

**Comments Of The  
Alkylphenols & Ethoxylates Research Council  
On The  
July 10, 2007 Federal Register Notice Regarding The  
TSCA Section 21 Petition on Nonylphenol and Nonylphenol Ethoxylates  
Docket ID Number: EPA-HQ-OPPT-2007-0490  
Submitted July 25, 2007**

**I. INTRODUCTION**

The Sierra Club, the Environmental Law & Policy Center, the Pacific Coast Federation of Fishermen's Association, the Washington Toxics Coalition, Physicians for Social Responsibility, and UNITE HERE jointly filed a citizen's petition pursuant to section 21 of the Toxic Substances Control Act (TSCA) that seeks to direct EPA to initiate rulemakings on nonylphenol (NP) and nonylphenol ethoxylates (NPE). The petition calls on EPA to:

- Require manufacturers and importers to conduct specific health and safety studies pursuant to TSCA section 4;
- Require labeling on products containing NP/NPE pursuant to TSCA section 6; and,
- Limit the use of NP/NPE in certain circumstances pursuant to TSCA section 6.

In accordance with the 90-day statutory deadline, EPA must either grant or deny the petition by September 4, 2007.

TSCA section 21 authorizes any person to petition the Administrator to initiate a proceeding under defined sections of the Act. The statute further specifies, "if the Administrator grants the petition, the Administrator shall promptly commence an appropriate proceeding..." On the other hand, if the Administrator denies the petition, EPA is obligated to publish in the Federal Register the Administrator's reasons for such denial.

If the Administrator denies a petition (or actually fails to deny or grant within the specified 90-day period) the petitioner may commence a civil action in a district court of the United States to compel the action requested in the petition. Any such request for judicial review must be filed within 60 days after the Agency's denial. To be successful in Court, the petitioner must demonstrate "to the satisfaction of the court by a preponderance of the evidence" that:

- 1) In the case of a petition to initiate a proceeding under TSCA section 4 that:
  - "the information available to the Administrator is insufficient to permit a reasoned evaluation of the health and environmental effects of a chemical substance" to be subject to such rule or order; and,

- “in the absence of such information, the substance may present an unreasonable risk to health or the environment, or the substance is or will be produced in substantial quantities and it enters or may reasonably be anticipated to enter the environment in substantial quantities or there is or may be significant or substantial human exposure to it.”
- 2) In the case of a petition to initiate a proceeding for the issuance of a rule under section 6 or 8 that “there is a reasonable basis to conclude” that the issuance of such a rule or order is necessary to protect health or the environment against an unreasonable risk.

In a July 10 Federal Register notice (72 Fed. Reg. 37520), the Agency solicited information on the issues raised by the petition. The following comments are submitted on behalf of the Alkylphenols & Ethoxylates Research Council (APERC). APERC represents the major US manufacturers of NP and NPE, and as such has significant interest in this proceeding.

As described further below, APERC contends that EPA should deny the petition in its entirety, given the extensive effort that EPA has already devoted to reviewing NP/NPE and the more than adequate information that already exists to permit a “reasoned evaluation of the health and environmental effects” from exposure to these compounds. Additionally, the available information does not support the conclusion that additional TSCA regulations are necessary to protect human health or the environment.

## **II. GENERAL ISSUES**

### **A. There Is No Justification To Initiate A Section 4 Rulemaking on NP/NPE**

NP and NPE are some of the most studied compounds in commerce today. Interest in the biodegradation profile and environmental fate and effects (including weak estrogenic activity of NP) has prompted literally hundreds of studies by government, industry and academia - the majority of which have been published in the peer-reviewed literature. These studies assess fate and effects for mammalian and non-mammalian species, environmental levels and possible exposure to humans. It is significant to note that one of the most extensive studies on the effects of repeat exposure to NP is a rat study conducted by the National Center for Toxicological Research, which evaluated exposure to NP over three generations - far longer than studies that exist for most other materials.

The adequacy of the available test data has been the subject of review for over 10 years by the TSCA Interagency Testing Committee (ITC). ITC is charged with identifying “chemicals regulated by TSCA for which there are suspicions of toxicity or exposure and for which there are few, if any, ecological effects, environmental fate or health effects

testing data.”<sup>1</sup> Chemicals are recommended for testing to meet the data needs of the ITC's 16 US government member organizations.<sup>2</sup>

Through the ITC, the review of the adequacy of information on NP/NPE has been examined by the majority of federal regulatory and research agencies as well as by EPA. Furthermore, to facilitate discussions, ITC established a Subcommittee on alkylphenols that has had an ongoing dialogue with APERC. Those discussions have provided a constructive framework for EPA, and the other agencies, to maintain a current awareness of the state of the science on NP/NPE and numerous other alkylphenolic compounds. The alkylphenol industry remains committed to working with ITC including continuing discussions, if need be, on possible data needs, which in many instances go well beyond the type of data needs that EPA can address pursuant to TSCA section 4.

Assessing the adequacy of data has also been addressed by other governments and international organizations. Where a convincing case has been shown regarding the need for additional data, the alkylphenol industry has voluntarily allocated the resources to develop the necessary information.

It would be a waste of taxpayer resources to initiate a TSCA section 4 proceeding to assess the adequacy of data given the large number of studies available on these compounds, which undoubtedly far exceeds that for the vast majority of compounds governed by TSCA. Moreover, many of the issues that the petitioners have raised cannot be addressed by routine testing of individual compounds, but rather are generic scientific questions that have been and will continue to be the subject of research by the industrial and scientific community.

#### B. EPA Has Already Assessed And Addressed Risk Management Controls On NP/NPE

In addition to assessing data adequacy, several EPA offices have assessed the need for regulatory control of NP and NPE. Of particular relevance is the Risk Management review (RM1) conducted by the TSCA Office in the late 1990's that considered the information available at that time on effects and exposure.<sup>3</sup> Among the data reviewed were the results of the “30 Rivers Study” conducted to determine the levels of NP and NPE in water and sediments within the US.<sup>4</sup> The study employed a statistical design, developed in collaboration with the Office of Toxic Substances (OTS), to define the

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<sup>1</sup> TSCA Interagency Testing Committee (ITC). <http://www.epa.gov/opptintr/itc/>.

<sup>2</sup> Members Agencies of the ITC: Agency for Toxic Substances and Disease Registry (ATSDR); Council on Environmental Quality (CEQ); Consumer Product Safety Commission (CPSC); US Department of Commerce (DOC); Department of Defense (DoD); US Department of the Interior (DOI); US Environmental Protection Agency (EPA); Food and Drug Administration (FDA); National Cancer Institute (NCI); National Institute of Environmental Health Sciences (NIEHS); National Institute for Occupational Safety and Health (NIOSH); National Library of Medicine (NLM); National Science Foundation (NSF); National Toxicology Program (NTP); Occupational Safety & Health Administration (OSHA); US Department of Agriculture (USDA).

<sup>3</sup> Rodier, D. (1996). RM-1 Document for para-Nonylphenol. US EPA, CSRAD, Washington, DC.

<sup>4</sup> Radian Corporation. (1990). Nonylphenol and Nonylphenol Ethoxylates in River Water and Bottom Sediments. Prepared for Chemical Manufacturers Association, Washington, DC.

number of sampling points necessary to characterize the ambient levels of NP and NPE in US surface waters.

EPA's RM1 report highlights the low levels of NP concentrations in the environment. The assessment found that the highest values were associated with broader pollution in contaminated rivers. The RM1 report concluded that while 4-NP is toxic to aquatic organisms, "overall, the risks due to acute and chronic effects to pelagic organisms do not appear to be widespread." It is significant to note that while considerable more data are available today than at the time the OTS undertook its RM1 evaluation, there is no basis to change the conclusions from the RM1 assessment.

These compounds have also been the subject of extensive reviews (some of which are continuing) by EPA's Office of Pesticide Programs (OPP) and most comprehensively, by the Office of Water (OW).

Because of the use of NPE as an inert ingredient in pesticide formulations, NPE was recently reviewed by OPP as part of its statutorily mandated Tolerance Reassessment program. That assessment focused on potential human health effects to workers handling pesticide products containing NPE as well as consumers that may come in contact with residues of NPE from the use of these pesticides on food crops. That review, supported NPE's exemption from the requirement for a tolerance for residues under the Food Quality Protection Act (FQPA).<sup>5</sup> The assessment, completed in August 2006, concluded that there is "reasonable certainty of no harm to any population subgroup with special emphasis on infants and children."

Perhaps the most significant review of the available data was conducted by the Office of Water in support of its Aquatic Life Ambient Water Quality Criteria (WQC) for NP (71 Fed. Reg. 9337; February 23, 2006).<sup>6</sup> Section 304(a)(1) of the Clean Water Act requires that EPA establish water quality criteria "that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water." As noted by EPA, the WQC assessment is "based upon consideration of comments received from independent peer reviewers and the public."

Ambient WQC establish levels of a pollutant or other measurable substance in water that, when met, will protect aquatic life. In 2006, EPA set both acute and chronic criteria for both fresh and saline waters:

Acute WQC for NP - 28.0 µg/L (fresh water) and 7.0 µg/L (salt water); and,  
Chronic WQC for NP - are 6.6 µg/L (fresh water) and 1.7 µg/L (salt water).

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<sup>5</sup> US EPA, Office of Prevention, Pesticides and Toxic Substances. (2006, July 31). Action Memorandum from Pauline Wagner, Chief, Inert Ingredient Assessment Branch, Registration Division to Lois Ross, Director, Registration Division. Inert Reassessments: Four Exemptions from the Requirement of a Tolerance for Nonylphenol Ethoxylates. <http://www.epa.gov/opprd001/inerts/nonylphenol.pdf>.

<sup>6</sup> US EPA. (2006, February 23.) Notice of Availability of Final Aquatic Life Ambient Water Quality Criteria for Nonylphenol. Federal Register, Vol. 71, No. 36. <http://www.epa.gov/fedrgstr/EPA-WATER/2006/February/Day-23/w2558.htm>.

The supporting document for the WQC assessment was finalized in December 2005 and as such reflects much of the recent information on these compounds.<sup>7</sup> EPA used whole organism endpoints such as reproduction and growth effects to derive the WQC values. Since these endpoints reflect the culmination of molecular, biochemical and tissue-level effects at the whole organism level, the NP WQC also addresses all mechanisms of action - including estrogenic effects. In addition, the WQC document for NP EPA concludes:

*the ability of nonylphenol to induce estrogenic effects has seldom been reported at concentrations below the freshwater Final Chronic Value of 6.5965 µg/L.*

The establishment of the WQC for NP set in motion an elaborate risk management program under the Clean Water Act to ensure that levels of NP in US waters are below the federal WQC values. Under the Clean Water Act, states with primacy are obligated, as appropriate and necessary, to adopt local corresponding WQCs and to impose discharge limitations through National Pollutant Discharge Elimination System (NPDES) permits or other similar instruments to ensure that ambient levels remain below the WQC.

Since the adoption of the WQC in 2006, many states have not yet begun to adopt state specific standards. At the same time, based on the available monitoring results, there is little reason to believe that environmental levels in the US are above the federal WQC for NP. (See section III item 9 below.)

### **III. SPECIFIC ISSUES RAISED IN THE PETITION**

The petition seeks eleven specific actions relating to testing and risk management activities under TSCA sections 4 and 6. Following are APERC's comments in response to the various actions.

#### **Items 1 and 2. Filling the gaps for chronic toxicity of NPE oligomers and testing the additive toxicity of NP and NPE oligomers to [aquatic] species**

The petition recommends additional chronic aquatic toxicity testing on NPE oligomers along with studies using test systems with combined exposure to NP, NPE1 and NPE2 since they may co-occur in surface waters. The petition specifically recommends aquatic toxicity studies for the purpose of filling the gaps for chronic toxicity of NPE oligomers:

*Aquatic toxicity of nonylphenol ethoxylates increases with decreasing length of the ethoxylate chain. To date, there is a lack of data on chronic toxicity of short-chain NPE. Such data is necessary for development of protective chronic water quality criteria and standards that account for the full range of negative impacts from NP and NPEs.*

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<sup>7</sup> US EPA. (2005, December). Aquatic Life Ambient Water Quality Criteria - Nonylphenol. Final Report 822-R-05-005. <http://www.epa.gov/waterscience/criteria/nonylphenol/final-doc.pdf>.

The petition also recommends filling the gaps regarding the additive toxicity of NP and NPE oligomers to [aquatic] species:

*Research supports that NP, NP1EO and NP2EO occur in mixtures in the aquatic environment. These substances have a common mode of toxicity, narcosis, such that their effects are treated properly as additive” (CEQG, 2002) Thus to adequately protect water quality criteria and standards should be set by summing the impacts of these chemicals. In order to set a water quality standard based on additivity, states such as Illinois require studies of combinations of substances on at least three species of ecologically diverse taxa. EPA recently released a study on the additive acute toxicity of NP, NP1EO and NP2EO to Pimephales promelas (fathead minnow) and Ceriodaphnia dubia. Petitioners request that EPA require research on a third species from a different taxa e.g. Hyalella azteca (an amphipod) to serve as the basis for additive NP and NPE water quality standards.*

Since the testing recommended under items 1 and 2 in the petition relate to degradation intermediates of NPE found in surface waters, it is useful to understand their presence in the environment. All NPE products are complex chemical substances that contain oligomer species of varying levels of ethoxylation that fall in a normal distribution with both the mean and average at the target ethoxylation level. (As such when commercial NPE products are used as test substances in toxicity studies, the test organisms are exposed to the entire distribution of oligomers.) The most common NPE product in commerce is manufactured to a target of nine moles of ethoxylation (NPE9) with a range of oligomers ranging from NPE1 to NPE>17. Due to their use patterns, the vast majority of NPE are discharged into wastewater treatment systems after use, which has been shown to be effective in reducing loads entering the environment.<sup>8,9,10</sup> Biodegradation has been shown to be the dominant mechanism for removal of NPE and during its degradation pathway, the low mole ethoxylate degradants, NPE1 and NPE2 predominate.<sup>11,12,13,14</sup> NP develops primarily via anaerobic biodegradation pathways and is therefore only a minor metabolite in aerobic wastewater treatment systems (Maguire et al., 1999; Melcer et al., 2007).

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<sup>8</sup> Drewes, J.E., Hemming, J., Ladenburger, S.J., Schauer, J., and Sonzogni, W. (2005). An Assessment of Endocrine Disrupting Activity Changes during Wastewater Treatment Through the Use of Bioassays and Chemical Measurements. Water Environ. Res., 77 (1), 12-23.

<sup>9</sup> Esperanza, M., Suidan, M.T., Nishimura, F., Wang, Z., Sorial, G., Zaffiro, A., McCauley, P., Brenner, R., and Sayles, G. (2004). Determination of Sex Hormones and Nonylphenol Ethoxylates in the Aqueous Matrices of Two Pilot-Scale Municipal Wastewater Treatment Plants. Environ. Sci. Technol., 38 (11), 3028-3035.

<sup>10</sup> Melcer, H., Klečka, G., Monteith, H., and Staples, C. (2007). Wastewater Treatment of Alkylphenols & Their Ethoxylates: A State of the Science Review. Water Environment Federation, Alexandria, VA.

<sup>11</sup> Ahel, M., Giger, W., and Koch, M. (1994). Behavior of Alkylphenol Polyethoxylate Surfactants in the Aquatic Environment – I. Occurrence and Transformation in Sewage Treatment. Wat. Res., 28 (5), 1131-1142.

<sup>12</sup> Staples, C.A., Williams, J.B., Blessing, R.L., and Varineau, P. (1999). Measuring the Biodegradability of Nonylphenol Ether Carboxylates, Octylphenol Ether Carboxylates and Nonylphenol. Chemosphere, 38 (9), 2029-2039.

<sup>13</sup> Staples, C.A., Naylor, C.G., Williams, J.B., and Gledhill, W.E. (2001). Ultimate Biodegradation of Alkylphenol Ethoxylate Surfactants and their Biodegradation Intermediates. Environmental Toxicology and Chemistry, 20, 2450-2455.

<sup>14</sup> Melcer, H. (2007).

Numerous and robust chronic ecotoxicity data exist for NP and many are summarized in EPA's technical Support Document for the NP Water Quality Criteria.<sup>15</sup> Chronic studies for NP as well as other NPE degradates, including NPE1 and NPE2 are well summarized by Staples et al. (2004) and Servos et al. (1999).<sup>16,17</sup> Attachment I presents a list of the studies that were reviewed in the Staples et al. (2004) paper and which examined chronic population-level effects to aquatic organisms with various NPE products and their degradates including NPE1, NPE2, NP and nonylphenol ether carboxylate (NPEC). For each study, the table provides a description of the study conditions, the reported effects and relevant concentrations as well as the study quality scores derived by Staples et al. (2004). As is readily apparent, studies of the chronic ecotoxicity effects of NPE1, NPE2 and NPE1.5 (a 1:1 mixture of NPE1 and NPE2) exist in at least four aquatic species.

For those compounds that do not have specific data, the US EPA and other regulatory bodies in Canada and the EU have embraced the concept of "read-across" for the extrapolation of toxicity among compounds with similar structural features and modes of action. The concept is simply that structural features such as ethoxylate chain length relates to toxicity in a predictable fashion. As noted by the petitioners, for NPE this relationship is diminishing acute toxicity with increasing ethoxylate chain length. For example, the acute toxicity of untested NPE oligomers or mixtures can be estimated using read-across from the abundant data that are available for many NPE. Key studies demonstrating this relationship of acute toxicity with NP and NPE1-40 include Yoshimura (1986) and Hall et al. (1989).<sup>18,19</sup> More recently, Sun and Gu (2005) and TenEyck (2006) also conducted acute tests with NP and low-mole NPE1 and NPE2 that further support the relationship.<sup>20,21</sup>

Staples et al. (2004) documented the relationship of diminishing toxicity with increasing ethoxylate chain length for the NPE compounds tested in longer term studies. As Staples et al. (2004) noted, chronic values for NPE9 and higher to at least NPE18, based on a variety of population-level endpoints related to survival, growth and development, and reproduction, ranged from about 900 to 14,000  $\mu\text{g/L}$ . Chronic values for the relatively insensitive NPEC1 were similar to those of NPE9, ranging from 3,216 to 11,950  $\mu\text{g/L}$ . For NPE1 and NPE2 chronic values varied from 11 to 500  $\mu\text{g/L}$ . Collectively, these data

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<sup>15</sup> US EPA (2005).

<sup>16</sup> Staples, C., Mihaich, E., Carbone, J., Woodburn, K., and Klecka, 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, 999-1017.

<sup>17</sup> Servos, MR. (1999). Review of the Aquatic Toxicity, Estrogenic Responses and Bioaccumulation of Alkylphenols and Alkylphenol Polyethoxylates. Water Qual. Res. J. Canada, 34 (1), 123-177.

<sup>18</sup> Yoshimura, K. (1986). Biodegradation and Fish Toxicity of Nonionic Surfactants. JAOCS, 63 (12), 1590-1596.

<sup>19</sup> Hall, W.S., Patoczka, J.B., Miranda, R.J., Porter, B.A., Miller, E. (1989). Acute Toxicity of Industrial Surfactants to *Mysidopsis bahia*. Arch. Environ. Contam. Toxicol., 18, 765-772.

<sup>20</sup> Sun, H. and Gu, X. (2005). Comprehensive Toxicity Study of Nonylphenol and Short-chain Nonylphenol Polyethoxylates on *Daphnia magna*. Bull. Environ. Contam. Toxicol., 75, 677-683.

<sup>21</sup> TenEyck, M. and Markee, T. (2006). Additive Toxicity of Nonylphenol, Nonylphenol Monoethoxylate, and Nonylphenol Diethoxylate to Selected Freshwater Species, *Pimephales promelas* (Fathead Minnow) and *Ceriodaphnia dubia*. Unpublished study by Lake Superior Research Institute, University of Wisconsin-Superior, WI. Prepared for EPA Region 5.

can be used to assess the chronic aquatic toxicity of NP and NPE without having to conduct studies of the individual degradates that have not been tested.

It is also feasible to rely on acute toxicity values in order to predict possible effects from chronic exposure. For example, the chronic WQC by EPA relied on both acute and chronic toxicity data.<sup>22,23</sup> The chronic WQC for NP was calculated from the acute WQC using an acute-to-chronic ratio based on data for the same species (e.g., fathead minnows, *Daphnia magna*). Thus collectively, the database of acute and chronic aquatic toxicity studies is sufficient and can be reliably used to assess chronic effects of any of the biodegradation intermediates found in surface waters. (See Staples et al. (1998), Servos (1999) and Staples et al. (2004) for a comprehensive list of literature assessing the aquatic toxicity of NPE and their degradation intermediates.)

The petitioners also request the development of test data to assess the additive toxicity of different oligomers to [aquatic] species. They attempt to justify this testing need by citing the guidance provided by the State of Illinois, which calls for testing organisms simultaneously exposed to two or more chemicals of interest.

The question of how to address the toxicity of mixtures is certainly of interest to states and to EPA; however, there is no justification for requiring the conduct of additional toxicity studies to assess environmental effects given that there are sufficient data available to predict aggregate effects of these compounds. Various traditional methods are available to address the cumulative toxicity of two or more constituents of a mixture of NPE oligomers.

One method is the use of the Toxic Units approach that was employed by Sun and Gu (2005) in their assessment of the cumulative toxicity of mixtures of NP, NPE1 and NPE2 to medaka.<sup>24</sup> A similar approach was adapted by Environment Canada (EC) and the Canadian Council of Ministers of Environment (CCME) in their comprehensive regulatory assessment of NPE and their biodegradation intermediates.<sup>25,26</sup> This approach involves the use of relative toxicity factors, i.e., relating the toxicity of the very comprehensively studied NP for which a chronic toxicity threshold has been established, relative to NPE degradation intermediates (lower NPE, higher NPE, and NPECs). Environment Canada referred to the relative toxicity factors as toxic equivalency quotients (TEQ). Canada concluded that there was adequate data to establish TEQ for

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<sup>22</sup> Stephan, C.E., Mount, D.I., Hansen, D.J., Gentile, J.H., Chapman, G.A., and Brungs, W.A. (1985). Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. PB85-227049. US EPA, Washington, DC.

<sup>23</sup> US EPA (2005).

<sup>24</sup> Sun, H. and Gu, X. (2005).

<sup>25</sup> Environment Canada and Health Canada (EC and HC). (2001). Priority Substances List Report: Nonylphenol and its Ethoxylates. <http://www.ec.gc.ca/substances/ese/eng/psap/final/npe.cfm>.

<sup>26</sup> Canadian Council of Ministers of the Environment (CCME). (2001). Canadian Water Quality Guidelines for the Protection of Aquatic Life: Nonylphenol and its Ethoxylates. Excerpt from Publication No. 1299; ISBN 1-896997-34-1. <http://www.c2p2online.com/documents/CEQGnonylphenolwater.pdf>

NP, NPE1 and NPE2, as well as higher NPE and NPEC. In addition, Fenner et al. (2002) used a similar approach to assess the toxicity of NP and NPE in Swiss rivers.<sup>27</sup>

EC/CCME used data from two fish species: fathead minnows *Pimephales promela* and the killifish *Oryzias latipes*, and three invertebrate species: two water fleas *Daphnia magna*, and *Ceriodaphnia dubia*, and the mysid shrimp *Mysidopsis bahia*. The fish and mysids are sexually reproducing organisms, while the daphnid species reproduce asexually most of the time.<sup>28,29</sup> EC (2001) was also able to use the available data to develop a method for calculating the additive toxicity of these compounds.

In addition, as noted in the petition, EPA Region 5 recently sponsored a study of the relative toxicity of NP, NPE1 and NPE2 with *Pimephales promelas* (fathead minnow) and *Ceriodaphnia dubia*<sup>30</sup> and other studies of the relative toxicity and/or additivity of NPE degradation intermediates exist in the published literature and generally support the Canadian TEQ approach.<sup>31,32,33,34</sup>

In summary, adequate aquatic studies exist to allow the Agency to assess the potential acute and chronic toxicity of NPE and their biodegradation intermediates. In addition, the abundant available data can also be used to assess the cumulative toxicity of mixtures of NPE degradation intermediates through the use of established assessment methods. Therefore, there is no justification for additional studies on the chronic aquatic toxicity of NP and NPE.

### **Item 3. Research on individual endocrine disruption impacts and on the relationship between individual endocrine disruption impacts and pollution-level impacts**

The petition's suggestion that studies should be conducted to understand "individual endocrine impacts" and their relationship to "population-level impacts," while academically interesting, is not a chemical-specific issue. This request relates to generic research to test the hypothesis that screening assays for endocrine activity are predictive of effects relating to apical endpoints in individual organisms and/or population-level effects. These questions relate to many compounds, including the natural hormones found in the human waste stream.

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<sup>27</sup> Fenner, K., Kooijman, C., Scheringer, M., and Hungerbuhler, K. (2002). Including Transformation Products into the Risk Assessment for Chemicals: The Case of Nonylphenol Ethoxylate Usage in Switzerland. Environ. Sci. Technol., 36, 1147-1154.

<sup>28</sup> EC and HC (2001).

<sup>29</sup> CCME (2001).

<sup>30</sup> TenEyck, M. and Markee, T. (2006).

<sup>31</sup> Yoshimura, K. (1986).

<sup>32</sup> Hall, W.S. (1989).

<sup>33</sup> Ankley, G.T., Peterson, G.S., Lukasewycz, M.T., and Jensen, D.A. (1990). Characteristics of Surfactants in Toxicity Identification Evaluations. Chemosphere, 21, 3-12.

<sup>34</sup> Balch, G. and Metcalfe, C. (2006). Developmental Effects in Japanese Medaka (*Oryzias latipes*) Exposed to Nonylphenol Ethoxylates and their Degradation Products. Chemosphere, 62, 1214-1223.

EPA has an existing and extensive research program partially examining these questions. In 1996, EPA's Office of Research and Development (ORD) identified endocrine disruption as one of its top six research priorities. ORD developed a risk-based research program based on a peer-reviewed Research Plan published in 1998 that includes the following long-term goals:<sup>35</sup>

- *Providing a better understanding of the science underlying the effects, exposure, assessment, and risk management of endocrine disruptors.*
- *Determining the extent of the impact of endocrine disruptors on humans, wildlife, and the environment.*

Understanding the relationship between mechanistic endpoints (e.g., vitellogenin gene expression) or biomarkers (e.g., vitellogenin induction) and adverse effects in individuals (e.g., reproduction or developmental effects) or population effects (e.g., declining species populations), while interesting and useful in investigating the endocrine issue generically, is not necessary for the assessment of either the hazard or the risk of a particular compound. This is especially true in the case of NP, a compound that has been extensively tested using existing, validated test protocols and studied in risk assessment processes that adequately define the hazards to individuals and/or their risk at the population level. In fact, in the case of NP, governmental assessments have found that traditional apical endpoints are more sensitive than endocrine-mediated endpoints.<sup>36,37,38</sup> These existing higher-level studies, which are based on dose-response and adverse health or ecotoxicity effects, account for effects resulting from all modes of action (including endocrine). Even if the mode of action of a substance is not completely established, these more traditional studies provide a better measure of the hazard and/or risk of any compound - including NP/NPE. Neither the petition nor the extensive available data on NP/NPE support a conclusion that NP/NPE represent a risk to the environment for any reason - including endocrine mediated effects.

#### **Item 4. Testing for vitellogenin gene expression**

The petition notes "EPA's Office of Research and Development has developed a vitellogenin gene expression assay as an indicator of estrogen exposure" and requests the Agency initiate a rulemaking to "require that the assay be applied to NP and each individual NPE."

As noted in the petition, the purpose of vitellogenin gene expression tests is to serve as an indicator of an estrogen agonist exposure; these assays have not been demonstrated to be predictive of other estrogenic mediated effects. More importantly, existing studies are

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<sup>35</sup> US EPA. (1998). Endocrine Disruptors Research Initiative. <http://www.epa.gov/endocrine/>.

<sup>36</sup> US EPA (2005).

<sup>37</sup> EC and HC (2001).

<sup>38</sup> European Union (EU). (2002). European Union Risk Assessment Report: 4-Nonylphenol (branched) and Nonylphenol. [http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK\\_ASSESSMENT/REPORT/4-nonylphenol\\_nonylphenolreport017.pdf](http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/4-nonylphenol_nonylphenolreport017.pdf).

available using other robust proven systems to test NPE and their environmentally relevant degradation intermediates for endpoints mediated by estrogenic activity.

Attachment II provides a table summarizing many of the available studies that have addressed vitellogenin (VTG) related endpoints. Uncertainties about the chemical purity of the test material in some of the VTG studies published in the early 1990s called into question these early results. Noting “the relative estrogenicity of nonylphenol and its ethoxylates has not been clearly demonstrated” in the previous literature, Dussault et al. (2005) tested the relative estrogenic activity of NP, NPE1 and NPEC. They used commercially representative material and accepted methodologies for measuring estrogenicity using *Oncorhynchus mykiss* (rainbow trout) in a 21-day vitellogenin induction study.<sup>39</sup> This study, which was sponsored in part by Environment Canada, found that plasma VTG induction occurred in fish only at water concentrations of NP that were well in excess of EPA’s chronic WQC of 6.6 µg/L. Specifically, the study found plasma VTG induction in fish exposed to NP started to appear at water concentrations of 13 µg/L but more typically occurred at water concentrations of 25 µg/L of NP. Based on the results of this study, the authors were also able to estimate estrogenic equivalency (EEQ) and the cumulative rank (%) of NP, NPE1 and NPEC.

Nichols et al. (2001) tested the commercial product NPE9 and found no induction of VTG at any concentration tested in a 42-day flow-through study with fathead minnows (*P. promelas*).<sup>40</sup> Balch and Metcalfe (2005) also evaluated the relative *in vivo* toxicity and estrogenicity of NPE9 as well as NPE4, NPE1 and NP using *Oryzias latipes* (medaka fish) to determine 100-day NOECs for standard toxicity related endpoints (i.e., survival, weight and length) along with other estrogenically mediated end points (i.e., sex ratio, mixed secondary sex characteristics and gonadal intersex.) No endocrine effects were observed for the two commercial products NPE4 and NPE9.<sup>41</sup>

Considering that existing studies are available that measure vitellogenin induction and other estrogen mediated endpoints for NPE and its environmentally relevant degradants, the need for additional testing utilizing another vitellogenin gene expression method cannot be justified.

### **Item 5. Testing related to levels of NP and NPE in humans and estrogenic effects in humans**

The petition states:

*Data on human exposure to NP and NPE are scarce. Research that does exist on levels of NP in humans is limited by testing of a single isomer, as well as the lack*

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<sup>39</sup> Dussault, E.B., Sherry, J.P., Lee, H.-B., Burnison, B.K., Bennie, D.T., and Servos, M.R. (2005). *In Vivo* Estrogenicity of Nonylphenol and Its Ethoxylates in the Canadian Environment. Human and Ecological Risk Assessment, 11, 353-364.

<sup>40</sup> Nichols, K.M., Snyder, E.M., Snyder, S.A., Pierens, S.L., Miles-Richardson, S.R., and Giesy, J.P. (2001). Effects of Nonylphenol Ethoxylate Exposure on Reproductive Output and Bioindicators of Environmental Estrogen Exposure in Fathead Minnows, *Pimephales promelas*. Environmental Toxicology and Chemistry, 20, 510-522.

<sup>41</sup> Balch, G. and Metcalfe, C. (2005).

*of knowledge regarding the relevance of an oxidative metabolism pathway for NP in humans. Another gap in current knowledge is the extent of dermal absorption of NP and NPEs contained in consumer products. Thus, Petitioners request that EPA require research to establish the relevance of oxidative metabolism of NP in humans and to identify the urinary metabolites of NP commercial mixtures. Petitioners also request research on dermal absorption of NP and NPEs, as well as of NP and NPE exposure in a nationally representative sample of the US population.*

The petition suggests there is a need for data on human exposure to NP and NPE but fails to recognize the considerable information available about the use patterns and potential human exposure to these compounds. In fact, human exposure to NPE in current industrial and consumer uses is minimal and NP is only used in industrial applications where it is further reacted into derivative products such as NPE.

The specific request from the petitioners for a dermal absorption study has already been addressed by Monteiro-Riviere et al. (2003).<sup>42</sup> That study was conducted as an outgrowth of the joint risk assessment conducted by Health Canada (HC) and Environment Canada in 2001 that evaluated human exposure and risk to NP and NPE. That assessment concluded that NP and NPE “are not considered a priority for investigation of options to reduce human exposure.”<sup>43</sup> The Canadian assessment relied on worst case assumptions about exposure to these compounds via skin contact and assumed that NP and NPE were “absorbed across the skin to the same extent as via the gastrointestinal tract,” which greatly overestimated absorption through the skin. The Canadian assessment recommended additional work on the dermal absorption of these compounds to allow for further refinement of the human exposure assessment. The Monteiro-Riviere study, which was published two years later in 2003, determined that the skin absorption of NP and commercial NPE was less than 1%.

The Canadian assessment also considered the potential for these chemicals to demonstrate estrogenic activity and concluded that “NP was estrogenic only at relative high doses.” It further concluded that NPE of longer chain lengths (NPE4, NPE9 and NPE12), which are the NPE of commercial interest, were not estrogenic in *in vivo* (conducted on living animals) studies and in a sensitive *in vitro* (laboratory) test.<sup>44</sup> These longer chain NPE are the ingredients found in the products that workers and consumers use.

The petitioners also requested “that EPA require research to establish the relevance of oxidative metabolism of NP in humans and to identify the urinary metabolites of NP commercial mixtures.” An available study of the metabolism of NP in a mammalian system confirmed that ingested NP is rapidly broken down into compounds that are not

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<sup>42</sup> Monteiro-Riviere, N.A., Van Miller, J.P., Simon, G.S., Joiner, R.L., Brooks, J., and Riviere, J.E. (2003). *In Vitro* Percutaneous Absorption of Nonylphenol (NP) and Nonylphenol Ethoxylates (NPE-4 and NPE-9) in Isolated Perfused Skin. *Journal of Toxicology: Cutaneous and Ocular Toxicology*, 22 (1&2), 1-11.

<sup>43</sup> EC and HC (2001).

<sup>44</sup> EC and HC (2001).

estrogenic and are eliminated within 24 hours.<sup>45</sup> This study, conducted on rats, also confirmed that no significant accumulation of NP occurs in any body organ or tissues following dosing at levels exceeding real-world exposure estimates. More recent assessments conducted by Environment Canada<sup>46</sup> and the State of Washington<sup>47</sup> have also confirmed that NP and NPE do not bioaccumulate.

Perhaps more relevant than these existing studies on the metabolic pathway and fate of NP/NPE, are the abundant robust studies on the chronic, reproductive and developmental effects of NP and NPE. These traditional toxicological studies in rats that measure chronic effects in parents and offspring often include an evaluation of reproductive and developmental effects that is indicative of an endocrine mode of action. Numerous studies - some conducted over two or three generations - have evaluated whether the alleged weak estrogenic activity of NP affected reproductive or developmental end points in rats.<sup>48,49,50,51, 52,53</sup> These studies uniformly concluded that there are no effects on reproductive function or performance from NP at any of the doses tested. These findings are consistent with and support the results of a multi-generation rat study conducted by the US National Institute of Environmental Health Sciences, which concluded that “NP was not a selective reproductive or developmental toxicant.”<sup>54</sup> Another study recently published by Tyl et al. (2006) determined that there were no adverse effects on sperm following three generations of exposure in rats.<sup>55</sup>

As previously noted, the well-established understanding of the human safety of NPE is also demonstrated in the recent OPP inerts assessment, which classified this compound as exempt from the requirement for a tolerance under the FQPA.

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<sup>45</sup> Green, T., Swain, C., Van Miller, J.P., and Joiner, R.L. (2003). Absorption, Bioavailability, and Metabolism of *para*-nonylphenol in the Rat. Regulatory Toxicology and Pharmacology, 38, 43-51.

<sup>46</sup> Environment Canada. (2005). Decision on Categorization of Nonylphenol, Octylphenol and their Ethoxylates. <http://www.aperc.org/docs/environmentcanadadecision112105.pdf>.

<sup>47</sup> Washington State, Department of Ecology. (2006, January 13). Chapter 173-333 WAC - Persistent Bioaccumulative Toxic Substances (PBTs) - New rule. <http://www.ecy.wa.gov/pubs/wac173333.pdf>.

<sup>48</sup> Latendresse, J.R., Weis, C.C., Mellick, P.W., Newbold, R.R., and Delclos, K.B. (2004). A Five Generation Reproductive Toxicity Assessment of *p*-Nonylphenol (NP) In CD Sprague-Dawley Rats. Toxicologist, 1066, 219.

<sup>49</sup> Nagao, T., Wada, K., Marumo, H., Yoshimura, S., and Ono, H. (2001). Reproductive Effects of Nonylphenol in Rats after Gavage Administration: A Two-Generation Study. Reproductive Toxicology, 15, 293-315.

<sup>50</sup> Odum, J. and Ashby, J. (2000). Neonatal Exposure of Male Rats to Nonylphenol Has No Effect on the Reproductive Tract. Toxicological Sciences, 56, 400-404.

<sup>51</sup> Odum, J., Pyrah, I.T.G., Soames, A.R., Foster, J.R., Van Miller, J.P., Joiner, R.L., and Ashby, J. (1999). Effects of *p*-Nonylphenol (NP) and Diethylstilboestrol (DES) on the Alderly Park (Alpk) Rat: Comparison of Mammary Gland and Uterus Sensitivity Following Oral Gavage or Implanted Mini-pumps. Journal of Applied Toxicology, 19, 367-378.

<sup>52</sup> Cunny, H.C., Mayes, B.A., Rosica, K.A., Trutter, J.A., and Van Miller, J.P. (1997). Subchronic Toxicity (90-Day) Study with *para*-Nonylphenol in Rats. Regulatory Toxicology and Pharmacology, 26, 172-178.

<sup>53</sup> Tyl, R.W., Myers, C.B., Marr, M.C., Castillo, N.P., Seely, J.C., Sloan, C.S., Veselica, M.M., Joiner, R.L., Van Miller, J.P., and Simon, G.S. (2006). Three-Generation Evaluation of Dietary *para*-Nonylphenol in CD (Sprague-Dawley) Rats. Toxicological Sciences, 92 (1), 295-310.

<sup>54</sup> Chapin, R.E., Delaney, J., Wang, Y., Lanning, L., Davis, B., Collins, B., Mintz, N., and Wolfe, G. (1999). The Effects of 4-Nonylphenol in Rats: A Multigeneration Reproduction Study. Toxicological Sciences, 52, 80-91.

<sup>55</sup> Tyl, R.W. (2006).

Considering that there are abundant high-quality studies available that support the conclusions, that there is no unreasonable risk to humans from the current uses of NP/NPE, the petitioners' request for additional mammalian studies are unnecessary. In addition, the US Center for Disease Control has included NP on the list of compounds for its National Report on Human Exposure to Environmental Chemicals and has an active program for the development of appropriate analytical methods for biomonitoring in human matrices well under way.<sup>56</sup>

#### **Item 6. Testing for health impacts on workers handling the chemicals at industrial laundries**

As discussed under item 5 above, the human safety of NPE in current uses is well supported by existing toxicological data and exposure information. Human exposure to NPE due to their use as an ingredient in laundry detergent is minimal. It is APERC's understanding that there has been a general acceptance of automated delivery systems for wash systems using the liquid detergents within the industrial laundry industry. Considering the abundant available studies for NPE, the minimal worker exposure to NPE in industrial laundries and the negligible dermal absorption of these compounds if skin contact does occur, the petitioners' request that EPA require epidemiological studies of the health impacts on workers in industrial laundries under TSCA section 4 is not warranted.

#### **Item 7. Testing for determine[ing] exposure to NPE in residential indoor air**

The petitioners request:

*that EPA adopt a Section 4 rule requiring manufacturers to conduct testing to assess the concentrations of NP and NPEs in indoor air and dust and to assess the health threats posed by the levels found. With respect to NP and NPEs, there is no reason to suspect that homes in Cape Cod are unusual. Every home in the United States may have these levels.*

There is no need for additional studies to assess the concentrations of NP or NPE in indoor air since adequate studies on the exposure to NPE/NP in household dust have already been conducted.<sup>57,58,59,60</sup> Considering that NPE have been widely used in various

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<sup>56</sup> Center for Disease Control and Prevention, Department of Health and Human Services. (2003, September 30). Notice of Candidate Chemicals for Possible Inclusion in Future Releases of the National Report on Human Exposure to Environmental Chemicals. Federal Register, Vol. 68. No. 189.

[http://www.cdc.gov/exposurereport/pdf/fr\\_093003.pdf](http://www.cdc.gov/exposurereport/pdf/fr_093003.pdf).

<sup>57</sup> Butte, W. and Heinzow, B. (2002). Pollutants in House Dust as Indicators of Indoor Contamination. Rev. Environ. Contam. Toxicol., 175, 1-46.

<sup>58</sup> Rudel, R.A., Camann, D.E., Spengler, J.D., Korn, L.R., and Brody, J.G. (2003). Phthalates, Alkylphenols, Pesticides, Polybrominated Diphenyl Ethers, and other Endocrine-Disrupting Compounds in Indoor Air and Dust. Environmental Science & Technology, 37 (20), 4543-4553.

<sup>59</sup> Costner, P., Thorpe, B., and McPherson, A. (2005, March). Sick of Dust: Chemicals in Common Products - A Needless Health Risk in Our Homes. Clean Production Action.

<http://www.safer-products.org/downloads/Dust%20Report%20with%20Appendices.pdf>.

household applications for close to fifty years, it is not surprising that trace levels of these compounds are found in household dust. In fact, the levels of NP and NPE found in these four studies are relatively consistent further indicating no need for additional studies.

Even conservative estimates that consider the lowest doses that resulted in any type of effect in rats - and included a 100-fold safety factor – indicate that concentrations of AP/APE in household dust are roughly thirty thousand times lower than safe levels determined for NP in mammalian systems.<sup>61</sup> Considering these margin of exposure estimates along with the abundant and robust mammalian data available on NP/NPE that have provided the basis for governmental assessments concluding no risk for human health, the petitioners' requests for mammalian and/or human testing and monitoring and household dust monitoring are not warranted under TSCA section 4.

The petition also requested that EPA take action under TSCA section 6(a) to:

**Item 8. Require labeling on all products containing the chemical**

A section 6 rulemaking to require labeling is not justified and there is no evidence that such a requirement is needed to address any concern regarding human health or environmental effects.

**Item 9. Restrict the use of the chemicals where the user cannot verify that the chemical will receive proper treatment from an activated sludge treatment process designed to nitrify**

The petitioners' call for restricting the use of NP/NPE unless the user can verify proper treatment is not only unworkable under TSCA section 6 but also unnecessary in light of the low level of these compounds found in the environment.

The primary issue of concern from the use of products containing these compounds is potential environmental effects to aquatic species. As already noted, issues associated with the aquatic toxicity of these compounds have been extensively reviewed, including most recently by EPA in support of its ambient WQC for NP.

The adoption of the WQC for NP and the associated provisions of the Federal Water Pollution Control Act, a.k.a. the Clean Water Act, sets in place an effective means to assure that there is proper treatment and disposal of products containing these compounds. This act is a comprehensive statute aimed at restoring and maintaining the chemical, physical and biological integrity of the nation's waters. The recent adoption by

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<sup>60</sup> Santillo, D., Labunska, I., Davidson, H., Johnston, P., Strutt, M., and Knowles, O. (2003). Consuming Chemicals: Hazardous Chemicals in House Dust as an Indicator of Chemical Exposure in the Home. Greenpeace Research Laboratories Technical. Note 01/2003, GRL-TN-01-2003. <http://www.greenpeace.org.uk/MultimediaFiles/Live/FullReport/5679.pdf>

<sup>61</sup> Alkylphenols & Ethoxylates Research Council. (2005, April 20). Statement: Human Exposure to Alkylphenols and Their Ethoxylates in Household Dust. <http://www.aperc.org/duststudy042005.htm>.

EPA of the WQC for NP should assure that levels of NP are below the WQC in the nation's waters.

Given the long-standing interest in these compounds, NP as well as NPE and other degradates have been included in various monitoring programs that have resulted in a large database of environmental measurements ranging from studies in limited geographical areas to much more comprehensive multi-state evaluations.

A recent assessment by Klecka et al. (2007) of all available environmental monitoring results of NPE and their degradates in US surface waters was conducted to develop a statistical understanding of environmental exposures to these compounds.<sup>62</sup> Examination of frequency distributions for NP concentrations indicated that 99% of the levels in fresh surface waters are below the Chronic Aquatic Life Ambient Water Quality Criteria for NP of 6.6 ug/L.

The Klecka et al. assessment also included a conservative aggregate assessment of environmentally relevant NPE oligomers along with NP and NPEC, another environmentally relevant degradant, since these compounds typically occur together in the aquatic environment as mixtures. For the purpose of illustration, an analysis of aggregate concentrations of NPE and their degradants was performed using the relative toxicity values developed by Environment Canada.<sup>63</sup> Concentrations of the individual compounds were adjusted according to their toxicities relative to NP, and then summed into a single aggregate, NP equivalent concentration per sample. According to Environment Canada's relative toxicity values, the lower mole NPE (n=1-8) were estimated to be less toxic than NP by a factor of two, and the higher mole NPE (n≥9) and NPEC were less toxic than NP by a factor of 200. The majority (>67%) of the values reported for NP, NPE1, NPE2, NPE3 and NP1EC in the study were below the detection limit. Moreover, the aggregate NP-equivalent exposure for these compounds was compared to the Chronic Water Quality Criteria for NP. Although not all NPE compounds were analyzed in all of the studies, the majority did analyze for the substances of greatest toxicological interest. It is striking to note that over 97% of the aggregate NP-equivalent concentrations were below the chronic criteria value.

It is significant to appreciate that this assessment included monitoring results spanning over 15 years and as such includes results from time frames when there was much greater use of NPE-based products and prior to the general upgrade of wastewater treatment plants.<sup>64</sup> These aspects alone suggest that environmental levels in existence today can be expected to be less than the results analyzed in the Klecka et al. assessment. Moreover, any measurements would not have the benefit of even lower levels that might be achieved based on the adoption of the NP WQC.

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<sup>62</sup> Klečka, G., Zabik, J., Woodburn, K., Naylor, C., Staples, C. and Huntsman, B. (2007). Exposure Analysis of C8- and C9-Alkylphenols, Alkylphenol Ethoxylates, and Their Metabolites in Surface Water Systems within the United States. *Human and Ecological Risk Assessment*, 13 (4), 792-822.

<sup>63</sup> EC and HC (2001).

<sup>64</sup> Melcer, H., Klečka, G., Monteith, H., and Staples, C. (2007). Fate of Nonylphenol Ethoxylate Surfactants in Wastewater Treatment Plants. Manuscript published in connection with Water Environment Federation Compounds of Emerging Conference, Providence, RI, July 29, 2007.

Considering that the abundant available data on NP/NPE do not indicate unreasonable risk to human health or the environment, along with the fact that the petition does not provide any basis to conclude that levels in the environment may present an unreasonable risk, there is no need for EPA to initiate a TSCA section 6 proceeding to regulate NP/NPE.

**Items 10 and 11. Ban the use of the chemicals in industrial and consumer detergents and require pollution prevention planning by facilities that use 2000 kg or more of NP or NPE.**

For the same reasons addressed under item 9 above there is no justification for EPA to initiate a TSCA section 6 proceeding to restrict the use of NPE in industrial or consumer detergents; moreover, there is no need to use TSCA section 6 to require any additional pollution prevention planning activities.

**ATTACHMENT I to APERC COMMENTS ON TSCA SECTION 21 PETITION**

**Docket ID Number: EPA-HQ-OPPT-2007-0490**

**SUPPORTING DATA TABLE FROM:** Staples, C., Mihaich, E., Carbone, J., Woodburn, K., and Klecka, 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, 999-1017.

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
<u>Fish and Amphibians</u>					
NPE-18	Sheepshead minnow <i>Cyprinodon variegatus</i>	70-d FT, partial life cycle, sub-adults (F0) allowed to mature then bred, breeding trials and hatching trials were conducted in clean water, 40 fish per each of two replicates, spawning groups of 2 males and 5 females per replicates, 50 eggs used for hatching trials from each replicate, 50 fry from each replicate monitored for 7 days for survivability, mean measured concentrations were 72 to 103% of nominal for Ex 1 and 75 to 86% of nominal for Ex 2, Ex 1: 0, 900, 1870, 3890, 8700, 20500 µg/L, Ex 2: 0, <50, <50, 750, 4200, 8600 µg/L	Ex 1: F0 adult survival: 1870 (3890) F0 growth: 3890 (8700) F0 egg production: (900), F1 hatch: 900 (1870) F1 survival: 3890 (8700)  Ex 2: F0 adult survival: 4200 (8600) F0 growth: 4200 (8600) F0 egg production: 8600 F1 hatch: 750 (4200) F1 survival: 8600	Ex 1: 1,2,3,4,5,6 valid  Ex 2: 1,2,3,4,5,6 valid	Johnson et al. (2001)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
NPE-9	Fathead minnow <i>Pimephales promelas</i>	7-d, R, unspecified concentrations, USEPA methods, specific test conditions given, 1-d old fish used, non-GLP	mortality: 1800 (2000) growth: 1000 (2000)	2,3,4,5, use with care	Dorn et al. (1993)
NPE-9	Fathead minnow <i>P. promelas</i>	42-d, FT, 0, 0.21, 0.65, 2.1, 7.9 µg/L NPE-9, breeding trials with 3 males and females in each of 3 replicates per test concentration	mortality: 7.9 fecundity: 7.9	1,2,3,4,5,6 valid	Nichols et al. (2001)
NPE-2 NPEC-1 NP	Rainbow trout <i>Oncorhynchus mykiss</i>	21-d, FT at nominal 30 µg/L for all compounds, 2-yr old fish (Ex 1), dose response test with NP (Ex 2), 2-yr old fish, 0, 0.24, 1.06, 1.85, 5.02, 20.3, and, 54.3 µg/L, The source of NPEC1 test material is uncertain as NPEC1 is not a commercially available product and must be specially synthesizes for use in testing.	Ex 1: reduced testis weight NPE-2: (38.3) NP: (36.8) Ex 2: reduced testis weight, NP: 20.3 (54.3)	1,2,3,5,6 valid	Jobling et al. (1996)
NPE-2 NPEC-1 NP	Rainbow trout <i>O. mykiss</i>	Ex 1: 108-d FT (22-d exposure), female juvenile fish, 0, 1, 10, 50 µg/L, concentrations unmeasured Ex 2: 466-d FT (31-d exposure), female juvenile fish, 0, 1, 10, 30 µg/L, concentrations unmeasured	Stability of test concentrations unknown, inconsistent in direction and small (<10%) variations in weight and length observed, use of a serial sampling scheme questioned by authors	1,3 not valid	Ashfield et al. (1998)
NP	Rainbow trout <i>O. mykiss</i>	18-week, FT, female 2-y old fish, mean measured concentrations of 0.7, 8.3, 85.6 µg/L	survival: 85.6 OSI: 8.3 (85.6) HSI: 8.3 (85.6)	1,2,3,5,6 valid	Harris et al. (2001)
NP	Rainbow trout <i>O. mykiss</i>	90-d, FT, ELS test, fertilized eggs allowed to hatch (~day 34) grow out until day 90,	mortality: 10.3 (23.1) hatch success: 114	1,2,3,4,5,6 valid	Brooke (1993a)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
		two replicates for each concentration, measured test concentrations of 0, 6.0, 10.3, 23.1, 53.0, 114 µg/L, ASTM and USEPA methods	growth: 6.0 (10.3)		
NP	Rainbow trout <i>O. mykiss</i>	1 year exposure during embryonic, larval, and juvenile stages, FT, mean measured concentrations of 0, 1.05, 10.17 µg/L, endpoints were survival, hatch rate, weight, sex ratio	no effects at either concentration for any endpoint	1,2,3,5,6 valid	Ackerman n et al. (2002)
NP	Fathead minnow <i>P. promelas</i>	42-d paired-breeding assay, 21-d clean water exposure, then 21-d exposure to NP at 0, 100 µg/L nominal (Ex 1) or 0, 1, 10, 100 µg/L nominal, replicate tanks, methanol cosolvent, concentrations measured at days 0 and 21, 48 to 99% nominal achieved, critical issue: authors state this may be insufficient for confidence in accuracy, study otherwise study well reported	Ex 1: mortality: 71 growth: 71 fecundity: (71) Ex 2: mortality: 57.7 growth: 57.7 fecundity #eggs spawned: 8.1 (57.7) #spawnings: 0.65 (8.1) egg batch size: 8.1 (57.7)	1,3,4,5 use with care	Harries et al. (2000)
NP	Fathead minnow <i>P. promelas</i>	33-d, FT, ELS test (growth measured at day 28), measured test concentrations, ASTM and USEPA methods	mortality: 7.4 (14) length: 23	1,2,3,4,5,6 valid	Ward and Boeri (1991b)
NP	Fathead minnow <i>P. promelas</i>	28-d, FT, ELS test, 20 4-week old fry per each of two replicates, measured test concentrations of 0, 9.3, 19.2, 38.1, 77.5, 193 µg/L, ASTM and USEPA methods	survival: 77.5 (193) wet weights: 38.1 (77.5)	1,2,3,4,5,6 valid	Brooke (1993b)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
NP	Fathead minnow <i>P. promelas</i>	42-d, FT, measured test concentrations, adults exposed for 7-d, then breeding tiles added for 14 days (Ex 1=21 days), breeding tiles removed for 7 days, tiles added for 14 days (Ex 2=2nd breeding period), Ex 1: 0, 0.05, 0.16, 0.40, 1.6, 3.4 µg/ L, Ex 2: 0, 0.09, 0.10, 0.33, 0.93, 2.4 µg/L	Ex 1: mortality: 3.4 inhibition of egg production per female: 1.6 (3.4) solvent control egg production ceased at day 11 of breeding Ex II: complete inhibition of reproduction in solvent control makes it impossible to show effects due to NP	Ex 1: 1,2,3,4,5 valid  Ex 2: 1,2,3,4 not valid due to solvent control performance	Giesy et al. (2000)
NP	Bluegill sunfish <i>Lepomis macrochirus</i>	28-d, FT, 4-week old juveniles, mean measured concentrations of 0, 5.6, 12.4, 27.6, 59.5, 126 µg/L, ASTM and USEPA methods	mortality: 59.5 (126) wet weight: 126	1,2,3,4,5,6 valid	Brooke (1993b)
NPE-9 NPE-4 NPE-1 NPEC1 NP	Japanese medaka <i>Oryzias latipes</i>	100-d, R, NPE-9: 30 to 1000 µg/L, NPE-4: 10 to 1000 µg/L, NPE-1: 10 to 300 µg/L, NPEC1: 100 to 3000 µg/L, NP 1 to 100 µg/L, endpoint: sex ratio	NPE-9: 1000 NPE-4: 1000 NPE-1: 300 NPEC1: 3000 NP: 100	1,2,3,5,6 valid	Balch and Metcalf (2003)
NP	Japanese medaka <i>O. latipes</i>	30-d, FT, 5to 8 days post hatch fish exposed for 30 days followed by a 30 day grow out period, 0, 0.54, 0.77, 1.93 µg/L	mortality: 1.93 growth: 1.93 sex differentiation: 1.93 OSI: 1.93	1,2,3,4,5,6 valid	Nimrod and Benson (1998)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
			TSI: 1.93 egg production: 1.93 egg viability: 1.93 hatching success: 1.93		
NP	Japanese medaka <i>O. latipes</i>	90-d, R, 1-2 day post hatch fish, selected but only indirect analysis of test concentrations, nominal 0, 10, 50, 100 µg/L	40% control mortality invalidates this study	1,3,4,5 not valid	Gray and Metcalfe (1997)
NP	Japanese medaka <i>O. latipes</i>	14-d, R, males exposed then bred with unexposed females in clean water for 7-d, nominal concentrations only, acetone cosolvent, 0, 0.03, 0.10, 0.30 µM/L nominal or 0, 6.6, 20, 66 µg/L (NP mwt~220 g/mol)	egg production: 66 hatching success: 66	1,3,5 use with care	Shioda (2000)
NP	Japanese medaka <i>O. latipes</i>	1.5-generation, FT, <24-h embryos continuously exposed, raised to adults, bred, eggs hatched, raised for 60-d to sexual maturity, mean measured concentrations 0, 4.2, 8.2, 17.7, 51.5, 183 µg/L, several parameters (GSI, fecundity, fertility, F1 generation test) were only examined at lower concentrations due to the few number of surviving adults	F0 hatching: 51.5 (183) F0 time to hatch: 183 F0 swim-up success: 51.5 (183) F0 day 60 mortality: 8.2 (17.7) F0 sex ratio: 17.7 (51.5) F0 somatic growth: 51.5 (183) F0 TSI: 17.7 F0 OSI: 4.2 (8.2) F0 egg production: 17.7 F0 fertility: 17.7 F1 hatchability: 17.7 F1 mortality: 17.7 F1 somatic growth: 17.7	1,2,3,4,5,6 valid	Yokota (2001)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
			F1 sex ratio: 8.2 (17.7)		
NP	Swordtail <i>Xiphophorus helleri</i>	60-d, S, 20 juvenile fish per treatment, nominal concentrations only, 0, 0.2, 2.0, 20 µg/L	total body length and sword lengths measured in males, no confidence in concentrations as use of a 60-d static system invalidates this test	1,3 not valid	Kwak et al. (2001)
NP	Flounder <i>Platichthys flesus</i>	21-d, FT, wild adult fish, mean measured concentrations of 0, 7.2, 24.5 µg/L	reduced testis weight: 24.5 reduced liver weight: 7.2 (24.5)	1,2,3,5,6 valid	Allen et al. (1999)
NP	Atlantic salmon <i>Salmo salar</i>	28-d feeding study, 5 month old juvenile salmon dosed via feed	sex ratio: 1500 mg/kg dw LSI: (300 mg/kg, dw)	1,3,5 use with care	Norrgren et al. (1999)
NP	Atlantic salmon <i>S. salar</i>	30-d aqueous exposure of ethanol and 5, 10, 15 and 20 µg/L, 12-h R, nominal only, endpoint was survival	survival: 20 µg/L	3,5,6 use with care	Moore et al. (2003)
NP	Chinook salmon <i>Oncorhynchus tshawytscha</i>	29-d exposure, R, nominal only, grow-out period of 103 days post-hatch, egg through adult stages, endpoints: survival, sex ratio, gonad development	survival: 10 sex ratio: 10 female gonad development: 10 male gonad development: 10	1,3,5,6 use with care	Afonso et al. (2002)
NP	African clawed frog <i>Xenopus laevis</i>	Exposure from stage 10.5 (11-h) to stage 37 (49-h), 0.002 to 1000 µg/L, nominal-only, examined body shape, length, interocular distance, melanocyte differentiation, apoptosis	body shape, length, interocular differences: 20 (100) apoptosis, melanocyte differentiation: 2 (20)	1,3,5,6 use with care	Bevan et al. (2003)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in  $\mu\text{g/L}$  unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
NP	African clawed frog <i>X. laevis</i>	12-week, R, single replicate test systems, duplicate controls, tadpoles at developmental stage 38/40 (2 to 3 days post hatch), exposed until metamorphosis was accomplished in ~90% of all animals, nominal concentrations only, 0, $10^{-8}$ , $10^{-7}$ M (0, 2.2, 22 $\mu\text{g/L}$ using a mwt of ~220 g/mol)	temperature control vital influence on <i>Xenopus</i> development but design lacked temperature control; absence of explanation or discussion of temperature and of skewed control sex ratio invalidates study	3,5 not valid	Kloas et al. (1999)
<u>Invertebrates</u>					
NPE-9	Water flea <i>Daphnia magna</i>	R, 7-d, unspecified concentrations, USEPA methods, specific test conditions given, <24-h old neonates used, non-GLP	mortality: 10,000 (20,000) growth: 10,000	2,3,4,5, use with care	Dorn et al. (1993)
NPE	Water flea <i>D. magna</i>	2 generation (21-d F0 and 14-d F1), R, ASTM method, <24-h neonates used, nominal only, 0, 310, 620, 1200, 2500, 3000 $\mu\text{g/L}$ (1 <sup>st</sup> generation), 0, 1200, 2500 $\mu\text{g/L}$ (2 <sup>nd</sup> generation)	Absence of information as to the specific test chemical used and it's source/purity invalidate this test.	3,4,5,6 not valid	Baldwin et al. (1998)
NPE-1.5	Cladoceran <i>Ceriodaphnia dubia</i>	7-d, R, USEPA methods, <24-h old instar, mean measured concentrations were 0, 8.35, 25.7, 84.4, 285, 886, 2600 $\mu\text{g/L}$ , GLP	mortality: 285 (886) time to first brood: 285 (886) young per female per day: 285 (886)	1,2,3,4,5,6 valid	England (1995a)
NPE-1.5	Mysid shrimp <i>Mysidopsis bahia</i>	28-d, FT, life-cycle test, US TSCA and EPA methods, mean measured concentrations of 0, 2.2, 4.0, 7.7, 16, 32 $\mu\text{g/L}$ , <24-h juveniles, GLP	mortality: 32 growth: 16 (32) reproductive success: 7.7 (16)	1,2,3,4,5,6 valid	Sousa (1999)
NPEC-1	Cladoceran	7-d, R, USEPA methods, <24-h old instar,	mortality: 8400 (17,000)	1,2,3,4,5,6	England

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
	<i>C. dubia</i>	mean measured concentrations were 0, 520, 1100, 2200, 4700, 8400, 17,000 µg/L, GLP	time to first brood: 2200 (4700) young per female per day: 2200 (4700)	valid	and Bucksath (1997)
NP	Cladoceran <i>C. dubia</i>	7-d, R, USEPA methods, <24-h old instar, mean measured concentrations were 0, 2.89, 10.4, 25.9, 88.7, 202, 377 µg/L, GLP	mortality: 202 (377) time to first brood: 88.7 (202) young per female per day: 88.7 (202)	1,2,3,4,5,6 valid	England (1995b)
NP	Water flea <i>D. magna</i>	21-d, R, OECD method, <24-h instar, mean measured concentrations of 0, 14, 24, 39, 71, 130, 250 µg/L, GLP	mortality: 24 (39) growth: 39 (71) reproduction: 24 (39)	1,2,3,4,5,6 valid	Comber et al. (1993)
NP	Water flea <i>D. magna</i>	21-d, R, ASTM method, mean measured concentrations of 0, 44.3, 63.1, 116, 215, 500 µg/L, GLP	mortality: 215 (500) growth: 116 (215) young per surviving adult: 215 (500) young per starting adult: 116 (215)	1,2,3,4,5,6 valid	Brooke (1993a)
NP	Water flea <i>D. magna</i>	2 generation (21-d F0 and 21-d F1), R, ASTM method, <24-h neonates used, nominal only, 0, 6.2, 12, 25, 50, 100 µg/L (1 <sup>st</sup> generation), 0, 6.2, 25 µg/L (2 <sup>nd</sup> generation)	1 <sup>st</sup> generation: mortality: 100 reproduction: 50 (100) 2 <sup>nd</sup> generation: mortality: 25 reproduction: 25	1,3,4,5 use with care	Baldwin et al. (1997)
NP	Water flea <i>D. magna</i>	21-d, R, atypical light regime of 8 h light: 16 h dark used to stimulate production of males, daphnids fed ample (Ex 1) or inadequate (Ex 2) amount of food, nominal	Ex 1 (high food): fecundity: 25 sex differentiation: 25 Ex 2:	1,3,5 use with care	Baer & Owens (1999)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in  $\mu\text{g/L}$  unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
		concentration of 0, 25 $\mu\text{g/L}$ . Controls in Ex 2 had higher % males and reduced total number offspring compared to Ex 1. Effects more pronounced with exposure to NP.	fecundity: 25 sex differentiation (increased % males): (25)		
NP	Water flea <i>D. magna</i>	21-d, R, OECD method used, <24-h instar, nominal concentrations only 0, 1, 10, 100 $\mu\text{g/L}$	Absence of data on control, solvent control performance, test concentration stability, concentration-specific reproduction data, or water quality invalidate this study.	3,4 not valid	Kopf (1997)
NP	Water flea <i>D. magna</i>	21-d, R, nominal concentrations only, varied by experiment, Ex I: typical assay examining mortality, reproduction and abnormal offspring, a second part with 20 test concentrations 90% of the next, Ex II: direct embryo-larval exposure measuring developmental effects, timing uncertain, concentrations unstated, assume nominal only, Ex III: embryo toxicity after maternal exposure	Absence of full test concentration data (nominal or measured), control performance, timing of all testing invalidates this study.	3 not valid	LeBlanc et al. (2000)
NP	Water flea <i>D. magna</i>	14-d S, nominal concentration only (100 $\mu\text{g/L}$ ), females exposed to 16h light/8h dark or 8h light/16h dark, female endpoints were survival, #molts, #live neonates, #deformed neonates, males exposed to 16h light/8h dark, male endpoints were survival and	females, 16h light: no effect on survival, #molts and abdominal process length stimulated, #deformed neonates increased	1,3,5,6 use with care	Gibble and Baer (2003)

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Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
		#molts	females 8h light: no differences males: no effects on survival, #molts or body length, length of first antennae reduced		
NP	Water flea <i>D. galeata</i>	2-generation, R, nominal-only, 0, 3 to 100 µg/L, endpoints: survival and #neonates, used to calculate the EC50 for reduction by 50% of the intrinsic rate of increase (r) of the population	Gen. 1: Survival: 70 (100) #neonates: 50 (70) EC50 (for r) = 65.2 Gen 2: Survival: 50 (70) #neonates: 70 (>70) EC50 (for r) = 81.5	1,3,5,6 use with care	Tanaka and Nakanishi (2002)
NP	Midge larvae <i>Chironomus tentans</i>	For all three experiments, 2 <sup>nd</sup> instar larvae were used and procedures were based on ASTM and EPA methods, mortality and growth of midge larvae measured using different exposure routes. Ex 1: 14-d, FT, aqueous exposure with minimal sand substrate, mean measured water column concentrations of 0, 23, 44, 76, 150, 320 µg/L, Ex 2: 14-d, FT, aqueous exposure with natural sediment substrate, some surface adsorption onto sediment occurred but were not at equilibrium, mean measured water	Ex 1: mortality: 76 (150) growth: 76 (150) Ex 2: - mortality (water column exposure): 38.7 (81.1) - growth (water column exposure): 20.5 (38.7) Ex 3: - mortality (sediment exposure): 20.1 (34.2 mg/kg dry)	1,2,3,4,5,6 valid	England and Bussard (1993)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
		column concentrations of 0, 7.19, 20.5, 38.7, 81.1, 146 µg/L, estimated pore water concentrations of 0, <0.823, 1.94, 1.95, 2.56, 3.67 µg/L, mean measured non-equilibrium sediment concentrations were 0, 0.0649, 0.0916, 0.193, 0.326, 0.475 mg/kg dry. Ex 3: 14-d, FT of clean water, sediment dosed with chemical, mean measured interstitial concentrations of 0, 15.3, 35.4, 78.3, 143, 252 µg/L, mean measured sediment concentrations of 0, 2.34, 4.79, 9.51, 20.1, 34.2 mg/kg.	- growth (sediment exposure): 20.1 (34.2 mg/kg dry) - mortality (pore water exposure): 143 (252) - growth (pore water exposure): 143 (252)		
NP	Midge <i>C. tentans</i>	Life-cycle test from hatch to emergence (53 days), concentrations of 0, 12.5, 25, 50, 100, 200 µg/L, mean measured concentrations ~one half nominal, endpoints: survival, growth, reproduction, emergence, viability of F1	larval mortality: 42 (91) growth: 91 emergence: 91 reproduction: 91 viability of F1: 91	1,2,3,4,5,6 valid	Kahl et al. (1997)
NP	Benthic crustacean <i>Leptocheirus plumulosus</i>	28-d life-cycle test using dosed sediment, mean measured concentrations of 2.1 to 61.5 µg/g dw, endpoints were survival and #young/female	survival: 61.5 µg/g dw #young/female: 61.5 µg/g	2,3,5,6 use with care	Zulkosky et al. (2002)
NP	Copepod <i>Tisbe buttagliai</i>	Ex 1: 53-d, R, nauplii <1 day old, mean measured concentrations of 0, 20, 40.5, 74, 300 µg/L, F0 eggs hatch, become adults, lay eggs (F1), grow out to be sexed Ex 2: 39-d, R, nauplii <1day old, mean	Ex 1: mortality: 20.0 (40.5) fecundity: 20.0 (40.5) sex ratio (F0 and F1): 20.0 (40.5)	1,2,3,5,6 valid	Bechmann (1999)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
		measured concentrations of 0, 20, 40.5 µg/L, F0 eggs hatch, become adults, lay eggs (F1), grow out to be sexed	Ex 2: mortality: 20.0 (40.5) fecundity: 20.0 (40.5) sex ratio (F0 and F1): 20.0 (40.5)		
NP	Mysid shrimp <i>M. bahia</i>	28-d, FT, life-cycle test, US TSCA and EPA methods, mean measured concentrations of 0, 3.9, 6.7, 9.1 µg/L, <24-h juveniles, GLP	mortality: 6.7 (9.1) growth: 3.9 (6.7) reproduction: 6.7 (9.1)	1,2,3,4,5,6 valid	Ward and Boeri (1991a)
NP	Mussel (marine) <i>Mytilus edulis</i>	Ex 1: 35-d, R, adults, 17 deg. C Ex 2: 30-d, R, adults, 17 deg. C Ex 3: 30-d, FT, adults, 10 deg. C Ex 4: 72-h, R, eggs from one adult and sperm from one adult, 17 deg C	Ex 1: 15-d LC50 = 500 35-d LC50 = 140 Ex 2: byssal strength: 18 (56) energy budget (stress indicator): 18 (56) Ex 3: byssal strength: 32 (56) energy budget (stress indicator): 32 (56) Ex 4: fertilization success: 200 early development: 200	1,3,5 use with care	Granmo et al. (1989)
NP	Rotifer <i>Brachionus calyciflorus</i>	72-h static, life cycle test, newly hatched mimetic females, 25 deg. C, acetone cosolvent, nominal concentrations only, 0, 0.21, 0.35, 0.59, 0.98, 1.63, 2.72, 4.54 µM/L (0, 46, 77, 130, 216, 359, 598, 1000	72-h EC50 = 2.63 µM/L (0-21 95% CI), or, 72-h EC50 = 565 µg/L	1,3,5 use with care	Radix et al. (2002)

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Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
		$\mu\text{g/L}$ using a NP mwt of 220 g/mol), only determinable endpoint was the ratio of ovigerous to non-ovigerous females			
NP	Nematode <i>Caenorhabditis elegans</i>	3-d lifecycle test, S, measured concentrations 0, 40.2 to 235.2 $\mu\text{g/L}$ , endpoints were body length, #offspring per worm	length: 40.2 (65.6) #offspring/worm: (40.2)	1,2,3,5,6	Hoss et al. (2002)
NP	Zooplankton community - Cladocera: 8 sp. - Copepoda: 8 sp. - Rotifera: 16 sp. - Ostracoda: species not named	20-d, R, littoral enclosures in a natural pond, mean measured water concentrations of 0, 5, 23, 76, 243 $\mu\text{g/L}$ , followed population abundance and community diversity of pelagic zooplankton	abundance (cladocera): 23 (76) abundance (copepoda): 5 (23) abundance (rotifera): 23 (76) abundance (ostracoda): 76 (243) community diversity: 23 (76)	1,2,3,5,6 valid	O'Halloran et al. (1999)
NP	Benthic macroinvertebrates - Chironomids: 2 sp. - Oligochaetes: 2 sp. - Mollusca: 2 sp.	20-d, R, littoral enclosures in a natural pond, mean measured water concentrations of 0, 5, 23, 76, 243 $\mu\text{g/L}$ , followed population abundance of benthos	abundance Tanytarsini: 76 (243) abundance Chironomini: 243 abundance Naididae: 23 (76) abundance Tubificidae: 243 abundance Bivalvia: 23 (76) abundance Gastropoda:	1,2,3,5,6 valid	Schmude et al. (1999)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in µg/L unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
			76 (243)		
<u>Algae</u>					
NPE-30 NPE-9 NPE-6	Green algae <i>Selenastrum capricornutum</i>	21-d, S, nominal concentrations only of 0, 100000, 200000, 300000, 400000, 500000 µg/L	Absence of test concentration measurements in a 21-d static test and because populations would not be in log growth phase for 21 days invalidates tests	not valid	Nyberg (1988)
NPE-10	Natural algal assemblages	13-d, S, exposure to algae collected from sewage-impacted and non-impacted streams, endpoints: rate of algal biomass accumulation, total algal biovolume, algal community structure, Ex. 1: 200 µg/L, Ex. 2: 5, 50, 500 µg/L, nominal-only	Ex. 1: rate of accumulation: 200 total biovolume: (200) community structure: (200) Ex. 2: total biovolume: (5) community structure: (5)	3,5,6 use with care	Wilson et al. (2003)
NPE-9	Green algae <i>S. capricornutum</i>	96-h, S, unspecified concentrations, USEPA methods, specific test conditions given, non-GLP	growth (cell counts): 8000 (16,000)	2,3,4,5 use with care	Dorn et al. (1993)
NP	Green algae <i>S. capricornutum</i>	96-h, S, ASTM and EPA methods, mean measured concentrations of 0, 26.6, 66.7, 136, 329, 694, 1480, 2720 µg/L	growth (biomass): 694 (1480)	1,2,3,4,5,6 valid	Brooke (1993a)
NP	Green algae <i>S. capricornutum</i>	72-h, S, growth (biomass) test, US TSCA and EPA methods, mean measured concentrations, GLP	Growth (biomass): 92	1,2,3,4,5,6 valid	Ward Boeri (1990b)
NP	Green algae <i>Scenedesmus</i>	72-static, DIN-EN28692	Only summary data presented, no data on	not valid	Kopf (1997)

Table A. Chronic Population-Level Effects of NPE, NPEC, and NP to Aquatic Organisms. All units in  $\mu\text{g/L}$  unless otherwise specified.

Material	Species	Test Conditions	Reported effects NOEC (LOEC)	Acceptance Scoring & Study Validity	Reference
	<i>subspicatus</i>		replicates given, no control data given, no measurements of concentrations given		
NP	Green algae <i>Skeletonema costatum</i>	96-h, S, ASTM and EPA methods, mean measured test concentrations of 0, 10, 20, 38, 110, 160 $\mu\text{g/L}$ , GLP	growth (cell number): EC10 = 12	1,2,3,4,5,6 valid	Ward Boeri (1990a)
NP	Duckweed <i>Lemna minor</i>	96-h, FT, ASTM and EPA methods, mean measured concentrations of 0, <88.0, 109, 375, 901, 2080 $\mu\text{g/L}$	frond production: 901 (2080)	1,2,3,4,5,6 valid	Brooke (1993a)
NP	Duckweed <i>L. minor</i>	Ex 1: 6-d, S, nominal concentrations only of 0, 500, 2500, 5000 $\mu\text{g/L}$ , NOECs and LOECs determined qualitatively Ex 2: 4-d, R, nominal concentrations only of 0, 125, 250, 500, 1250, 2500 $\mu\text{g/L}$ , NOECs and LOECs determined qualitatively	Ex 1: growth (frond number): 2500 (5000) Ex 2: frond multiplication: 250 (500)	1,3,6 use with care	Prasad (1989)
NP	Macrophyte <i>Salvina molesta</i>	9-d, S, nominal concentrations only of 0, 2500, 10000, 25000 $\mu\text{g/L}$ , NOECs and LOECs determined qualitatively	growth (frond number): (2500)	1,3,6 use with care	Prasad (1989)

**Abbreviations:** S = static test system, R = renewal test system, FT = flow-through test system, NOEC = no observed effect concentration, LOEC = lowest observed effect concentration, ECx = effect concentration causing x percent effect, LCx = lethal concentration causing x percent lethality, ELS = early life stage test, NPE = nonylphenol ethoxylates, NPEC = nonylphenol ether carboxylates, NP = nonylphenol, NPE1.5 = mixture of NPE1 and NPE2. GSI = gonadosomatic index (ratio of gonad weight to body weight), TSI = testis-somatic index, OSI = ovasomatic index, ASTM = American Society for Testing and Materials, USEPA = U.S. Environmental Protection Agency, OECD = Organization for Economic Cooperation and Development, GLP = Good Laboratory Practices, mwt = molecular weight (g/mol).

**Note:** NOEC are always shown without parentheses. LOEC are always shown with parentheses. If a NOEC is not accompanied by a LOEC, then no effects were reported at the highest concentration tested. If a LOEC is not accompanied by a NOEC, then effects were reported at all concentrations tested.

**Note:** Full citations for referenced studies are listed in the manuscript Staples, C., Mihaich, E., Carbone, J., Woodburn, K., and Klecka, 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, 999-1017

**ATTACHMENT II - APERC COMMENTS ON TSCA SECTION 21 PETITION**

**Docket ID Number: EPA-HQ-OPPT-2007-0490**

**SUPPORTING DATA ON VITELLOGENIN AND RELATED STUDIES FROM:** Staples, C., Mihaich, E., Carbone, J., Woodburn, K., and Klecka, 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, 999-1017.

**Note:** Full citations for study references are available in the Staples et al. (2004) manuscript.

Table B. Secondary and Supplemental Endpoints - Vitellogenin and Related Studies.

Material	Test System	Endpoint Description	Comments	Reference
<u>In Vitro Tests</u>				
NPE40 NPE9 NPE2 NP	VTG induction in male rainbow trout hepatocytes	relative potency as compared to 17- <i>B</i> estradiol (which equals 1)	NPE40: no activity NPE9: 2E-7 NPE2: 6 E-6 NP: 9E-6	Jobling and Sumpter (1993)
NPE2 NP	VTG induction in cultured male rainbow trout hepatocytes	dose response assessment of VTG production	No statistics used, however, substantial increase in VTG induction at 1E-5 M concentrations of NPE2 and NP compared to 1E-6 and 1E-7 M	White et al. (1994)
<u>In Vivo Tests</u>				
NPE2 NP	Exposed male adult rainbow trout ( <i>O. mykiss</i> ) for 3 weeks	VTG induction in blood plasma from exposure to nominal 30µg/L	100- to 1000-fold increase in serum VTG concentration for both NPE2 and NP, as compared to controls	Jobling et al. (1996)
NP	Exposed male adult rainbow trout ( <i>O. mykiss</i> ) for 3 weeks	Dose response assessment of VTG induction, GSI measured in same fish	VTG induced at 20.3, but not 5.02 µg/L. GSI reduced at 54.3, but not 20.3 µg/L	Jobling et al. (1996)

Table B. Secondary and Supplemental Endpoints - Vitellogenin and Related Studies.

Material	Test System	Endpoint Description	Comments	Reference
NP	Exposed female adult rainbow trout ( <i>O. mykiss</i> ) for 18 weeks	Dose response assessment of VTG induction, plasma levels of follicle stimulating hormone (FSH) and gene expression of FSH in the pituitary, plasma and pituitary levels of luteinizing hormone (LH) and gene expression in pituitary, plasma E2 levels, GSI measured in same fish	VTG induction at 8.3 µg/L and higher, plasma E2 reduced at 85.6 µg/L, plasma FSH and pituitary gene expression reduced at all concentrations, pituitary LH reduced at 85.6 µg/L, LH gene expression reduced at 8.3 µg/L, transitory slight increase in plasma LH, GSI reduced at 85.6, but not 8.3 µg/L	Harris et al. (2001)
NP	Exposed Atlantic salmon ( <i>S. salar</i> ) smolts for 30 days	Solvent control, 5 to 20 µg/L	No effects on plasma VTG, plasma Na <sup>+</sup> or Cl <sup>-</sup> ions, gill Na <sup>+</sup> K <sup>+</sup> ATPase activity at any concentration	Moore et al. (2003)
NP	Exposed male adult flounder ( <i>P. flesus</i> ) for 3 weeks	VTG induction, gonad histopathology, GSI and HSI measured in same fish	No induction of VTG at highest mean measured concentration of 24.5 µg/L, no effects on gonad histology	Allen et al. (1999)
NPE-9	42-d flow-through with fathead minnows ( <i>P. promelas</i> )	Plasma VTG, 17β-estradiol (E2), testosterone (T)	No induction of VTG at any concentration tested (highest 7.9 µg/l), No differences between treatments and control for plasma E2 or T	Nichols et al. (2001)
NP NPE1 NPEC	21-d flow-through rainbow trout ( <i>Oncorhynchus mykiss</i> )	Plasma VTG	Plasma VTG induction in fish exposed to NP started to appear at water concentrations of 13 µg/L but more typically occurred at water concentrations of 25 µg/L of NP. Measurements of the relative potency of NP1EO and NP1EC compared to NP, yielded ratios of 0.22 and 0.03, respectively	Dussault et al (2005)

Table B. Secondary and Supplemental Endpoints - Vitellogenin and Related Studies.

Material	Test System	Endpoint Description	Comments	Reference
NPE-9 NPE-4 NPE-1 NPEC1 NP	Japanese medaka <i>Oryzias latipes</i>	100-d, R, endpoints: secondary sex characteristics, male papillary processes, testis-ova	NPE-9 and NPE-4: all endpoints NOEC 1000 µg/L, highest concentration tested NPE-1: secondary characteristics NOEC (LOEC) 100 (300) µg/L, all other endpoints NOEC 300 µg/L NPEC1: all endpoints NOEC 3000 µg/L, highest concentration tested NP: secondary characteristics NOEC (LOEC) 10 (30) µg/L, male processes NOEC 100 µg/L, testis-ova 30 (100)	Balch and Metcalfe (2005)
NP	28 to 31-d exposure to carp ( <i>Cyprinus carpio</i> )	Measured serum sex steroids, VTG induction, gonadal histology, mean measured concentrations up to 5.36 µg/L	As compared to controls, no effects on somatic growth or liver or gonad weights, no differences in plasma E2 or testosterone, no differences in plasma VTG, no histopathological changes observed in testes, hepatopancreas, brain or gills	Villeneuve et al. (2002)
NP	Paired-breeding assay with fathead minnows ( <i>P. promelas</i> ), flow-through	Secondary sex characteristics, serum VTG	Reduction in number of tubercles but not the size of the fat pad noted at 8.1 to 57.7 µg/L. Serum VTG induced at 8.1 to 57.7 µg/L.	Harries et al. (2000)
NP	42-d flow-through with fathead minnows ( <i>P. promelas</i> )	Plasma VTG, 17β-estradiol (E2)	Dose related decrease in plasma VTG in females. No dose related change in plasma VTG in males. ~Equal elevation of plasma E2 at all doses except the highest (3.4 µg/L) in females and males.	Giesy et al. (2000)

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NPE-9 NP	42-d flow-through, fathead minnows ( <i>P. promelas</i> ).	Observations of secondary sex characteristics and gonadal histology.	NPE-9 - No effects on tubercle or fat pad size, on survival, or on testicular or ovarian histology at highest level (5.5 µg/L). Ex I: NP - No effects on tubercle or fat pad size, or survival at highest level (3.4 µg/L). Increase in severity score of male gonads at 1.6 but not 0.4 µg/L. Ex II: NP - data not usable as solvent control completely inhibited egg production (see companion paper, Giesy et al. 2001)	Miles-Richardson et al. (1999)
NP	9-d flow-through, rainbow trout ( <i>O. mykiss</i> )	Induction of plasma VTG	Significant induction of VTG at 76 µg/L	Pederson et al. (1999)
NP	1 year flow-through, rainbow trout ( <i>O. mykiss</i> )	Induction of VTG, ZRP expression in liver	VTG induction: (1.05) µg/L ZRP expression: 1.05 (10.17) µg/L	Ackermann et al. (2002)
NP	3-d renewal, swordtail fish ( <i>X. helleri</i> )	Induction of VTG nRNA in liver, scoring of male gonadal cells for necrosis/apoptosis	VTG induced at 4 to 100 µg/L. Increases in numbers of necrotic/apoptotic male gonad cells at 4 to 100 µg/L.	Kwak et al. (2001)
NP	Sheepshead minnow <i>Cyprinodon variegatus</i>	Following a 16-d renewal exposure, hepatic VTG nRNA regulation and plasma VTG clearance rates were measured	hepatic VTG rapidly diminished following cessation of exposure, plasma VTG clearance concentration and time dependent, plasma clearance slower than hepatic clearance	Hemmer et al. (2002)

E2 is 17 $\beta$ -estradiol, EE2 is ethinylestradiol, a synthetic estrogen, VTG is vitellogenin, FSP means female-specific proteins, T means testosterone, NOEC means no observed effect concentration, LOEC means lowest observed effect concentration, EC<sub>x</sub> is an effect concentration caused at x percent level, F0 is the parental generation and F1 is the offspring generation of the F0, NPE are nonylphenol ethoxylates, NP is nonylphenol, NPEC are nonylphenol ether carboxylates, GSI is gonadosomatic index, LSI is the liver-somatic index, GLP refers to the use of Good Laboratory Practices.