic organisms in the lab or out in the environment. However, it is interesting to note that EPA's *in vitro* estrogenic screening results for NP continue to support EPA's chronic WQC for NP with effect concentrations that are 7 to 23 times greater than the chronic freshwater (6.6 µg/L) criteria and 28 to 88 times greater than the chronic saltwater criteria (1.7 µg/L).[16]

The US EPA WQC for NP were based on study results on ecological effects of this compound available through 2005. Studies published since that time continue to support the US EPA WQC.[17]

EPA's WQC for NP, in conjunction with the extensive body of data available for NPE, provide EPA with a more than adequate basis to assess the risk of these compounds in the aquatic environment.

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The mission of the APE Research Council, which is composed of manufacturers, processors and raw material suppliers of alkylphenols (AP) and alkylphenol derivatives (e.g., alkylphenol ethoxylates (APE)), is to promote the safe use of AP and AP derivatives through research, product stewardship and outreach efforts, within the framework of responsible chemical management. For more information about AP and APE go to www.aperc.org.

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[1] U.S. Environmental Protection Agency (US EPA). (Accessed 2010, June 30). Statement: EPA's Toxicity Testing of Dispersants. http://www.epa.gov/bpspill/dispersants-testing.html

[2] U.S. Environmental Protection Agency (US EPA) Office of Research and Development (ORD). (2010, June 30). Analysis of Eight Oil Spill Dispersants Using In Vitro Tests for Endocrine and Other Biological Activity. http://www.epa.gov/bpspill/reports/EPADispersantInVitroReport30june2010FINALx.pdf

[3] Routledge, E.J., & Sumpter, J.P. (1996). Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry, 15 (3), 241-248.
[4] Lee, P.C., & Lee, W. (1996). In vivo estrogenic action of nonylphenol in immature female rats. Bulletin of Environmental Contamination and Toxicology, 57, 341-348.
[5] Islinger, M., Pawlowski, S., Hollert, H., Völkl, A., & Braunbeck, T. (1999). Measurement of vitellogenin-mRNA expression in primary cultures of rainbow trout hepatocytes in a non-radioactive dot blot/RNAse protection-assay. The Science of the Total Environment, 233, 109-122.
[6] Jobling, S., & Sumpter J.P. (1993). Detergent components in sewage effluent are weakly oestrogenic to fish: An in vitro study using rainbow trout (Oncorhynchus mykiss) hepatocyte. Aquatic Toxicology, 27, 361-372.
[7] Dussault, E.B., Sherry, J.P., Lee, H.B., Burnison, B.K., Bennie, D.T., & Servos, M.R. (2005). In vivo estrogenicity of nonylphenol and its ethoxylates in the Canadian environment. Human and Ecological Risk Assessment, 11 (2), 353–364
[8] Balch, G., & Metcalfe, C. (2006). Developmental effects in Japanese medaka (Oryzias latipes) exposed to nonylphenol ethoxylates and their degradation products. Chemosphere, 62 (8), 1214-1223.
[9] Metcalfe, C.D., Metcalfe, T.L., Kiparissis, Y., Koenig, B.G., Khan, C., Hughes, R.J., Croley, T.R., March, R.E., & Potter, T. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry, 20, 297–308.
[10] US Environmental Protection Agency (US EPA). (2006, February 23). Notice of availability of final aquatic life ambient water quality criteria for nonylphenol. Federal Register, 71 (36), 9337-9339. http://www.epa.gov/EPA-WATER/2006/February/Day-23/w2558.htm.
[11] Staples, C.A., Weeks, J., Hall, J.F., & Naylor, C.G. (1998). Evaluation of aquatic toxicity and bioaccumulation of C8- and C9-alkylphenol ethoxylates. Environmental Toxicology and Chemistry, 17, 2470-2480.
[12] Staples, C., Mihaich, E., Carbone, J., Woodburn, K., & Klečka, G. (2004). A weight of evidence analysis of the chronic ecotoxicity of nonylphenol ethoxylates, nonylphenol ether carboxylates, and nonylphenol. Human and Ecological Risk Assessment, 10 (6), 999-1017.
[13]US EPA. (2006, February 23).
[14] US EPA. (2006, February 23).
[15] US Environmental Protection Agency (US EPA). (2005). Aquatic life ambient water quality criteria - nonylphenol. Report 822-R-05-005. US Environmental Protection Agency, Washington, DC, USA. http://www.epa.gov/waterscience/criteria/nonylphenol/final-doc.pdf
[16] In-vitro test results are expressed as AC50 or EC50, the concentration associated with the activity at the 50th percent point on the concentration response curve. Based on the concentration-response curves obtained by EPA using the Attagene, Inc. ERα assay, the AC50 values for branched NP were 0.68 and 0.61 μM for the ERα trans and ERα cis assays, respectively. Using a molecular weight of 220 g/mol for 4-br-NP, concentrations of 134 to 150 μg/L are calculated. Based on the results in EPA's ER Agonist Assay T47D-KBluc, the log EC50 for branched NP was reported as -6.657 M. Using a molecular weight of 220 g/mol for 4-br-NP, concentrations of 48.46 μg/L are calculated.
[17] Coady, K., Staples, C. Losey, B., and Klecka, G. (2009). A Hazard Assessment of Aggregate Exposure to Nonylphenol and Nonylphenol Mono- and Di-ethoxylates in the Aquatic Environment. Human and Ecological Risk Assessment. In press