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Mr. Frank Gostomski
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Dear Mr. Gostomski:

The Alkylphenols & Ethoxylates Research Council (APERC)[1] has reviewed the comments that EPA received in response to the January 5, 2004 proposal for Ambient Aquatic Life Water Quality Criteria (WQC) for Nonylphenol (NP) (69 Fed. Reg. 340) and appreciates this opportunity to submit supplemental comments for the Agency's consideration.

Some of the commenters question the adequacy of the database that EPA relied on to develop the proposed WQC for NP and encourage EPA to undertake additional reviews and analyses before finalizing the NP WQC. APERC is concerned that if EPA decides to undertake additional literature searches and to recalculate the WQC, it could significantly delay the issuance of the WQC and will not result in any substantive change in the values. For this reason, APERC maintains the position expressed in our previous comments[2] that EPA proceed expeditiously to finalize these criteria so they can be considered by states and tribes in setting their state specific standards. The Agency could subsequently undertake additional literature searches and recalculations at a later stage, and if warranted, propose revisions to the WQC.

Adequacy of EPA's Literature Search and Selection of Studies

In support of its proposed aquatic life WQC for NP, EPA conducted an exhaustive literature review and included one hundred and eighty eight references in its assessment of the acute and chronic effects of NP to a broad range of aquatic species. While it is true that NP is the subject of numerous new scientific publications every year, this does not necessitate recalculation of the WQC. Acute and chronic effect levels for NP in aquatic species have been generally consistent over the years indicating that the current database of studies adequately represents the range of effects for this compound in a broad range

[1] Members of APERC include: Dover Chemical Corporation; Crompton Corporation; Great Lakes Chemical Corporation; Huntsman LLC; Rhodia Inc.; Rohm and Haas Company; Schenectady International, Inc.; and, The Dow Chemical Company.
[2] Alkylphenols & Ethoxylates Research Council (APERC) Comments (OW-2003-0080-0015).

of species. Since the WQC calculations are statistically based, the available database of fresh water aquatic effects is sufficiently large and robust that there would be little impact from incorporating additional studies in the WQC calculations. In fact, even if the results of some of the newer studies were materially different, it would not substantially change the WQC values. Indeed, the draft Criteria document that underlies the current proposal contains more than 100 references that were not considered in the draft April 1997 Criteria document, with only a very small change to the WQC values.

Nonetheless, we have reviewed each of the studies that the US Fish and Wildlife Service asserts were either overlooked by EPA or were published after the analysis was completed. Brief comments on each of these 18 studies are included in Attachment I. Based on our assessment, we do not believe that these studies warrant reconsideration of the proposed WQC values. Several of the studies do not conform to EPA's Guidelines for Deriving Numerical National WQC.[3] Of particular note, several of these studies relate to nonstandard protocols or studies reporting histological effects in the absence of standard acute or chronic endpoints.

Additionally, as noted in APERC's previous comments, EPA should modify the "endocrine" section (pages 4-5) of the NP WQC document which currently includes statements that are not grounded with the same degree of scientific support as the rest of the document. Commenter suggestions that EPA should consider studies that show "possible endocrine effects at levels below the draft criteria value,"[4] indicate that there is confusion about the difference between histological responses and/or other "endocrine" effects versus standard endpoints. In its previous comments, APERC provided several specific suggestions to clarify the endocrine discussion in the NP WQC document for EPA's consideration.

Analytical Methods

The National Park Service[5] points out the need for analytical methods "to ensure that comparisons with the criteria are valid" and recommends that other methods and QA/QC issues should be discussed. Similarly, the Hampton Roads Sanitary District, Virginia Beach, VA suggests that "EPA must provide promulgated procedures that are capable of producing reliable data at the concentration of these criteria before these criteria can be finalized or the NPDES permitting process for this parameter will be unreliable and inconsistent and fail to adequately support the intent of the Clean Water Act."[6] These are very legitimate concerns given the proliferation of new methods and test kits to measure NP in environmental waters, some of which may be subject to

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[3] Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. US Environmental Protection Agency. PB85-227049.
[4] US Fish and Wildlife Service Comments (OW-2003-0080-0021).
[5] National Park Service Comments (OW-2003-0080-0002).
[6] Hampton Roads Sanitation District (Virginia Beach, VA) Comments (OW-2003-0080-0012).

interferences. APERC agrees that providing references to analytical methods that are suitable for the measurement of NP in environmental waters would be a useful addition to the WQC document for NP.

Fortunately, there are well established, validated analytical methods that are suitable for measuring NP in environmental waters already available. APERC, EPA Region V, the US Department of Agriculture and the US Geological Survey have each developed analytical methods for the measurement of NP in environmental waters and/or wastewater effluents.[7,8,9,10] While a formal round robin comparison has never been conducted, field monitoring results derived using these methods generally provide consistent results.[11,12,13] In anticipation of the need for states and municipalities to measure aquatic levels of NP relative to EPA's proposed WQC, APERC and the above mentioned Agencies have been coordinating on the validation of a simple, robust, analytical method that is both time and cost-effective. The draft method, "Standard Test Method for Determination of Nonylphenol and Nonylphenol Ethoxylates in Environmental Waters" is under the review of ASTM Subcommittee D-19. Additional information on the ASTM analytical method and validation process is located at www.astm.org. In addition, analytical standards for NP are available through commercial sources.

Summary

EPA's proposed WQC for NP were calculated using statistical methods, based on an exhaustive literature review. It is unlikely that incorporating additional studies will have a significant impact on the final derived values. The existing database used to calculate the NP WQC adequately reflects the latest scientific knowledge about the effects of this compound.

In addition, adequate analytical methods exist to provide reliable monitoring results relative to the proposed WQC for this compound. Therefore, APERC encourages

[7] Method of Test and Standard Operating Procedure for Determination of Nonylphenol and Nonylphenoxyethanol in Environmental Water by Steam Distillation and High-performance Liquid Chromatography. Huntsman Method No. ST-38.34-94. 1994.
[8] EPA CRL Method GCMS004.
[9] Brown, G.K., S.D. Zaugg and L.B. Barber. 1998. Wastewater Analysis by Gas Chromatography/Mass Spectrometry. (http://toxics.usgs.gov/pubs/wri99-4018/Volume2/sectionD/2506_Brown/pdf/2506_Brown.pdf)
[10] Loyo-Rosales, J.E., I. Schmitz-Afonso, C.P. Rice and A. Torrents. 2003. Analysis of Octyl- and Nonylphenol and Their Ethoxylates in Water and Sediments by Liquid Chromatography-tandem Mass Spectrometry. Anal. Chem. 75:4811-4817.
[11] Weeks, J.A., W.J. Adams, P.D. Guiney, J.F. Hall and C.G. Naylor. 1996. Risk Assessment of Nonylphenol and its Ethoxylates in U.S. River Water and Sediment. The Alkylphenols & Alkylphenol Ethoxylates Review. 1:64-74.
[12] Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber and H.T. Buxton. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in US Streams, 1999-2000: A National Reconnaissance. Environ. Sci. Technol. 36:1202-1211.
[13] Rice, C.P., I. Schmitz-Afonso, J.E. Loyo-Rosales, E. Link, R. Thoma, L. Fay, D. Altfater and M.J. Camp. 2003. Alkylphenol and Alkylphenol-Ethoxylates in Carp, Water, and Sediment from the Cuyahoga River, Ohio. Environ. Sci. Technol. 37:3747-3754.

EPA not to delay issuance of the NP WQC so that states, tribe and local regulatory agencies will have the benefit of clear guidance about protective levels of this compound in the aquatic environment.

Please let me know if we can provide any further clarification on any of these issues.

Sincerely,

Robert J. Fensterheim
Executive Director

ATTACHMENT I

Brief comments on literature cited by US Fish and Wildlife Services

1. Arsenault, J.T.M., W.L. Fairchild, D.L. MacLatchy, L. Burridge, K. Haya and S.B. Brown. 2004. Effects of Water-borne 4-nonylphenol and 17-estradiol Exposures During Parr-smolt Transformation on Growth and Plasma IGF-I of Atlantic Salmon (*Salmo salar* L.). Aquatic Toxicology. 66:255-265. – This paper reports on the effects of NP during parr-smolt transformation on growth and plasma IGF-I effects in the marine Atlantic salmon (*Salmo salar*). Effects were reported only at 20 μg/L NP and higher. The draft saltwater criteria is 1.4 μg/L.

2. Arukwe, A., B.M. Nilsen, K. Berg and A. Goksoyr. 1999. Immunohistochemical Analysis of the Vitellogenin Response in the Liver of Atlantic Salmon Exposed to Environmental Oestrogens. Biomarkers. 4:373-380. – This paper reports on the immunohistochemical analyses of liver vitellogenin (VTG) induction in the marine Atlantic salmon (*Salmo salar*). VTG is a biomarker of exposure to potential endocrine active substances, but is not a toxicological effect.

3. Beechman, R.K. 1999. Effect of the Endocrine Disruptor Nonylphenol on the Marine Copepod *Tisbe battagliai*. The Science of the Total Environment. 233:33-46. – The marine copepod *Tisbe battagliai* was exposed in a life cycle study to NP. Life table parameters were calculated. The NOEC was 31 μg/L for all life table parameters, as well as parent generation and F1 offspring sex ratios. The draft saltwater criteria is 1.4 μg/L.

4. Brown, R.J., M. Conradi and M.H. Depledge. 1999. Long-Term Exposure to 4-nonyphenol Affects Sexual Differentiation and Growth of the Amphipod *Corophium volutator* (Pallas, 1766). The Science of the Total Environment. 233:77-88. – The marine amphipod *Corophium volutator* was acutely exposed to NP for 96 hours with a reported 96-h LC50 of 1670 μg/L. Juvenile offspring were raised to adults with exposure to NP reporting LOECs for density and growth of 10 μg/L. The draft saltwater criteria is 1.4 μg/L.

5. Christensen, L.J., B. Korsgaard and P. Bjerregaard. 1999. The Effect of 4-nonylphenol on the Synthesis of Vitellogenin in the Flounder *Platichthys flesus*. Aquatic Toxiocology. 211-219. – This paper reports on the induction of VTG in flounder *Platichthys flesus*. VTG is a biomarker of exposure to potential endocrine active substances, but is not a toxicological effect.

6. Hecht, S. and B.L. Boese. 2002. Sensitivity of an Infaunal Amphipod, *Eohaustorius estuarius*, to Acute Waterborne Exposures of 4-nonylphenol: Evidence of a Toxic Hangover. Environmental Toxicology and Chemistry. 21:816-819. – The acute toxicity of NP to the estuarine amphipod *Eohaustorius estuarius* was measured, reporting a 96-h

LC50 of 227 μg/L. The sublethal effect of reburial into sediment was measured at 1-h, 24-h, and 48-h, with EC50 values of 138 to 163 μg/L reported. The draft saltwater criteria is 1.4 μg/L.

7. Hecht, S.A., J.S. Gunnarsson, B.L. Boese, J.O. Lamberson, C. Schaffner, W. Giger and P.C. Jepson. 2004. Influences of Sedimentary Organic Matter Quality on the Bioaccumulation of 4-nonylphenol by Estuarine Amphipods. Environmental Toxicology and Chemistry. 23:865-873. – The bioaccumulation potential of NP to the estuarine amphipod *Eohaustorius estuarius* was reported. Bioaccumulation factors do not affect the calculation of a water quality criteria, but rather are important supplemental information.

8. Hoyt, P.R., M.J. Doktycz, K.L. Beattie and M.S. Greeley Jr. 2003. DNA Miroarrays Detect 4-nonylphenol-induced Alterations in Gene Expression During Zebrafish Early Development. Ecotoxicology. 12:469-74. – DNA microarrays were used to detect alterations in gene expression during the early development stages in the freshwater fish zebrafish. These endpoints are biomarkers of potential exposure to compounds that affect growth and development, but are not themselves toxicological effects.

9. Moore, A., A.P. Scott, N. Lowera, I. Katsiadaki and L. Greenwood. 2003. The Effects of 4-nonylphenol and Atrazine on Atlantic Salmon (*Salmo salar* L.) Smolts. Aquaulture. 222:253-263. – The induction of VTG in Atlantic salmon *Salmo salar* was reported as was the induction of altered gill ion-activity. These endpoints are biomarkers of exposure to potentially endocrine active compounds, but are not themselves toxicological effects.

10. Nice, H.E., M.C. Thorndyke, D. Morritt, S. Steele and M. Crane. 2000. Development of *Crassostrea gigas* Larvae is Affected by 4-nonylphenol. Marine Pollution Bulletin. 40:491-496. – Larvae of Pacific oysters *Crassostrea gigas* were exposed for 72 hours to NP in concentrations ranging from 0.1 to 100 μg/L. Development rate was reduced at 1 μg/L and higher in the first 56 hours, but had completely recovered by 72 hours at all treatments except 100 μg/L. There were no significant delays in development by 72 hours in the next three lower concentrations, 0.1 to 10 μg/L. The saltwater criterion of 1.4 μg/L would be protective of oysters.

11. Nice, H.E., D. Morritt, M. Crane and M. Thorndyke. 2003. Long-term and Transgenerational Effects of Nonylphenol Exposure at a Key Stage in the Development of *Crassostrea gigas*. Possible Endocrine Disruption? Marine Ecology Progress Series. 256:293-300. – Pacific oysters *Crassostrea gigas* were raised from fertilization until sexually mature (10 months). Larval oysters were exposed to NP (1 and 100 μg/L) from days 7 to 8 under static conditions. At one month and again at two months, oysters were transferred to larger and larger flow through tanks. No effects on growth rate, length, wet weight or sex ratio were reported. Hermaphroditic oysters were 17% and 30% of the total oysters from the 1 and 100 μg/L exposure, respectively. However, the number of oysters on which the histology was performed was n=6 (1 μg/L) and n=17 (100 μg/L). Thus, only one oyster was hermaphroditic among those exposed to 1 μg/L and five

oysters among those exposed to 100 μg/L. Gametes from the exposed adult oysters were significantly less viable at both concentrations. NP disappeared in the 1μg/L tanks by 6 hours. It is unclear that these oysters were actually exposed to NP. In addition, Pacific oysters can change their sex and low levels of hermaphrodism occur in wild populations. Due to the very small sample sizes and uncertain exposure, especially at the lower concentration, it is unclear how to interpret these data.

12. Rice, C.P., I. Schmitz-Afonso, J.E. Loyo-Rosales, E. Link, R. Thoma, L. Fay, D. Altfater and M.J. Camp. 2003. Alkylphenol and Alkylphenol-ethoxylates in Carp, Water, and Sediment from the Cuyahoga River, Ohio. Environmental Science and Technology. 37:3747-3754. – The concentrations of nonylphenol ethoxylates and their biodegradation intermediates were analyzed in water, sediment and biota collected in the Cuyahoga River in Ohio. Environmental concentrations are useful for conducting risk assessment but are not used to calculate a water quality criteria.

13. Uguz, C., M. Iscan, A. Erguven, B. Isgor and I. Togan. 2003. The Bioaccumulation of Nonylphenol and its Adverse Effect on the Liver of Rainbow Trout (*Onchorynchus mykiss*). Environmental Research. 92:262-270. – The bioaccumulation of NP was measured in the freshwater rainbow trout *Oncorhynchus mykiss*. In addition, hepatic VTG induction was determined. Bioaccumulation factors do not affect the calculation of a water quality criteria, but rather are important supplemental information. VTG induction is a biomarker of exposure to potentially endocrine active compounds, but is not a toxicological effect.

14. Zhang, L. and K.N. Baer. 2001. The Effects of 4-nonylphenol on Reproduction and Embryo Development in *Daphia magna*. Toxicologist. 60:163. – The pelagic invertebrate, waterflea *Daphnia magna* was exposed to NP for either 35 days for chronic studies and 3 days for embryo development studies. In the chronic study, exposure to NP at 50 μg/L significantly increased total fecundity and neonate deformities. However, no significant increases in sex ratios were reported. NP at 200 and 800 μg/L caused neonate deformities in 3-day embryo studies, while at 1000 μg/L, embryo development was arrested. The draft criteria for freshwater is 5.9 μg/L. This document is only an abstract from a recent Society of Toxicology meeting.

15. Ashfield, L.A., T.G. Pottinger and J.P. Sumpter. 1998. Exposure of Female Juvenile Rainbow Trout to Alkylphenolic Compounds Results in Modifications to Growth and Ovosomatic Index. Environmental Toxicology and Chemistry. 17:679-686. – Rainbow trout *Oncorhynchus mykiss* were exposed to NP for 30 days and then allowed to grow for over 400 days. Inconsistent, slight and transitory results on growth were reported at 10 but not 1 μg/L. The authors state that they are not sure the findings are meaningful.

16. Bennie, D.T. 1999. Review of the Environmental Occurrence of Alkylphenols and Alkylphenol Ethoxylates. Water Quality Research Journal of Canada. 34:79-122. – This paper is a comprehensive review of environmental monitoring data for nonylphenol

ethoxylates and their biodegradation intermediates. Environmental concentrations are useful for conducting risk assessment but are not used to calculate a water quality criteria.

17. Miles-Richardson, S.R., S.L. Pierens, K.M. Nichols, V.J. Kramer, E.M. Snyder, S.A. Snyder, J.A. Render, S.D. Fitzgerald and J.P. Giesy. 1999. Effects of Waterborne Exposure to 4-nonylphenol and Nonylphenol Ethoxylate on Secondary Sex Characteristics and Gonads of Fathead Minnows (*Pimephales promelas*). Environmental Research (Series A). 80:S122-S137. – Breeding fathead minnows *Pimephales promelas* were exposed for 42 days to NP. Two experiments were conducted. At day 42 of each study, gonadal histology was examined in males using a qualitative scoring criteria. Apparently significant differences in male gonadal histology were found at the highest concentrations tested (3.4 and 2.4 μg/L for experiments 1 and 2) as compared to controls. However, the sample size of the 3.4 μg/L treatment was n=4 fish (exp. 1) and of the 2.4 μg/L treatment was n=2 fish (exp. 2). In exp. 1, survival was 100% at all concentrations and controls. In exp. 2, survival was reduced to 50% in controls as well as the highest concentration of NP. The second experiment would likely not be considered an acceptable study to use for calculation of a criteria due to the high control mortality. In addition, gonadal histology is not typically considered a population-level endpoint that are used to develop criteria. There already are GLP studies available for NP with fathead minnows. The addition of another fathead minnow study would not alter the draft criteria significantly, if at all.

18. Schwaiger, J., U. Mallow, H. Ferling, S. Knoeer, T. Braunbeck, W. Kalbfus and R.D. Negele. 2002. How Estrogenic is Nonylphenol: A Transgenerational Study Using Rainbow Trout (*Oncorhynchus mykiss*) as a Test Organism. Aquatic Toxicology. 59:177-189. – Rainbow trout *Oncorhynchus mykiss* were exposed to NP (0, 1, 10 μg/L) for 10 days per month for four months. VTG analyses in adult males was significantly increased at 1 and 10 μg/L. Gonadal histology was not affected at any concentration. Eggs from the exposed adults were used to breed F1 trout. Apparently, significant effects on survival of the earliest stages of egg development occurred at 1 μg/L, however this mortality was only 10%. Later-staged eggs had significantly reduced survival and hatch only at 10 μg/L. Survival of later-staged eggs was actually greater than in controls (10% vs. 15%), although not significantly better. Egg hatch rate was reduced in F1 eggs from F0 adults exposed to 10 μg/L (60% vs. 80% hatch rate in controls). Hatchlings were raised to adults (3 years). No effects on survival, growth or development were reported. Histology revealed no testis-ova formation or change in sex ratio in F1 offspring (from exposed F0 vs. control F0 adults). Some fluctuations (increases or decreases as compared to controls) of sex steroid levels occurred in F1 offspring from F0 adults exposed to 10 μg/L. Thus the most significant effects were the reduced hatch success at 10 μg/L. These results are similar to another GLP chronic early life stage test with trout that reported NOEC/LOEC of 6/10 μg/L. The proposed freshwater criteria of 5.9 μg/L is protective of rainbow trout.