

Comments
of the
Alkylphenols and Ethoxylates Research Council
on the
Canadian Environmental Protection Act
Draft Priority Substance List Assessment Report
for Nonylphenols and its Ethoxylates

Robert J. Fensterheim
Executive Director
Alkylphenols and Ethoxylates Research Council

Of Counsel:
Carolyn R. Hathaway
Latham & Watkins
1001 Pennsylvania Avenue, N.W.
Suite 1300
Washington, DC 20004

May 30, 2000

Alkylphenols and Ethoxylates Research Council
1250 Connecticut Avenue, N.W.
Suite 700
Washington, D.C. 20036

Comments of the
Alkylphenols and Ethoxylates Research Council
on the
Canadian Environmental Protection Act
Draft Priority Substance List Assessment Report
for Nonylphenol and its Ethoxylates

The Alkylphenols and Ethoxylates Research Council (APERC) appreciates this opportunity to submit comments on the draft Canadian Environmental Protection Act (CEPA) Priority Substances List (PSL) Assessment Report for Nonylphenol and its Ethoxylates (March 2000) (“Assessment Report”) by Environment Canada and Health Canada. APERC is comprised of major North American manufacturers and processors of alkylphenols (APs) and alkylphenol ethoxylates (APEs), including nonylphenols (NP) and nonylphenol ethoxylates (NPE).¹ APERC and its member companies have conducted extensive research on the fate, effects and exposure to NP and NPE.

APERC is not submitting a separate set of comments on the Environment Canada Supporting Document for Nonylphenol and its Ethoxylates (March 2000) (“Supporting Document”). However, these comments on the Assessment Report are generally applicable to and should be incorporated into the Supporting Document as well. In addition, specific comments on the data needs identified in the Supporting Document are included in Part IV of these comments.

I. OVERVIEW OF CEPA TOXIC DESIGNATION

The PSL Assessment Report presents a reasonably comprehensive and generally accurate summary of the extensive health and environmental effects, fate and exposure databases for NP and NPE. Based on this information, the PSL Assessment Report proposes that:

Under CEPA 64(b) Nonylphenol and its ethoxylates are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends.

Under CEPA 64(c) On the basis . . . of the margin of exposure between effect levels and reasonable worst cases estimates of intake from environmental media, nonylphenol and its ethoxylates are not considered a priority for investigation of options to reduce public exposure through control of sources that are addressed under CEPA.

Thus, NP and NPE are not considered to be CEPA toxic under paragraphs 64 (b) or (c) of the Canadian Environmental Protection Act. The PSL Assessment Report notes, however, that in a limited number of instances, NP and NPE concentrations in partially treated or untreated effluents from textile mills, pulp and paper mills and municipal wastewater treatment plants

¹ Members of APERC are: Dover Chemical Corporation, GE Plastics, Huntsman Corporation, Mitsubishi Chemical Corporation, Rhodia Inc., Schenectady International, Inc., Sunoco, Inc, and Union Carbide Corporation.

(MWWTPs) may exceed the chronic effect level calculated by Environment Canada. Based on this information, the PSL Assessment Report proposed that:

Under CEPA 64(a) Nonylphenol and its ethoxylates are entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity.

Therefore, the Assessment Report proposed that “nonylphenol and its ethoxylates be considered ‘toxic’ as defined in CEPA paragraph 64(a).”

Based on the analysis presented in the PSL Assessment Report, however, higher ethoxylated, commercial NPE (NP3EO-NP100EO) should not be included in the conclusion of “CEPA Toxic.” The estimated no effect value (ENEV) for the higher ethoxylates in receiving waters is 200 µg/L. Comparisons of environmental concentrations of NP9EO with the ENEV are presented in Table 12 and Figure 10 of the Assessment Report. These comparisons show that there are no freshwater sites in which the estimated exposure value exceeds the ENEV for the higher ethoxylates. Although the ENEV for NPE is apparently exceeded in untreated textile mill effluent, it is inappropriate to assume that aquatic organisms are chronically exposed to untreated effluents. These data do not justify a conclusion that higher ethoxylated, commercial NPE is CEPA Toxic.

The Assessment Report should therefore limit the conclusion of “CEPA Toxic” to NP, NP1EO and NP2EO. As the data demonstrate and Environmental Canada has recognized, NPE may be treated adequately in well-functioning wastewater treatment plants. Where treatment is not present or is inadequate, however, levels of NP and low mole NPE in wastewater and receiving streams near wastewater outfalls may exceed effect levels for sensitive aquatic species. Thus, APERC has consistently advocated the use of NPE in conjunction with effective wastewater treatment.

II. GENERAL COMMENTS

As noted above, APERC believes that, in general, the PSL Assessment Report and Supporting Document provide a fairly comprehensive and accurate summary of the biodegradation, bioaccumulation and exposure data for NP and NPE. The reviews of mammalian and aquatic toxicity include most of the high quality data that are available. The toxicity databases also include some data of poor quality, which should not be used.

Specific comments on the data summaries are included in Part III, below. There are, in addition, several general issues that should be addressed. These are presented below:

(1) The Assessment Report should not include data on octylphenol or octylphenol ethoxylate: In a number of places, the Assessment Report includes, in the data summaries, selected studies on octylphenol (OP) and octylphenol ethoxylate (OPE). The PSL Assessment Report states, “The scope of this Assessment Report is limited to nonylphenol and its ethoxylates. However, because of the similar toxicological properties of octylphenol and its ethoxylates (OP/OPEs) and because they are present in similar environmental compartments, relevant data on these compounds have been reviewed.”

APERC believes strongly that it is inappropriate to selectively include data on OP/OPE in the Assessment Report. In particular, the document makes selective use of data for OP and OPE for comparison of environmental fate and estrogenic activity. If these data are appropriate to make such comparisons, then data for OP and its ethoxylates should be included in discussions regarding human health. The availability of data for OP kinetics, OPE metabolism, and, most importantly, a two-generation study with OP, combined with the strong weight-of-evidence from NP and NPE studies, provide overwhelming evidence that appropriate use of commercial APEs poses no risk to human health.

(2) The assessment of environmental effects based on conventional endpoints is adequate to evaluate potential endocrine effects: In the review of endocrine endpoints, the Assessment Report concludes that endocrine modulation-based effects require an environmental health assessment that is different than an assessment based on the conventional endpoints of mortality, growth and reproduction. This is not appropriate and should be removed from the Assessment Report. While CEPA '99 does direct the Ministers to conduct research on endocrine disrupting substances, it does not require the performance of a risk assessment specifically for these types of compounds using only these “mode of toxicity” related endpoints. Biomarkers, whether they are involved in the endocrine system or are indicative of exposures to other types of compounds, are only useful in risk assessment if they are linked to adverse effects. Neither biomarkers of exposure (e.g., vitellogenin expression) to compounds with apparent estrogenic activity nor transient histopathologic observations are adverse endpoints that indicate actual effects to populations of aquatic organisms. The conventional endpoints of mortality, growth and particularly reproduction integrate the cumulative impacts (i.e., whether changes in blood steroid levels, alterations to gonads, redirected energy use to produce vitellogenin in males, etc.) caused by exposure to a compound. These data show that the higher ethoxylated NPEs most commonly used in commercial products are not estrogenic to aquatic species. The abundant database assessing conventional endpoints for NP, supplemented by the growing database of effects for NPE and NPEC, allows the confident interpretation of aquatic effects caused by these compounds. There is no basis to assume that these conventional data are missing effects caused by possible modulations to the endocrine system.

(3) Data show that commercial NPEs are not estrogenic in mammalian species: The Synopsis of the Assessment Report states that “While NP and some short-chain NPEs have estrogenic activity in mammalian systems, the results of available studies indicate that this occurs at relatively high dose levels.” APERC agrees that this statement is accurate relative to NP and further suggests that the estrogenic responses of NP are predictable, based on dose and response, and consistent (repeatable). It is unclear, however, what data support the statement for “short-chain NPEs.” It is scientifically inappropriate and misleading to suggest that a response in an *in vitro* cell system represents “estrogenic activity in mammalian systems.”

The Assessment Report frequently makes reference to “estrogenic responses” and receptor binding of NPE and APEs. These statements are misleading and incorrectly suggest that the weak responses of low mole ethoxylates represent the activity of NPE (or APEs) as a class. Commercial NPEs (NP9EO and NP4EO) are not estrogenic in mammals based on *in vivo* uterotrophic assays. Further, they are negative in *in vitro* binding assays. Therefore, under all current and proposed screening/testing regimes, they would not be considered estrogenic

(“endocrine disrupters”) or advance past Tier I screening. The Assessment Report should clearly reflect the lack of estrogenic effects that have been seen for commercial NPEs.

(4) The Assessment Report should distinguish data on NP from those on NPE:

The discussion in the Assessment Report fails to clearly distinguish NP from NPE and, in some cases, to distinguish between environmental and human health issues. Since there is at least indirect evidence that NPE is not metabolized to NP to any significant extent in mammals, these distinctions are critical. As noted in Section 2.4.3, commercial NPEs (i.e. NP4EO and NP9EO) do not have estrogenic activity as measured by the uterotrophic assay at the highest possible doses (Williams *et al.*, 1996). The doses employed in this study would have resulted in a positive uterotrophic response if only 10% of the dose was converted to bioavailable NP. However, no uterotrophic response was observed. These key data should be included in the discussions of estrogenicity of NP and NPE.

Similarly, the Assessment Report refers, in a number of places, to “identified effect levels for NP/NPEs” and then uses the lowest-observed-effect-level (LOEL) for NP. While APERC concurs that NP can be considered to be the “worst case” for evaluation of potential effects for these products, the Assessment Report should clearly state that NPE has not been shown to have effects similar to NP (i.e. kidney mineralization in male rats). To avoid this distinction causes confusion and unwarranted concern regarding exposure to commercial NPE.

(5) Skin absorption data are available to refine estimates of exposure:

APERC agrees that more detailed skin absorption data are useful in refining exposure estimates as included in the attached manuscript from studies sponsored by APERC at North Carolina State University. (Attachment 1). The manuscript indicates that absorption of NP, NP4EO and NP9EO at concentrations of 0.1 to 10% do not penetrate skin to any significant extent, even when left in contact for eight hours. Further studies, using perfused porcine skin flaps as a check for viability of the skin samples, have been completed (manuscript in preparation). These studies confirm the minimal absorption (< 1%) of NP and NPE. Based on these data, the worst-case estimate of skin absorption for NP and NPE should be 1% of the dose and the Margins of Exposure should be recalculated.

(6) NPE undergoes rapid biodegradation in wastewater treatment and

continues to degrade in the environment. The Assessment Report understates the biodegradability of NP and NPE. Further, the report overstates the potential for generation of metabolites of NPE. Studies of the degradation of NPE in MWWTP, of NP and NPE in receiving waters and in labs, and of NP in sludges show high rates of NPE degradation during treatment, low ppb or non-detect levels downstream of MWWTP, and continuing degradation of NPE and NP in soils and the environment. Based on these data, the Soap and Detergent Association monograph “Alkylphenol Ethoxylate” (SDA, New York, 1999) concluded that “APE undergoes rapid breakdown during conventional wastewater treatment and continues to degrade in water and soil.”

III. SPECIFIC COMMENTS

A. Environmental Fate (Section 2.3.1.)

The Assessment Report does not adequately characterize the degradation pathway for NPE. Figure 2 (The Biological degradation pathway for NPEs) suggests that NPE degradation stops at NP. This is not correct. With sufficient oxygen present, biodegradation of NPE proceeds by sequential removal of ethoxylate units to form low mole ethoxylates and ether carboxylates. These, in turn, undergo ring cleavage and formation of carbon dioxide, carboxylate ring-opened fragments and cellular matter. This should be reflected in the diagram of the degradation pathway. Included as Figure 1 is a revised diagram of the degradation pathway for NPE.

The discussion of degradation in municipal wastewater treatment plants, in Section 2.3.1.2.2, states that “In general, primary biodegradation of NPEs in MWWTPs is readily achievable, but ultimate biodegradation is not.” This is inconsistent with data from OECD 301B and 301F tests, which demonstrated that NPE and the common metabolites of NP9EO are rapidly degraded. In the 28-day test, NP was degraded to carbon dioxide by 62% and nearly met OECD standards for classification as readily degradable. NP9EO (which represent 80% of NPE in commerce), degraded to components which are themselves degradable. Wastewater treatment will generate minor quantities of surfactant metabolites; these metabolites are degradable in the receiving environment.²

The PSL2 report further concludes, based on only one study, that “more than 60% of the higher-chain APEs that enter MWWTPs exit as stable metabolites (e.g., APs and short-chain APEs) in either their effluents or their sludges (Ahel *et al.*, 1994a).” Subsequent work referenced in the PSL2 document has shown that APE’s metabolites are degradable in rivers and soils, and should not be considered to be “stable.” Data cited in the Assessment Report show that metabolites are present at ppb levels in MWWTP effluents while MWWTP influents have NPE at ppm levels. Total NPE removal is typically above 90%, from product in the influent to mineralization. As stated by Maguire (1999), “The intermediate and final products of metabolism are more persistent than the parent NPEs, but there is no doubt that such chemicals will also be ultimately biodegraded.” This should be reflected in the Assessment Report.

The Assessment Report also states that, “The application of NP-containing sludges to agricultural land may result in potential exposure in terrestrial environments.” We agree that there is always the potential for exposure. However, studies cited in Section 2.3.3.4 of the report indicate that NP will not persist. In addition, Marcomini (1989) showed greater than 80% reduction of the total NP, NP1EO, and NP2EO in sludge-amended soils in Switzerland within three weeks. Degradation will occur in aerobic soils - precisely the conditions where sludges are applied. This discussion of environmental fate in soil, Section 2.3.1.3, should also include the study by Hughes (1996) which demonstrates the rapid mineralization of NP9EO in soil (57% in 64 days) without formation of NP.³

² Staples, C. *et al.* 1999. *Chemosphere*, 38 (9):2029-2039.

³ Hughes, A.I., Fisher, J. and Brumbaugh, E. 1996. Biodegradation of NPE in Soil. Proceedings of the 4th CESIO World Surfactants Congress, Vol. 4, 1996.

B. Environmental Distribution (2.3.2)

The Assessment Report presents the results of distributional modeling for NP. The model inputs include half-lives for water of 1700-5500 hr (or 71 to 229 days) and 5500 to 17,000 hr. (or 229 to 708 days) in soil and sediment. These very high (slow) degradation half-lives are not supported by available data also presented in the Assessment Report. Many of the data given in the Assessment Report and the Supporting Document were analyzed by Naylor (personal communication) using the Larson equation from Swisher, 1987.⁴ The Larson equation develops the best-fit slope of the kinetic data from degradation tests. The calculated decay rate can be used to calculate a half-life, assuming first order kinetics. The resulting table, attached as Table 1, shows that true half-lives (measuring from the end of the lag phase to the beginning of the plateau near test end) range from about 6 to 26 days for water, soil, and even water sediment slurry. Thus, the scientifically appropriate water and soil half-lives would be 7 to 28 days or 168 to 672 hours.

C. Environmental Concentrations (2.3.3)

APERC agrees with Environment Canada's use of measured data to assess environmental exposure. APERC further agrees with Environment Canada that the collection of additional exposure data would be useful to further the assessment.

To avoid confusion and misuse of the data in the future, the Assessment Report should emphasize that the bioaccumulation factors (BAFs), reported in Section 2.3.3.6, are dry-weight based. BAFs typically are expressed on a wet or fresh weight basis and emphasizing that these are dry-weight data will ensure that the values are compared appropriately.

D. Effects Characterization

1. Ecotoxicity (2.4.1)

As noted above, the review of the ecotoxicity database for NP and NPE includes most of the high quality data that are available. However, the database also includes some data of poor quality. Although some of these studies were scored to reflect their quality, the studies were used in the assessment. Poor quality data should not be used. If additional studies are needed, they should be identified as data needs. The data summary also should not include data on OP and OPE.

Specific comments on selected ecotoxicity studies in Section 2.4.1.4 of the Supporting Document are presented below. This review is not comprehensive. Rather, these three studies are addressed as examples of data that should be excluded from the assessment.

(1) The study by Ashfield *et al.* (1998) is inadequately presented. Ashfield exposed fish for 30 days and then raised them for over 400 days. Effects on growth and gonad weights fluctuated slightly during the grow-out period with some treatments slightly higher than controls, then reverting to no difference or lower than controls. The study results followed no

⁴ Swisher, RD 1987. Surfactant Biodegradation, 2nd Ed., vol. 18 of Surfactant Science Series, Marcel Dekker, Inc. Ch. 5 Section V., p294.

dose response, used very few animals in individual comparisons, required cube-root transformation of some data to enable statistics to show an effect, no response was more than about 10% from the control, and the authors themselves questioned whether such results were significant. This study is very poor quality and cannot be used to assess potential risks.

(2) In Christiansen *et al.* (1998), exposure of eelpout fish was based on injection of NP. This route of exposure is irrelevant to assess effects of any contaminants. The OECD Fish Expert Group has reached consensus that exposure of aquatic organisms to compounds should be by an environmentally relevant route. The two routes identified as relevant are in the water or incorporated into food items. This should be noted.

(3) Gimeno *et al.* (1997) studied tert-pentyl phenol, a non-commercial compound, and not NP or NPE. It is irrelevant to a CEPA toxicity assessment of NP and NPE and should be removed.

2. Toxicity to Terrestrial Plants and Animals (Section 2.4.1.3)

A definitive avian dietary study was performed in 1999 by EBA, Inc., Snow Camp, North Carolina (EBA, 2000).⁵ The eight-day bobwhite quail study exposed quail chicks to NP9EO in the diet at concentrations from 0 to 5000 ppm for five days with three additional days of observation. No abnormal behavioral observations or mortalities were observed at any concentration tested, therefore, the LC₅₀ was determined to be greater than 5000 ppm. APERC will provide a copy of the final study report when it is available.

3. Endocrine Effects Data (Section 2.4.1.4)

The summary of the relative potency of NPEs and NP omits a number of factors that should lead to the conclusion that only the 1 and 2 mole ethoxylates show weak estrogenic activity, and these at much lower potency than NP, and that NP9EO does not have estrogenic activity.

The study by Jobling and Sumpter (1993) was one of the earliest conducted to look at the relative potency of these compounds. As acknowledged by Professor Sumpter in a number of public forums, the authors paid little attention to the purity of the materials tested and took few precautions to prevent contamination. Further, the data as presented are questionable at best. The EC50 values on which the potency calculations are based are extrapolations from single concentrations and assume maximum response for all chemicals. This is not appropriate. In addition, the assay purports linear response over approximately seven orders of magnitude (1 pM to 10 uM with extrapolation over yet another order of magnitude) without validation. Responses over such a range for standard assays would normally be considered aberrant and due to contamination or other non-specific effects. Finally, this is the only study to indicate estrogenic activity of NP9EO.

⁵ EBA 2000. Avian Dietary Toxicity Test with Nonylphenol Ethoxylate 9 (NPE9) in the Northern Bobwhite (*Colinus virginianus*). EBA, Inc. Study No. 019812.

The study by Routledge and Sumpter (1996) was much more carefully controlled. They did not however, test NP9EO as might be construed by the citation. The results are summarized in the following table:

| <u>Compound</u> | <u>Relative Potency Compared to 17β-Estradiol</u> |
|-----------------|---|
| NP | 0.00014 |
| NP1EC | 0.00004 |
| NP2EC | 0.00004 |
| NP2EO | 0.000002 |
| NP12EO | No Response |

Based on these data NP2EO is two orders of magnitude less potent than NP. Moreover, it would be impossible to predict a positive response for NP9EO from these data. This relative potency is consistent with the review for NP2EO from the Routledge and Sumpter paper cited in Section 2.4.3 of the Assessment Report.

The Assessment Report also states that Jobling *et al.* (1996) showed similar potency for induction of vitellogenin of NP2EO and NP1EC as for NP in trout hepatocytes. Jobling *et al.* did not evaluate *in vitro* trout hepatocytes. Similar *in vivo* vitellogenin blood concentrations were reported for 30 ppb of NP and NP2EO and NP1EC, which is at least four orders of magnitude less potent than 17β-estradiol (E2) (2 ppt).

A very recent study (C. Metcalfe, personal communication) has shown that NP1EC has no estrogenic activity in the yeast estrogen screen (YES) assay at concentrations up to 100,000 ppb. Both of the ether carboxylates were well characterized and contained no detectable alkylphenol. Based on the absence of response at the highest concentration tested, the ether carboxylates are more than 5.0×10^7 less potent than E2. In the same screen, NP1-2EO has no activity below 10,000 ppb, and slight activity at 10,000 ppb, which is above its solubility limit. Thus, NP1-2EO is about 10^6 less potent than E2. This calls into serious question the results of Jobling *et al.* (1996), in which the test materials were not characterized.

If OP and OPE data are to be included in the Final Assessment Report, it should be noted that Dr. Metcalfe also tested OP1EC with similar negative results.

4. Mammalian and Human Effects (Section 2.4.3)

In the discussion in the Assessment Report of the multigenerational study of NP in rats (NTP 1997, Chapin *et al.* 1999), the statement regarding the increase in gestational length can be misconstrued. The values for the F2 gestational length for the treated groups, 21.8, 21.6, and 22.0 were all similar to the F1 control value of 21.9 days and were only statistically significant because the F2 controls were slightly lower, 21.4 days. Data like these are anticipated

in studies with the large numbers of data points in a multigeneration study. They are a statistical aberration and should not be cited.

Finally, the inclusion of the reference to the studies by de Jager *et al.* (1999a, 1999b) should be reconsidered. The mortality observed at 100 (3/20) and 250 (15/20) mg/kg/day is inconsistent with the vast amount of data on NP in rats and suggests that the doses and/or the procedures were incorrect. Cunny *et al.* (1997), Chapin *et al.* (1999), Talmage (1994). In addition, the data from Lee *et al.* (1998) should be removed from the Assessment Report based on soon-to-be-published studies by J. Odium and J. Ashby at Zeneca Central Toxicology Laboratory (included, with permission of Dr. Ashby, in Attachment 2). These studies indicate that the cited results from Lee *et al.* (1998) were not reproducible (even when NP was administered intraperitoneally).

E. Environmental Risk Characterization 3.1.2

1. Overview (Section 3.1.2.1)

To calculate the estimated no effect value (ENEV) for aquatic species, an application factor (AF) of 10 was applied to the lower bound 95th percentile no-effect value. The AF of 10 was applied to account for (1) species differences and (2) reported sublethal effects. Neither of these considerations justifies the AF of 10. The PSL guidance permits the use of a lower AF when it is justified by the data. In this case, the data justify the use of an AF of 5.

(1) *Species differences* - For NP and NPE, there are data for at least 44 species of aquatic organisms, including fish, invertebrates, algae, and microorganisms. The species inhabit the surface layer, water column, and benthic zone. They cover warm and cold water bodies and both fresh water and marine systems. Examination of all of the chronic no-observed-effect-concentration (NOEC) and EC10 values for fish, invertebrates and algae shows that chronic values range from 2 to 77.5 µg/L. These data are presented in Table 2 (attached). In light of this abundant database, an AF of 5 is adequate. Markers of potential estrogenic activity are not relevant endpoints for assessing the effects on mortality, growth and reproduction of NP and NPE.

(2) *Reported sublethal effects* – These Assessment Report should not apply an assessment factor based on chronic data that measure sublethal effects. A further reduction in the ENEV because of reported sublethal effects, that are intended to be measured in chronic studies, is scientifically inappropriate.

When an AF of 5, rather than 10, is used, the Tier 3 ENEVs would be as follows. The recalculated ENEVs are protective of the full range of aquatic toxicity data that are reported.

ENEV_{water} (NP) = 2 µg/L (not 1 µg/L)

ENEV_{water} (NP1-2EO) = 4 µg/L (not 2 µg/L)

ENEV_{water} (NP3-16EO) = 400 µg/L (not 200 µg/L)

ENEV_{water} (NPEC) = 400 µg/L (not 200 µg/L)

2. Risk Characterization for NP (3.1.2.3)

In Table 6, K_d is probably intended to mean “distribution coefficient,” not “dissociation constant.”

In Table 7 (footnote), Staples *et al.* (1998) assumed 95% water content for algae and 85% for fish, not 95% for both.

Tables 11 and 12 should be clarified to explain why lake sites show exceedances at 1/5 sites on Table 11 and 2/5 on Table 12.

3. Endocrine Disruption in Aquatic Biota (3.1.2.3.6)

The endpoints of mortality, growth, and reproduction are the endpoints used for aquatic hazard assessment, which are in turn used to assess aquatic risks. Endpoints related to the assessment of mechanisms of toxicity or that are only indications of exposure are inadequate to perform hazard and risk assessment. However, the Assessment Report attempts to perform essentially two different risk assessments, one based on conventional apical endpoints and one based on a mechanism or mode of toxicity, that being endocrine modulation. While knowing the mode of action can be useful, the mode of action is itself not an effect or an adverse endpoint. The U.S. Environmental Protection Agency (EPA) does not consider endocrine disruption to be an adverse endpoint but rather a mode or mechanism of action leading potentially to other outcomes which are typically considered in reaching regulatory decisions.⁶ A similar finding was reached at the 5NR workshop on Risk Assessment for Endocrine Disrupting Compounds held in Huntsville, Ontario in February 2000.

Both the U.S. EPA and the CEPA '99 legislations take a risk-based approach to assessing chemicals. The goal of the U.S. EPA Office of Pollution Prevention and Toxics (OPPT) is to:

. . . prevent unreasonable risks to health and the environment as a result of the manufacture, processing, use, and disposal of industrial chemicals.

Similarly, CEPA '99 states that it takes a "risk-based approach to decision-making", at an "ecosystem level" of organization. The U.S. EPA risk assessment guidance states that the assessment endpoints used to assess potential impacts to the aquatic environment are:

. . . protection of aquatic organisms (algae, invertebrates, and fish) . . . any effects . . . would be exhibited at least up to the population level of organization.

The measurement endpoints that are used to enable the assessment of impacts to the aquatic environment are:

⁶ U.S. EPA 1997. Special report on environmental endocrine disruption: an effects assessment and analysis. EPA/630/R-96/012.

. . . mortality, growth and development, and reproduction . . . since populations are governed by these measurement endpoints, OPPT assumes that adverse effects to these endpoints would manifest themselves up to the population level of organization.

Measurement endpoints such as growth rate, fecundity, survival and growth of young, and behavioral endpoints such as mating and care-giving may all be used to quantify the assessment endpoints of survival, reproduction, and development and many of these are included in current testing guidelines. These tests are sensitive to effects from endpoints suggested by the U.S. EPA, the Endocrine Disrupter Screening and Testing Program and OECD for evaluation of estrogenic effects. Data from these higher tiered tests are available for NP.

For many years, OECD has been considering the issue of testing for endocrine disrupting substances. A detailed review paper, entitled “Appraisal of Test Methods for Sex Hormone Disrupting Chemicals”⁷ was prepared by the United Kingdom as a basis to begin to assess the suitability and availability of existing test methods. The list of endpoints to be developed in a fish chronic study by the OECD are similar to those already included in the existing U.S. EPA testing guidelines and include: embryo hatchability and viability (F₀ and F₁), larval survival, growth and development (F₀ and F₁), time to sexual maturity (F₀), secondary sexual characteristics (F₀), sex ratio (F₀ and F₁), egg production (F₀), spawning frequency/behavior (F₀), fertilization success (F₀ and F₁), histopathological findings (F₀ and F₁), and gamete maturation (F₀). Biochemical markers such as vitellogenin, steroids, and steroid enzyme (F₀ and F₁) were identified as optional. The conclusion/recommendation from the expert consultation was primarily an acknowledgment that the work underway with respect to the development of methods for detection of endocrine disrupters in fish, is still at the level of “pre-validation” or method optimization. However, the chronic study itself does allow for an understanding of effects on growth, reproduction and development based on the apical endpoints without the inclusion of the biomarkers. All of these data already exist for NP/NPE, so focusing on unvalidated biomarkers is not appropriate.

Many weak endocrine disrupting substances produce non-endocrine related adverse effects on reproductive and other biological functions that are much more significant than might be predicted by their minor mode of endocrine action. It is for this reason that toxicity testing for regulatory programs and ultimately the risk assessment paradigm focuses on identifying adverse effects to the organism rather than on defining mechanistic pathways. Cellular and sub-cellular changes caused by stressors are relevant in risk assessment only if they result in changes at the whole organism level.⁸ While the endocrine-related biomarker studies cited in the Assessment Report provide information on chemicals that may bind to the estrogen receptor, none of these endpoints are standardized, validated, or related to an adverse impact on the whole organism, let alone the population which is the basis for risk assessment decisions in

⁷ Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals Capable of Affecting the Reproductive Process. Prepared by the MRC Institute for Environment and Health for the UK Department of Environment, Transport and the Regions, 1998.

⁸ Fairbrother A, Ankley G, Birnbaum L, Bradbury S, Francis B, Gray E, Hinton D, Hohanson L, Peterson R, and Van der Kraak G. 1999. Reproductive and developmental toxicology of contaminants in oviparous animals, p. 283-362. In Di Giulio R and Tillitt D (ed.), Reproductive and developmental effects of contaminants in oviparous vertebrates, Society of Environmental Toxicology and Chemistry, Pensacola, FL.

both Canada and the United States. In addition, there are as many similar *in vitro/in vivo* studies showing no or contradictory effects. The inherent variability in both the methods and the outcomes observed in the cited studies suggests that they should not be used to quantify or manage risk in the environment. Even if there is *in vitro* information suggesting a potential mode of action, given the complexity of hormonal regulation in organisms, biochemical or histopathological changes at one time point may not be manifested as adverse reproductive or developmental effects. These endpoints can serve to augment a risk assessment but, unless they are able to predict higher level effects, they should not be used to derive population level no-effect-levels or to set regulatory criteria.

4. Terrestrial Risk Assessment (Section 3.1.2.5)

The terrestrial risk assessment relies on a Danish study by Krogh *et al.* (1996) that is unsupported by subsequent research by the same authors. The Assessment Report should address the bioavailability of NP from sludge applied material. The Danish treatment plant sludge of 140 µg/g dry weight (dw) was used in a series of toxicity tests measuring soil function (nitrification) and toxicity to various soil dwelling organisms following the application of 21 tonnes/hectare (t/ha) or nearly three times the allowable application of sludge in Canada. The field studies showed no negative effects of sewage sludge on soil microorganisms or fauna up to one year after application.

The ecotoxicity of NP in sewage sludge from municipal STPs has been the subject of considerable study in Denmark^{9,10}. The acute toxicity of NP added to soil and sewage sludge to various soil organisms has been reported. Small arthropods, microorganisms, and annelids were tested. The endpoints addressed included survival, growth and reproduction. EC₅₀ values ranging from 13.7 to 58.4 µg/g dw were reported (duration not given). An earthworm reproductive toxicity EC₁₀ value of 3.5 µg/g dw was apparently also reported¹¹. The researchers then examined the effect of NP on the same organisms in field studies by exposing the organisms to sludge-amended fields with known NP sludge concentrations. The concentrations of NP ranged up to 140 µg/g dw. The amount of sludge amendment was equivalent to 3.5, 7, and 21 t/ha. The maximum amount is nearly three times the Canadian limit of 8 t/ha per 5 years. The researchers monitored the abundance of earthworms (numbers of adults and numbers of cocoons), various small arthropods, collembola (several species), and the common laboratory test collembola *Folsomia fimetaria*. The authors also measured the extent of microbially mediated nitrification. No negative effects were observed to soil microorganisms or fauna up to one year after application. A follow up report examined possible reasons for the discrepancies between laboratory-derived effects (positive) and field studies (negative). The reasons included possible avoidance of sludge lumps, degradation of organic contaminants, sorption to solids (reducing

⁹ Jensen, J. and P.H. Krogh 1997. Ecotoxicological Assessment of Sewage Sludge Application. Proc. Management and Fate of Toxic Organics in Sludge Applied to Soil. Technical University of Denmark, Copenhagen, Denmark. April 30 - May 2, 1997.

¹⁰ Krogh, P.H., J. Jensen, M. Holmstrup, J.J. Scott-Fordsmann, and H. Lakkenborg Kristensen 1999. Why is sludge with toxicant load non-toxic in the field? Society of Toxicology and Chemistry - Europe, Leipzig, Germany, May 25-29, 1999.

¹¹ Krogh, P. H., M. Holmstrup, J., Jensen, and S.O. Petersen 1996. Økotoxikologisk vurdering af spildevandsslam i landbrugsjord. [Ecological assessment of sewage sludge on farm land- Report from the Danish EPA. In Danish with English summary]. Arbejdsrapport Nr. 43, 53 pp. Miljø-og Energiministeriet Miljøstyrelsen.

bioavailability), and the potential for stimulus of biological activity due to the increase in nutrients. Although the authors of the PSL2 assessment used the Danish laboratory data for the "hyper-conservative" and "conservative" assessments, the complete contradiction between laboratory and field toxicity results may be why the authors concluded that additional data are warranted on the "fate and effects of APs and APEs in sludge addition in the agricultural fields . . .".

For the "hyper-conservative" and "conservative" assessments the laboratory toxicity data were used. The Assessment Report document incorrectly used a soil density of 1340 kg/m³ (based on the stated use of the mass of one hectare being 2000 tonnes) and should use a more conventional density of 1700 kg/m³ for soil¹². Use of the corrected density proportionally reduces the exposure concentration. The EEV/ENEV ratios should then be recalculated accordingly and the use of conservative assumptions discussed (e.g., use of an assessment factor to worsen a toxicity value even though the laboratory data are directly refuted by field studies and application of 5 years worth of allowable sludge to be applied all at once).

The results do suggest that further data would be valuable to explain the discrepancies between the laboratory data and field studies, as is the conclusion of the PSL Assessment Report. To better understand the potential effects of NPE and its degradation products on soil invertebrates, APERC intends to conduct a chronic toxicity and reproduction test in the earthworm, *Eisenia fetida*, based on BBA Guideline VI, 2-2, and ISO/DIS 1126-2.

F. Human Health Risk Characterization (3.3.2)

APERC agrees with the conclusion of the Assessment Report that the margins of exposure between effect levels and reasonable worst case human intake indicate that NP and NPE are unlikely to present a significant risk to humans and that investigation of options to reduce public exposure is not a priority.

As discussed above, APERC also agrees that additional information regarding dermal absorption would permit a more meaningful assessment of potential risks from consumer products. The skin absorption studies included in Attachment 1 indicate that less than 1% of NP/NPE is absorbed by skin. The Margins of Exposure, (MOE) presented in table 14 should be recalculated using 1% absorption as a worst-case exposure to NP and NPE.

In addition, the MOE calculations should consider the available information to be sufficient to conclude that NPE is not significantly metabolized to NP. Thus, the assumption that 10% of the dose would be converted to biologically available NP (for comparison to the no-observed-adverse-effect-level (NOAEL) of 12 mg/kg/d) should be considered a worst-case scenario. As an alternative (or verification), the use of the chronic NOAEL of 40 mg/kg/d from the dog study (Smyth and Calandra, 1969) with NP4EO can be used. Since the effects at the next higher dose (200 mg/kg/d) were minimal, this is a very conservative NOAEL. Below are MOE values for the exposure scenarios (with MOE <100 in the Table) described in Table 14 recalculated using these worst-case assumptions. Similar calculations for the other scenarios result in higher MOEs but these uses are of minimal consequence to the exposure calculations.

¹² European Union 1994. Technical Guidance Document in support of the Commission Regulation (EC) 1488/94 on Risk Assessment for Existing Substances.

| <u>Exposure Medium</u> | <u>Health Canada MOE</u> | <u>Recalc. MOE*</u> | <u>Recalc. MOE**</u> |
|------------------------|--------------------------|---------------------|----------------------|
| Skin moisturizer | 0.5 | 530 | 180 |
| Fragrance | 8 | 7650 | 2550 |
| Household cleaner | 21 | 21000 | 7000 |
| Deodorant | 43 | 43000 | 14000 |

* Assumes 1% skin absorption and 10% metabolism to NP (NOAEL = 12 mg/kg/d)

** Assumes 1% skin absorption and NOAEL of 40 mg/kg/d (chronic)

Both recalculations assume worst case situations for the NPE exposure (lowest-mole commercial ethoxylate, NP4EO), absorption, and NOAEL

IV. COMMENTS ON RESEARCH NEEDS

Environment Canada identified a number of research needs during the development of the risk assessment for NP and NPE. These are included in the Supporting Document for Nonylphenol and its Ethoxylates. Environment Canada stated that "Addressing these knowledge or data gaps would reduce the uncertainties identified in this assessment and increase the understanding of NP/NPEs in the environment, which may be beneficial in risk management activities." APERC's comments on these data needs are presented below.

A. Treatability And Degradation (4.1)

According to the Supporting Document, "An improved understanding of the treatment and persistence (in the natural environment) of NP/NPEs would be greatly beneficial". Specific areas of additional research include:

- Determination of the degradation efficiency of NP/NPEs in MWWTPs that employ various treatment processes including the more advanced systems *e.g.*, ultraviolet oxidation, ozonation, adsorption "polishing", *etc.*

COMMENT: APERC agrees that efficient treatment of NPE residues is possible and the most effective means of reducing discharges. However, this information is not needed for an assessment of CEPA toxicity.

- Study of the production, treatability, persistence and environmental significance of halogenated derivatives of NPE degradation products

COMMENT: Halogenated derivatives are measurable only in vanishingly small concentrations in the environment. Results were presented by New York state authorities at the Society of Environmental Toxicology and Chemistry meeting (Philadelphia, PA, Nov. 1999). The authors searched for halogenated by-products of NP and NPE in NY Harbor sediments. The concentrations reported were either non-detectable or at trace levels only. The data suggest that halogenated derivatives are not a significant issue.

- Mass balance studies that take into account the relative importance of biodegradation, photodegradation, adsorption to suspended solids and bed

sediment, formation of unextractable residues, aerobic and anaerobic conditions and temperature effects in aquatic and terrestrial environments

COMMENT: A high quality study performed by the U.S. EPA (*see* March 1999 issue of Environmental Toxicology and Chemistry - five papers total) examined the fate of NP in littoral enclosures and showed that NP degraded rapidly in water, somewhat slower in sediment and posed effects at levels higher (less severe) than laboratory studies with similar species. It is unclear how further detailed studies will assist in the assessment of CEPA toxicity or the management of potential risks where few are seen now.

- Study of the atmospheric chemistry and fate of NPEs both in the atmosphere and terrestrial ecosystems because their use in aerially-applied pesticide formulations

COMMENT: NPE applied aerially is generally NP9EO. The atmospheric photo-oxidation rate of NP9EO can easily be calculated using SRC calculation methods. Hughes (1996) examined the terrestrial fate of NP9EO and showed that mineralization to carbon dioxide was rapid and extensive (57% in 64 days). Additional data repeating the study would not be a high priority.

B. Fate and Occurrence (4.2)

The Supporting Document states that, “although there is a large amount of data available on the distribution of APs, there are clearly some knowledge gaps on the fate and occurrence of NPEs in the Canadian environment.” These data gaps include:

- Additional work to determine the presence of the short chain NPEs and NPECs in effluents (particularly from textile mills) and in harbor sediments
- Additional study to determine the NP/NPE concentrations in pulp and paper mill effluents to confirm that levels have decreased recently
- Additional study of the fate of NPEs in textile mill effluents and their receiving environments in treatment systems and the environment, particularly at sites where there is little or no treatment

COMMENT: APERC supports the gathering and use of high quality monitoring data to support risk assessment and management activities. There are, however, river die-away studies using ring labeled NP9EO that show rapid primary biodegradation and some mineralization. (ABC Studies sponsored by APERC.) It is unclear how repeating these studies or performing additional studies on the environmental fate of NP and NPE are needed to assess CEPA toxicity or manage risks from textile mill effluents.

C. Biological Effects (4.3)

The Supporting Document states that “the emphasis of biological effects studies has been on NP and longer chain length NPEs with relatively few quality data available on the

chronic toxicity of lower chain length NPEs and NPECs.” Thus, the Supporting Document concludes that additional research is needed in the following areas:

- Studies comparing toxicity of APs, APEs and APECs using standardized methodology

COMMENT: This research need is refuted by the abundant data that are available for NP/NPE. Comparisons of these data are shown in Figure 5 of the Supporting Document. Figure 5 shows that there is a definite linear relationship between ethoxylate chain length and toxicity, suggesting that further testing is not a high priority.

- Bioaccumulation estimated for NPEs and NPECs, OP and OPEs.

COMMENT: The Supporting Document states, "the ability of NP and NPEs to bioaccumulate in the environment is low to moderate. . . Little data are available for NPE, but based on their structure they are not expected to bioaccumulate." This summary and conclusion are correct and supported by the available data on partitioning and metabolism. Therefore, additional data on NPE bioaccumulation would be a low priority. Data for OP/OPE should not be included in the assessment of CEPA toxicity and the management of risks for NP and NPE.

- Measurement of the partitioning properties (*e.g.*, K_{oc}) and bioavailability of APs, APEs and APECs.

COMMENT: Partitioning properties such as K_{oc} and K_{ow} can be reliably predicted from structure. APERC agrees that bioavailability data are not generally available for low mole NPE and NPEC. However, in studies of terrestrial fate and toxicity, bioavailability would be addressed.

- Extensive study of the relative estrogenic potency of the APs and APEs using validated standards for testing and performing tests both *in vitro* and *in vivo* to aid in the elimination of debate resulting from the inconsistency in relative potency reported for E_2 receptor binding, YES assay and Vg induction in trout hepatocytes.
- Potential endocrine-mediated effects of APs and APEs need to be studied for mechanisms other than estrogenicity.
- Validation that the assumption of additivity of AP, APE and APEC estrogenic responses in the environment is critical for interpretation of potential risk together in complex mixtures.
- Determination of the significance of estrogenic responses at the individual or population levels so that an assessment of the relative risk of this mode of action relative to other endpoints would be possible.

COMMENT: It is unclear why additional studies of endocrine endpoints and mechanisms are necessary to assess CEPA toxicity. E2 receptor binding, YES assay, and Vg induction are not adverse endpoints that can be used to assess environmental risks. Numerous studies of NP and NPE, which examine mortality, growth and reproduction, provide the necessary and relevant data for risk assessment purposes. In addition, the available data, though admittedly few, suggest the additivity of estrogenic responses. No studies with NP and NPE suggest otherwise. Finally, studies of APs and APEs other than NP and NPE should not be included in this review.

- Validation of the predicted responses in aquatic field studies, especially at textile and municipal effluent sites

COMMENT: It is not clear how this would be done, other than through studies like the U.S. EPA littoral enclosure study. In any case, APERC supports the collection of monitoring data to verify predicted concentrations, particularly where effects are predicted to occur.

- Study of the fate and effects of APs and APEs during sludge additions to the agricultural fields

COMMENT: APERC concurs on the need to assess the fate and effects of NP and NPE in sludge-amended soil. The toxicity study of NP9EO in feed fed to quail has been completed. No effects were seen at the highest dose of 5000 ppm in the diet.

- Determination of the relative contribution of APs and APEs to the toxicity and/or estrogenicity of complex environmental mixtures and effluents

COMMENT: The relative contribution of NP/NPE to the toxicity of complex environmental mixtures and effluents is beyond the assessment of CEPA toxicity. To aid in the management of risks potentially posed by NP/NPE in those media, all contaminants present in the media would need to be addressed, including other surfactants, PAHs, pesticides, PCBs, metals, mammalian and human hormones, phytoestrogens, and pathogens. This would be true for the management of any contaminant in an environmental mixture or effluent.

Figure 1

AEROBIC BIODEGRADATION PATHWAYS
OF NONYLPHENOL ETHOXYLATES

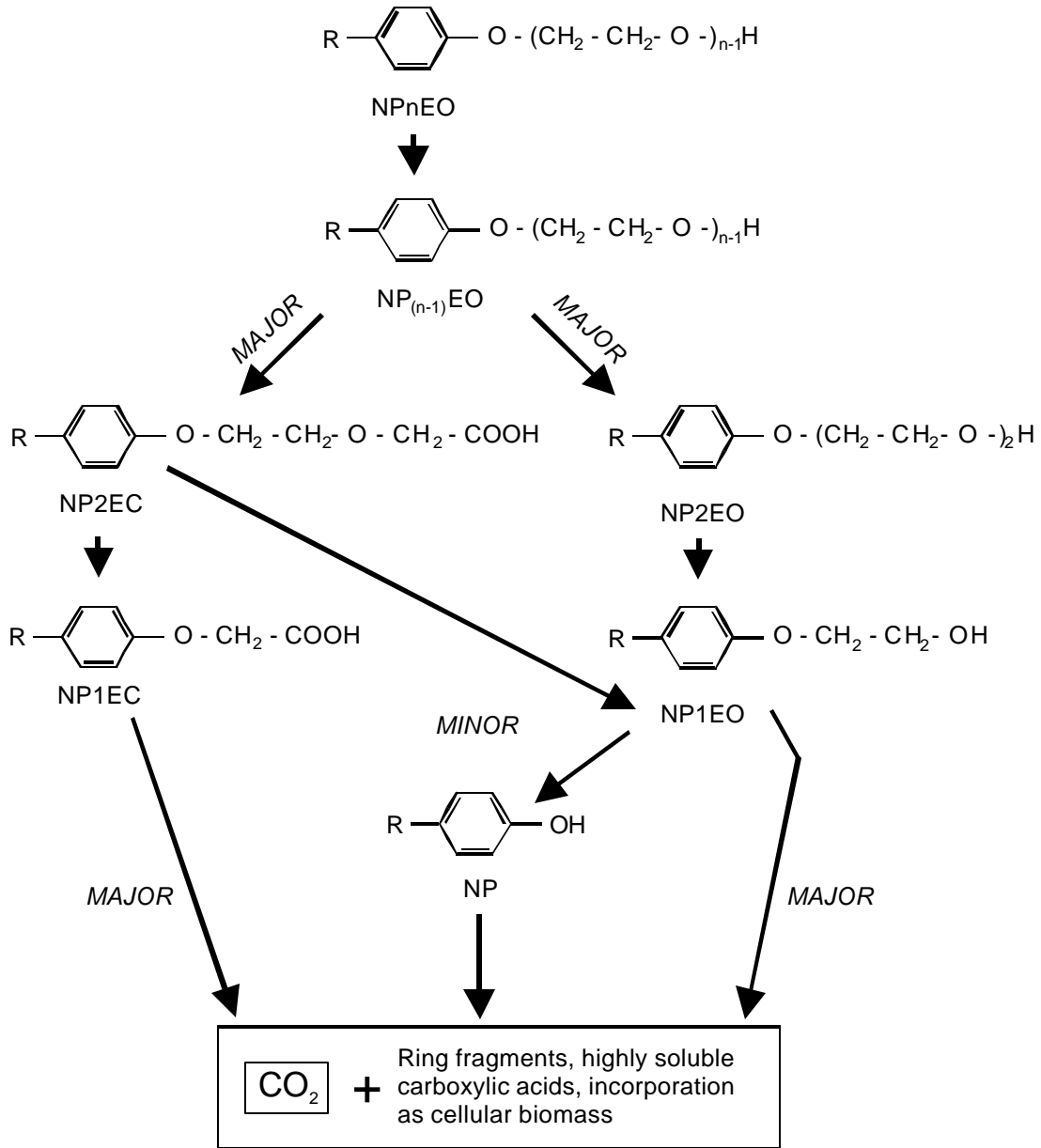


Table 1**Summary of Half-Life Calculations**

| Study | End point | Lag Time, days | Data Collection | Half-life, days |
|--|----------------------------|-----------------------|------------------------|------------------------|
| NPE in SCAS ABC 14C study (ABC, 1996) | CO2 trapped | 0 | day 30-day 47 | 2.7 |
| | 14C released from solids | 0 | day 30-day 47 | 5.8 |
| | 14C decrease in solution | 0 | day 30-day 47 | 21.3 |
| NPE in river die-away ABC 14C study (ABC, 1997) | CO2 trapped | 21 | day 28-day 127 | 18.6 |
| NPE in soil Amway study (Hughes, 1995) | CO2 trapped | 0 | day 3-day 64 | 18.2 |
| NP in sludge-amended soil Marcomini and Giger (| NP concentration decrease | 0 | day 5-day 41 | 7.3 |
| | | 0 | day 5-day 103 | 9.5 |
| NP in OECD 301F Exxon report (Lee, 1996) | Oxygen uptake | 3 | day 15-day 29 | 6.3 |
| NP in seawater Ekelund and Granmo (1993) | CO2 trapped, with sediment | 0 | day 7-day 56 | 26.3 |
| | without sediment | 28 | day 28-day 112 | 5.9 |
| NPE in river water Yoshimura, 1986 | Specific analysis by HPLC | 0 | day 4 - day 16 | 2.1 |

| | | | | |
|--|-------------------------|------|-----------------|-----------------|
| NP in soil Trocme | Specific analysis by GC | 6 | day 10 - day 40 | 10.4 |
| NP in soil (septic field) Terran Corp. study | | | 0 to 220 | 21 to 24 |
| <i>UCC OECD 301B tests</i> | CO2 trapped | | | |
| NPE1C, So. Charleston POTW | | 4.5 | day 5 - day 28 | 7.5 |
| NPE1C, Institute WWTP | | 4.1 | day 5 - day 28 | 6.6 |
| OPE1C, So. Charleston POTW | | 4.0 | day 5 - day 28 | 6.9 |
| OPE1C, Institute WWTP | | 3.9 | day 5 - day 28 | 5.7 |
| NPE2C, So. Charleston POTW | | 2.8 | day 5 - day 28 | 7.0 |
| OPE2C, So. Charleston POTW | | 4.5 | day 5 - day 28 | 7.9 |
| <i>Springborn OECD 301B tests</i> | CO2 trapped | | | |
| NP | | 2.5 | day 1 - day 35 | 7.6 |
| NPE1.5 | | 12.5 | day 1 - day 35 | 14.2 |
| NPE9 | | 1.5 | day 1 - day 35 | 11.9 |
| OP | | 5.8 | day 1 - day 35 | 10.4 |
| OPE1.5 | | 3.0 | day 1 - day 35 | 11.4 |
| OPE9 | | 1.7 | day 1 - day 35 | 13.5 |
| Benzoate | | 0.2 | day 1 - day 35 | 5.4 |
| DITA | | 1.9 | day 1 - day 35 | 13.5 |

Table 2

Chronic Toxicity of Alkylphenol Ethoxylates to Aquatic Organisms

| Species | Test Conditions | NOEC (LOEC) (µg/L) |
|----------------------------|--|---|
| <u>Fish</u> | | |
| Fathead minnow | 28-d early life, length: | 23 |
| <i>Pimephales promelas</i> | 33-d early life, survival: | 7.4 (14) |
| Fathead minnow | 28-d early life stage, length and mortality | 77.5 (193) |
| <i>Pimephales promelas</i> | | |
| Rainbow trout | 90-d, post-hatch, early life stage; flow-through | 6.0 (10) |
| <i>Oncorhynchus mykiss</i> | | |
| Rainbow trout | 3 week exposure up to ~50 ppb, 2-yr old fish, measured GSI, VTG, spermatogenesis | 20.3 (54.3) |
| <i>Oncorhynchus mykiss</i> | | |
| Bluegill sunfish | 28-d, length and mortality | 59.5 (126) |
| <i>Lepomis macrochirus</i> | | |
| Japanese medaka | 30 day following hatch, 30 day grow out, growth, GSI, mortality, reproduction, up to 2 ppb | >1.9 |
| <i>Oryzias latipes</i> | | |
| Japanese medaka* | 3 month renewal, 1-2 day post hatch; growth, sex ratios, testis-ova | 10 (50) (~40% control mortality) |
| <i>Oryzias latipes</i> | | |
| Zebrafish* | full life cycle test, fertilization rate, sex ratio | 2 (20) |
| DANIO RERIO | | |
| Japanese medaka* | 2-generation test, mortality, growth, reproduction | 2 (20) F0 and F1 based on mortality (most sensitive endpoint) |
| ORYZIAS LATIPES | | |
| <u>Invertebrates</u> | | |
| Water flea | 21-d growth: | 39 (71) |
| <i>Daphnia magna</i> | 21-d reproduction: | 24 (39) |
| Water flea | 7-d LC ₅₀ : | 120 |
| <i>Daphnia magna</i> | 14-d LC ₅₀ : | 120 |
| | 21-d LC ₅₀ : | 100 |
| Water flea | 21-d growth, static renewal | 116 (215) |
| <i>Daphnia magna</i> | | |
| Water flea | 21-d reproduction | >100 |
| <i>Daphnia magna</i> | | |
| Water flea | 2 generation survival & reproduction | 1 st gen: 50 (100) 2 nd gen: >25 |
| <i>Daphnia magna</i> | | |
| Cladoceran | 7-d reproduction: | 89 (202) |
| <i>Ceriodaphnia dubia</i> | 7-d mortality: | 202 (377) |
| Midge larvae | full life-cycle assay, survival, growth, emergence, fecundity, viability of offspring | ~100 (~200) |
| <i>Chironomus tentans</i> | | |

| Species | Test Conditions | NOEC (LOEC) (µg/L) |
|----------------------------------|--|--------------------|
| Mysid shrimp | 28-d growth: | 3.9 (6.7) |
| <i>Mysidopsis bahia</i> | 28-d reproduction: | 6.7 (9.1) |
| | 28-d mortality: | 6.7 (9.1) |
| Mussel | 35-d fertilization and early developmental success | >200 |
| <i>Mytilus edulis</i> | | |
| Mussel | byssus strength, renewal & flow-through | (56) |
| <i>Mytilus edulis</i> | | |
| Snail | mortality, growth, reproduction | 10 (100) |
| <i>Lymnaea stagnalis</i> | | |
| <u>Algae</u> | | |
| Duckweed | frond number, flow-through | 901 (2080) |
| <i>Lemna minor</i> | | |
| Green Algae | biomass, static | 694 (1480) |
| <i>Selenastrum capricornutum</i> | | |
| Green Algae | biomass (72-h EC ₁₀) | 3.3 µg/L |
| <i>Scenedesmus subspicatus</i> | growth rate (72-h EC ₁₀) | 25.1 µg/L |