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Californians for Alternatives for Toxics
315 P Street
Eureka, CA 95501

IPM-S-53
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June 13, 2008

Superintendent, Yosemite National Park
ATTN: Invasive Plant Management Plan
P.O. Box 577
Yosemite, CA 95389
Email: yose_planning@nps.gov

RE: Invasive Plant Management Plan for Yosemite NP.

Dear Yosemite NP

Californians for Alternatives to Toxics (CATs) is a public interest, non-profit organization concerned about the use of pesticides and alternatives to pesticides in California. Many CATs members live in the vicinity of or otherwise use and enjoy the Yosemite National Park. The activities that are planned for the Invasive Plant Management Plan for Yosemite NP are of particular concern to our members.

As written, this document is invalid as a vehicle of NEPA. It does not evaluate full potential of impacts, does not include a full range of alternatives, and is biased towards a preferred alternative that is both misleading and oversimplified.

Before addressing these concerns, however, would you please define for us the concepts that you are deriving your authority from. You state;

The Invasive Plant Management Plan tiers off the General Management Plan, and directly supports two goals of the plan:

- *Reclaim priceless natural beauty.*
- *Allow natural processes to prevail.*

You also state that you are fulfilling the following requirements of NEPA by deciding to use toxic chemicals;

- *Fulfill the responsibilities of each generation as trustee of the environment for succeeding generations*
- *Assure safe, healthful, productive, and aesthetically and culturally pleasing surroundings for all Americans*
- *Attain the widest range of beneficial use of the environment without degradation, risk to health or safety, or other undesirable and unintended consequences*

Though it is almost embarrassing to have to state this to you, it is obviously necessary. You do not reclaim priceless natural beauty and allow natural processes to prevail by applying dangerous toxic substances to them. Especially when one considers that there are natural alternatives available (See Range of Alternatives below). You are instead putting natural resources in an unnatural state that will produce both short and long term adverse effects that are either downplayed or ignored in the EA, in violation of NEPA.

Nor are you fulfilling your responsibility to future generations by continuing the shortsighted use of dangerous substances to replace natural methods of control.

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Nor are you assuring those whom you serve with a safe and healthful environment.

Range of Alternatives

You have failed to include two important alternatives, a non herbicide alternative that enlists the necessary support to effectively combat the problem of invasives in Yosemite NP, and a true no action alternative.

Effective Natural Alternative

You state that the use of pesticides will be incorporated "*if management objectives could not be achieved with the use of other control methods*". You also state that you would employ "*an extensive program staffed by park employees and volunteers*" and they "*would continue to use manual and mechanical techniques,*" but this would not meet management objectives.

You have not however given us an alternative that defines how manual and mechanical techniques might possibly work. How many personnel, how much money, etc. Without this alternative, you're excluding the alternative that actually upholds your general plan, preserving natural resources using natural methods and ensuring a safe and healthy environment.

Even if costs seem astronomical, this alternative needs to be explored. The bottom line is that management objectives could be met with a non herbicide objective if enough support and funding could be found. What that level is is unknown, because the Park Service would rather fund a quick fix using toxic chemicals in it's quest to provide "safe and healthful" surroundings.

True No Action Alternative

You do not have a true "No Action Alternative". Your no action alternative is really an action alternative that continues current strategies and methodologies. A true no action alternative leaves things as they are. Please include this as a baseline alternative.

NPS is Showing Clear Bias Towards the Preferred Alternative

The EA is highly defective when addressing impacts that could potentially be generated through the use of the dangerous chemicals proposed in this management plan. The following statements from the EA show a capricious attitude towards analysis of potential impacts from herbicide use.

"Under Alternatives 2 and 3, park staff would use low-toxicity herbicides."

"There would be a longterm moderate beneficial impact on soil microorganisms, soil chemistry, and hydrologic cycles, as invasive plant populations that could not be controlled without the use of herbicides are eradicated. The limited use of the herbicides would have a shortterm negligible adverse effect on soil quality. Overall, there would be long-term moderate beneficial impacts on soil microorganisms, soil chemistry, and hydrologic cycles as invasive plant populations are controlled and eradicated."

"The use of herbicides would..... Result.... in a short-term negligible adverse impact and a long-term moderate beneficial impact on wetlands in the park. Alternative 2 would meet integrated pest management goals because work crews would use the minimum amount of herbicide necessary to meet management goals."

“Alternative 2 would result in a long-term moderate beneficial impact on wildlife in the park. The use of terrestrial or aquatic formulations of glyphosate or aminopyralid (subject to labeling restrictions) without aerial application in terrestrial environments carries little to no risk to amphibians. Alternative 2 would result in a long-term moderate beneficial impact on wildlife in the park”

These statements are all inaccurate and misleading, put forth to present the preferred alternative as a safe vehicle for management goals. It is not. You are not applying low toxicity herbicides. NPE's (R11) alone are extremely toxic, both acutely and chronically, and are known endocrine disruptors that are considered by many scientists as the primary substance causing the feminization of fish throughout the world.

Your rosy portrayal of the use of highly toxic substances is an insult to knowledgeable Americans whose lands you manage. Waving off concerns with the flick of a wrist and painting such an unrealistic picture of potential impacts is a violation of NEPA and clearly an arbitrary and capricious action. The statement *“The use of herbicides would... result... in a short-term negligible adverse impact and a long-term moderate beneficial impact on wetlands in the park,”* is almost comical. As soon as the herbicide full formulation is applied, numerous species within the spray zone will be impacted. Acute toxic effects will be immediate. Endocrine disrupting effects would show themselves later in life, or to later generations. To claim that these impacts will be negligible is a lie.

Not Evaluating a Full Potential of Impacts

The NPS appears unaware of the dangers of using R11, whose primary component and its degradates are some of the most dangerous chemicals ever introduced into our environment. We do not have the time to bring you up to speed, but will cover as much as we can, before this capricious use of a highly dangerous substance is allowed to be applied throughout sensitive wetlands in our National Park.

The following is data supplied to the Forest Service concerning potential use of R11, the surfactant proposed for use in this management plan. Any references to the FS should be ignored. The data however is applicable to your proposal.

Herbicide Toxicity to Humans and Wildlife

Effects For R-11

Acute and Chronic Toxicity

Perhaps the greatest failing of the Yosemite EA (EA) can be found in its cursory and outdated analysis of potential adverse effects from the use of the surfactant R-11. In truth, it is hard to understand how this dangerous substance can be included in this project, considering the data that has been presented to other federal land managers, (and can be found in the administrative records for the Cottonwood Herbicide Project EA and QLG SEIS, all of which are to be incorporated herein by reference into the Yosemite EA administrative record). NPE and its metabolites have been shown to be both acutely toxic and endocrine toxic at environmentally relevant concentrations (low parts per billion).

Acute and chronic effects not related to endocrine disruption have been shown to occur at similar dose levels as endocrine disrupting effects (Environment Canada 2001, Lussier 1999, Hecht 2002, Zhang 2003). There is a large body of data that show LC50s to occur in the parts per billion range, and acute and chronic adverse toxic effects to occur in the low parts per billion range for NPE degradates. The following is from Environment Canada's CEPA toxic substances assessment for NPE and metabolites.

“There are a large number of studies reporting acute and chronic effects of NP in aquatic biota. There are, however, fewer studies reporting the toxicity of NPEs, and only a few studies that included the NPECs. Although studies described in the literature have used many species, different test methods and different chemicals, there is a consistent pattern in the toxicity reported. The range of acute toxicity for NP is similar for different organisms: for example, fish (17–1400 µg/L), invertebrates (20–3000 µg/L) and algae (27–2500 µg/L). Chronic toxicity values (No-Observed-Effect Concentrations, or NOECs) for NP are as low as 6 µg/L in fish and 3.9 µg/L in invertebrates. An acute to chronic toxicity ratio of 4:1 was determined based on the available literature.” (Environment Canada 2001)

The analysis incorporated into the risk assessment for acute and chronic non-endocrine toxicity is unrealistic, do not reflect current scientific understanding of expected effects and misrepresent potential risk from R-11.

Another issue that needs to be considered when attempting to analyze risk from any substance through the methodology used in the EA is the potential for differences in effects generated in surrogate species as opposed to listed species. Listed species have shown greater sensitivity than surrogate species in a number of studies. The following from USGS 2001 highlights this concern;

“Standard environmental assessment procedures are designed to protect terrestrial and aquatic species. However, it is not known if endangered species are adequately protected by these procedures. At present, toxicological data obtained from studies with surrogate test fishes are assumed to be applicable to endangered fish species, but this assumption has not been validated. Static acute toxicity tests were used to compare the sensitivity of rainbow trout, fathead minnows, and sheepshead minnows to several federally listed fishes (Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado pikeminnow, razorback sucker, Leon Springs pupfish, and desertpupfish). Chemicals tested included carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. Results indicated that the surrogates and listed species were of similar sensitivity. In two cases, a listed species had a 96-h LC50 (lethal concentration to 50% of the population) that was less than one half of its corresponding surrogate. In all other cases, differences between listed and surrogate species were less than twofold. A safety factor of two would provide a conservative estimate for listed cold-water, warm-water, and euryhaline fish species.” (USGS 2001)

Cancer and Mutations

Though not listed as carcinogens, NPE and metabolites have shown the potential to cause mutations and deformities (Atienzar 2002, Zhang 2003, Zumbado 2002) and are suspected of producing cancer effects (Yu 2003), possibly through endocrine mediated pathways. Garry et al 1999 found X-77, an NPE based surfactant, to be genotoxic.

Recent studies have also found similar results. Seiki et al found that, *“The total incidences of adenomas and carcinomas in the lungs of animals treated with nonylphenol and genistein were significantly higher than in the control group. 5-Bromo-2'-deoxyuridine labeling indices, reflecting cell proliferation, were also significantly elevated in the lungs of rats given 250 and 25 ppm nonylphenol.....These results indicate that nonylphenol and genistein have the potential to promote rat lung carcinogenesis, possibly via a mechanism involving stimulation of cell proliferation and DNA damage caused by oxygen radicals.” (Seiki 2003).*

Another recent study found that, *“DNA effects include DNA damage as well as mutations and possibly other effects at the DNA level that can be induced by chemical or physical agents that directly*

and/or indirectly interact with genomic DNA. Not only did exposure to NP and E2 induce changes in RAPD profiles in the exposed barnacle larvae when compared to control patterns, but also, and more importantly, there were similarities in the RAPD modifications in the exposed populations that had been treated to either chemical. We propose that NP and E2 induced some common DNA effects in barnacle larvae and that these specific modifications in RAPD patterns may arise as a consequence of hot spot DNA damage (e.g. DNA adducts) and/or mutations (point mutations or genomic rearrangements)" (Atienzar 2002).

Zhang et al reported, "The 96-h EC(50)'s for embryo lethality (arrested egg development) and deformities (curved or unextended shell spines and undeveloped second antennae) were 738 and 263 microg/L, respectively. Reproduction studies were conducted using conditions that stimulate male production (i.e., reduced photoperiod and food levels). An increase in neonate deformities was observed at 50 microg/L (without ethanol), but no changes were observed in fecundity or sex ratios. A decrease in sex ratios was observed at 25 and 50 microg/L (with ethanol) compared to the ethanol control. However, an increase in sex ratios was observed in the ethanol control compared to media controls. The use of ethanol as a solvent carrier confounds the effects of 4-NP on acute toxicity and male production" (Zhang 2003).

Zumbado 2002 found that, "These findings taken together suggest that the exposition to alkylphenols induce cell proliferation and spindle disturbances and that these compounds are capable of modulating the expression of putative membrane receptors for estrogens." (Zumbado 2002)

Yu 2003 concludes that, "the test compounds (n-4-nonylphenol, Bisphenol A and dibutylphthalate), like estradiol, markedly enhanced the proliferation of T47D cell and the metaphase of cell division, and the results showed time-dependent and dose-dependent model. These data showed that the tested chemicals could enhance the proliferation of human cervix cancer cell in vitro. This might hinted that these chemicals possessed estrogenic activity and they might play their estrogen through estrogenic receptor." (Yu 2003)

Neurological Effects

Though the body of data for neurological effects induced by NPE and metabolites is limited, there have been some findings that are very important and need to be included in the current risk assessment. The first of these studies involve surrogate species commonly used for analyzing effects to amphibians listed as sensitive by the FS.

"The developing nervous system is exquisitely sensitive to the effects of gonadal steroids, suggesting that inappropriate exposure to chemicals that mimic steroids may also have significant deleterious effects on the formation of neuronal structures. In this study researchers exposed *Xenopus* embryos to low doses of nonylphenol for approximately 48 hrs. The embryos exhibited significant deficits in overall morphology, increased numbers of apoptotic cells, and dramatic changes in the migration and morphological differentiation of pigment cells derived from the neural crest. NP was found to block nerve growth factor induced differentiation of spinal cord neurons isolated from early neural plate embryos and maintained in culture. Taken together, these data indicate that early exposure to EDCs can induce significant and deleterious effects in the development and differentiation of the nervous system" (Bevan et al 2001).

In another study, researchers investigated the effects of nonylphenol on synaptogenesis in primary cultures of fetal rat hypothalamic cells. They found that nonylphenol has different effects on dendritic

outgrowth and synaptogenesis. "MAP 2-positive area was increased by the 100 nM nonylphenol treatment, although other concentrations of nonylphenol did not alter the MAP 2-positive area. Nonylphenol also influenced synapsin I-positive area, but in a different manner. A significant increase in synapsin I-positive area was markedly reduced by 100 nM and 1 uM nonylphenol treatments. These results indicate that nonylphenol has different effects on dendritic outgrowth and synaptogenesis. According to the change in synapsin I-positive area, the synaptic density (synapsin I-positive area/MAP 2-positive area) was significantly increased by 10 nM nonylphenol treatment and decreased by 100 nM and 1 uM nonylphenol treatments. A significant decrease in the synaptic density was also observed after treatments with 1, 10 and 100 uM BPA. Thus, these results indicate that nonylphenol and BPA influence synaptogenesis in primary cultures of fetal hypothalamic cells." (Ohtani-Kaneko 2002).

A recent review on data from the National Center for Toxicological Research by Ferguson et al found that "Recent reviews have focused attention on the need for assessing the neurotoxicity of these compounds following developmental exposure.....Volume of the sexually dimorphic nucleus of the medial preoptic area was reduced by genistein, nonylphenol, and ethinyl estradiol exposure in males. The regulatory impact of these data and the directions for future research are discussed." (Ferguson 2000)

Sato 2002 found that nonylphenol, E2 and other xenoestrogens produced neurotoxic effects at extreme low doses but not through an estrogen receptor mechanism. "Xenoestrogens are man-made compounds that mimic the actions of estrogens through interactions with estrogen receptors (ERs). Although xenoestrogens have received a great deal of attention as possible causes of brain dysfunctions, little information concerning the effects of xenoestrogens on the central nervous system is available. In this study, we investigated the effects of 17beta-estradiol (E(2)) and four xenoestrogens (17alpha-ethynylestradiol, diethylstilbestrol, p-nonylphenol and bisphenol A (BPA)) on the neuronal survival using organotypic hippocampal slice cultures. When the cultured hippocampal slices were exposed to glutamate (1 mM, 15 min), the CA1-selective neuronal damage was induced. Pretreatment with E(2) and the xenoestrogens (24 h) selectively exacerbated the CA3 neuronal damage caused by glutamate. In spite of the marked difference of binding affinities to ERs, all compounds revealed maximal effects at 1 nM. ER antagonists, tamoxifen and ICI 182,780, did not affect responses to E(2) and the xenoestrogens, indicating that these effects are mediated through mechanisms other than ERs. In spite of the fact that BPA has little interaction with ERs at 1 nM, E(2) and BPA equally increased the expression of N-methyl-D-aspartate receptor in CA3 and upregulated the spine density of the apical portion of CA3 dendrites at 1 nM. These compounds also enhanced the sprouting of mossy fibers to CA3 neurons. These results suggest that exposure to E(2) and xenoestrogens during the developmental stage results in a marked influence on synaptogenesis and neuronal vulnerability through mechanisms other than ERs." (Sato 2002)

In Conclusion

As with the analysis of risk from endocrine mediated effects by R-11, non-endocrine effects, both acute and chronic, are seriously underestimated. Once again, the only real remedy is to start from scratch and use all data that exists, instead of a handful of poorly understood documents.

One important consideration for both endocrine and non endocrine effects from NPE concern using a compilation of NPE and its primary metabolites as a single source for toxic effects. This means estimating at different stages of the metabolic process what combination of substances will be present and analyzing their potential risk in an additive fashion. The following is from Environment Canada 2001. "Since NP and NPEs exist together in mixtures in environmental samples, the combined impact of the mixture was examined" (p 46).

Bibliography

Atienzar et al 2002, 4-n-Nonylphenol and 17-beta estradiol may induce common DNA effects in developing barnacle larvae. *Environ Pollut.* 2002;120(3):735-8.

Bevan et al 2001, Defects in embryogenesis and growth factor responsiveness induced by exposure to endocrine disrupting compounds. *Abstr Soc Neurosci* 2001;27(Pt 1):658

Environment Canada, 2001; Canadian Environmental Protection Act, Priority Substances List Assessment Report, Nonylphenol and its Ethoxylates. Available online at

Ferguson, SA; Scallet, AC; Flynn, KM; Meredith, JM; Schwetz, BA, 2000, Developmental Neurotoxicity of Endocrine Disruptors: Focus on Estrogens, *Neurotoxicology*, vol. 21, no. 6, pp. 947-956, Dec 2000, ISSN 0161-813X

Lussier et al, 1999; Acute Toxicity OF Para-nonylphenol TO Saltwater Animals. *Environmental Toxicology and Chemistry*: Vol. 19, No. 3, pp. 617-621.

Ohtani-Kaneko 2002, Endocrine disruptors influence synaptogenesis in primary cultures of fetal hypothalamic cells. *Environmental Sciences: an International Journal of Environmental Physiology and Toxicology* 2002;9(2-3):204-5

Sato K, Matsuki N, Ohno Y, Nakazawa K. 2002; Effects of 17beta-estradiol and xenoestrogens on the neuronal survival in an organotypic hippocampal culture. *Neuroendocrinology*. 2002 Oct;76(4):223-34.

Seike et al 2003, Enhancement of lung carcinogenesis by nonylphenol and genistein in a F344 rat multiorgan carcinogenesis model. *Cancer Lett.* 2003 Mar 20;192(1):25-36.

USGS 2001; Sappington et al. Contaminant sensitivity of threatened and endangered fishes compared to standard surrogate species. *Environ Toxicol Chem.* 2001 Dec;20(12):2869-76.

Yu Z, et al. 2003; Estrogenic activity of some environmental chemicals; *Sheng Yan Jiu.* 2003 Jan;32(1):10-2.

Zhang L et al. 2003; The effects of 4-nonylphenol and ethanol on acute toxicity, embryo development, and reproduction in *Daphnia magna*. *Ecotoxicol Environ Saf.* 2003 Jul;55(3):330-7.

Zumbado M, et al. 2002; Evaluation of acute hepatotoxic effects exerted by environmental estrogens nonylphenol and 4-octylphenol in immature male rats. *Toxicology.* 2002 Jun 14;175(1-3):49-62.

Effects and Environmental Fate of NPE and Degradates

A proper analysis of the potential effects from the use of NPE is not a part of the EA. Although

scientific understanding of endocrine disrupting chemicals (EDCs) is the fastest growing knowledge base in the field of toxicology, it is also one that is producing as many questions as answers. As an example one need only look at the growing list of endpoints and mechanisms of action related to EDCs in general and NPE and metabolites in particular.

Much of the data for endocrine disruption will be from the World Health Organization's and the US National Institute of Environmental Health Science's "Global Assessment of the State-of-the-Science of Endocrine Disruptors" (WHO, 2002). This report errs on the side of caution, but for data published prior to 2002, it is recognized as the most thorough government-sponsored review to date of endocrine disruption.

One of the problems experienced by land managers, when attempting to include EDs into the risk assessment process, is the use of limited data from a handful of sources and a cursory examination of other documents. This is especially true for the supporting documents used in the EA by the Syracuse Environmental Research Associates (SERA), none of which have been peer reviewed. This is not to say that the data provided is inaccurate, only that the data could have been selectively incorporated in a way that would produce a certain result. Because there is sometimes conflicting or unexpected results from one research project to the next when addressing endocrine effects, due to the wide range of variables involved, it is imperative that the entire body of data be incorporated into an analysis of potential effects. This concern has been stated quite succinctly in the WHO 2002.

"Concerns regarding EDCs have generated a vast number of divergent research studies conducted under various conditions and examining various outcomes. It is extremely rare that a single study could provide all the necessary relevant information to link a particular exposure scenario to a particular health outcome in wildlife or humans. Therefore, it is essential to evaluate the entire body of relevant knowledge" (WHO, 2002).

Endocrine Disruption

Overview

The endocrine system consists of a set of glands, the thyroid, parathyroids, testes, ovaries, adrenal, hypothalamus, pancreas, pineal, and pituitary glands, as well as other chemical regulators, and the hormones they produce, such as thyroxine, oestrogen, testosterone and adrenaline, which help guide the development, growth, reproduction, and behavior of animals, including human beings.

The currently recognized definition of endocrine disrupting chemicals is;

"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations. A potential endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations" (WHO, 2002).

EDCs have been associated with developmental, reproductive and other health problems in wildlife and laboratory animals. Some experts suggest these compounds may affect humans in similar ways.

"Analysis of the human data by itself, while generating concerns, has so far failed to provide firm evidence of direct causal associations between low-level (i.e., levels measured in the general population) exposure to chemicals with EDCs and adverse health outcomes. It is difficult to compare and integrate results from diverse human studies, because data are often collected at different time periods, using different experimental designs and under different exposure conditions. Often exposure data are completely lacking. Of particular concern is the lack of exposure data during critical periods of development that influence later functioning in adult life. Furthermore, the concentrations and potencies

of endogenous hormones and phytoestrogens are generally higher than those of exogenous chemicals. Despite these difficulties, exposure to EDCs has been suggested to play a role in adverse health outcomes, and concerns remain” (WHO 2002).

“Overall, the biological plausibility of possible damage to certain human functions (particularly reproductive and developing systems) from exposure to EDCs seems strong when viewed against the background of known influences of endogenous and exogenous hormones on many of these processes. Furthermore, the evidence of adverse outcomes in wildlife and laboratory animals exposed to EDCs substantiates human concerns. The changes in human health trends in some areas (for some outcomes) are also sufficient to warrant concern and make this area a high research priority, but non-EDC mechanisms also need to be explored” (WHO 2002).

Mechanisms of Action

Endocrine disruptors interfere with the functioning of the endocrine system, in at least three possible ways:

1) by mimicking the action of a naturally-produced hormone, such as oestrogen or testosterone, and thereby setting off similar chemical reactions in the body;

2) by blocking the receptors in cells receiving the hormones (hormone receptors), thereby preventing the action of normal hormones; or

3) by affecting the synthesis, transport, metabolism and excretion of hormones, thus altering the concentrations of natural hormones.

“Research has clearly shown that EDCs can act at multiple sites via multiple mechanisms of action. Receptor-mediated mechanisms have received the most attention, but other mechanisms (e.g., hormone synthesis, transport, and metabolism) have been shown to be equally important. For most associations reported between exposure to EDCs and a variety of biologic outcomes, the mechanism(s) of action are poorly understood. This makes it difficult to distinguish between direct and indirect effects and primary versus secondary effects of exposure to EDCs. It also indicates that considerable caution is necessary in extrapolating from in vitro data to in vivo effects, in predicting effects from limited in vivo data, and in extrapolating from experimental data to the human situation. A collective weight of evidence is essential in determining under what conditions observed effects resulting from exposure to EDCs occur via endocrine mediated mechanisms.”

“Despite an overall lack of knowledge of mechanisms of action of EDCs, there are several examples where the mechanism of action is clearly related to direct perturbations of endocrine function and ultimately to adverse in vivo effects. These examples also illustrate the following important issues:

a) Exposure to EDCs during the period when “programming” of the endocrine system is in progress may result in a permanent change of function or sensitivity to stimulatory/inhibitory signals.

b) Exposure in adulthood may be compensated for by normal homeostatic mechanisms and may therefore not result in any significant or detectable effects.

c) Exposure to the same level of an endocrine signal during different life history stages or during different seasons may produce different effects.

d) Because of cross talk between different components of the endocrine systems, effects may occur unpredictably in endocrine target tissues other than the system predicted to be affected” (WHO 2002, Executive Summary). (A more thorough discussion of mechanisms of action can be found in Chaps 2 and 3 of WHO 2002).

Due to these, and other confounding factors, results from every research project and data review needs to be thoroughly analyzed, through peer review and/or independent analysis, unlike APERC and

SERA documents, and USDA (Bakke) 2003. There has never been a time when the need for critical, objective analysis has been more important than with the issues surrounding endocrine effects.

Importance of Objective Analysis of Current Research

It is very important for risk assessors to thoroughly research issues and data that affect their decisions. This is especially true in the field of EDC research. When viewing conflicting data, it is important to analyze the source of the data and ask oneself if the potential for a conflict of interest is present. If so, it is important to understand the ramifications of a research project and/or public relations campaign funded by the manufacturer of the chemical in question.

An example can be found in the controversy that surrounded data showing low dose effects. When Fred vom Saal published two important bisphenol-A studies from his work group (Nagel 1997, Howdeshell 1999) that showed endocrine effects at extreme low doses (2ppb), industries response (including members of APERC) to these studies was both swift and damaging. Two industry supported studies attempting to duplicate these findings were quickly introduced. When these two studies were unable to duplicate vom Saal's findings a media barrage ensued claiming that findings of low dose effects by vom Saal were unreplicable and unreliable.

During the height of the controversy surrounding vom Saal's findings, data from the NTP "Low Dose Peer Review" was misrepresented by industry support groups with the intent of discrediting vom Saal's research team. The National Institute of Environmental Health Sciences was also concerned about industries inaccurate portrayal of the data and stated;

"Although Vom Saal's results were shown to be credible under the panel's statistical reanalysis, they were found not to be reproduced in other, equally credible studies. Interstudy differences in animal strain, diet, dosing regimens, and even housing conditions were all offered as possible explanations for the discrepancy.

Based on the inconsistency of the data, the panelists were not persuaded that a low-dose effect of bisphenol A has been conclusively established as a general or reproducible finding, an admission seized on by the plastics industry, which insists low-dose exposure to the chemical is safe.

"I believe Dr. Vom Saal is convinced of his findings, but he has not convinced his scientific peers," says Paul Foster, program director of endocrine, reproductive, and developmental toxicology at the CIIT Centers for Health Research in Research Triangle Park, North Carolina, a research organization sponsored by industry. "The inability to reproduce the findings of an increase in prostate weight [or any pathological responses associated with this weight change] in mice [of different strains] and rats indicates that this change is not robust, nor a universal phenomenon likely to have implications for human health risk assessment."

But Lucier cautions that the panel's statement on bisphenol A shouldn't be taken out of context. Vom Saal's data are of high quality, he says, and the evidence for a low-dose effect can't be discounted. Taken as a whole, the data for bisphenol A and other chemicals reviewed by the panel indicate nonmonotonic, linear, and even threshold responses are all possible outcomes of low-dose endocrine disruptor exposure. The fact that biologic effects were noted in the low-dose region below the NOEL for some data sets, he says, suggests that the EPA should review its current testing protocols to see if changes are required" (NIEHS 2001).

Recent research has now shown vom Saal's findings to be accurate. And a review of the industry studies revealed that it was their own incompetence which led to their failures. Welshons et al. published an analysis that showed that the two main industry attempts to replicate vom Saal's work failed because their control animals were inadvertently estrogenized by a contaminant, and thus unable to respond normally to endocrine disruptors (Welshons 2003).

In the last few years, independent laboratories have found results similar to vom Saal's, and these low dose effects are common with many different EDs, including NPE degradates (Yokota 2001, Matozo 2003, Tanaka 2001, Tanaka 2002, Zhang 2001, Zhang 2003, Ackerman 2002, Nice 2003, USEPA (Hemmer) 2002, Uguz 2003, Weber 2003, Hill 2003, Hahn 2002, Chitra 2002, Schwaiger 2002, Kwack 2002, Hecht 2002, Servos 1999, Pickford 2003, Seki 2003, Huang 2001, Fent, 2000, Meregalli, 2001).

Today, it is routine procedure to use ppb dosing levels in endocrine research. In fact, recent research has shown that, for both octylphenol and nonylphenol, exposure levels in the parts per trillion (ppt) range are, (or could potentially be) producing adverse endocrine effects (Nice 2003, Christian and Gillies 1999). As Sheehan theorizes (see below), for some situations there may be no NOAEL or amount small enough to avoid producing effects.

New Data is Constantly Redefining Risk Assessment Parameters for EDCs

Originally the scope of inquiry related to endocrine disruption only considered effects produced through an estrogen receptor mechanism. As stated above, this has now been expanded to include the blocking, synthesis, transport, metabolism and excretion of all hormones generated by all organs in the endocrine system.

As research continues to mount about the range of chemical-signaling systems vulnerable to disruption, it is apparent that endocrine disruption is most likely but one example of a broader class of contamination effects, termed "signal disruption" (Fox et al, 2001; McLachlan JA, 2001). All biotic systems use some form of signalling in their reproduction, growth, or other life functions. Natural chemical signals are important at all levels of organization of life; within cells, among cells, between organs, even between organisms, including from one species to another. Any of these chemical signals, in principle, are vulnerable to disruption. Scientists, for example, have just begun to look at the chemical signals that mediate communication between symbiotic organisms, such as nitrogen-fixing bacteria and the roots of the plants in which they live, and are examining how synthetic chemicals might interfere with these signals (See section "EDCs and Potential Effects to Flora" below). It is through this system of communication that cells talk to each other and produce the results needed to keep a living organism functioning properly. Disrupting these 'signals of life' could have important and far reaching ecosystem impacts. What if all the lights turn green. What if the train is told to continue on the track it's on even though another train is coming from the other direction.

This is the danger that organisms face through signal disruption. The end result is potentially disastrous, biota thrown into chaos. It is with this understanding that risk assessors must view potential effects from EDCs. It is not something trivial that can be cast aside with the wave of a few supporting documents.

There are numerous failings in the EA that are the result of a poor understanding of the issues involved with EDCs. The primary failing is found in the adherence to standard methods of assessing risk, i.e. that there is a linear dose/response relationship ("the dose makes the poison") and that this linear relationship can be assessed, using the standard mg/kg/per day exposure ratio, to establish a threshold below which there is no risk of adverse effects being generated. This paradigm exists because it has proven to be fairly accurate in other forms of toxicological risk assessment. For signal disruption and EDCs it is not always true for the following reasons.

- a) EDCs are entering a system that already has the signalling chemical (that they mimic), present at levels that produce effects.
- b) EDCs have shown that they can produce effects in a non-linear fashion, (ie in a non-monotonic curve).
- c) Timing of exposure can be a more important factor than rate and duration.

Signalling Chemicals Already Present

When risk assessors look at potential effects from different dose levels of a toxic substance, they are assuming that the system these chemicals might impact is not carrying a body burden of this substance. If this substance (or other substances that share a common mechanism) is already present in the system, then that is taken into account in an additive fashion. With EDs however, the equation is completely different. Hormone active substances (that is, hormones themselves) are already present in quantities sufficient to cause effect.

In WHO 2002 it was defined as *"The issue of dose-response relationships is perhaps the most controversial issue regarding EDCs. One of the reasons is that EDCs often act by mimicking or antagonizing the actions of naturally occurring hormones. These hormones (often more potent than exogenous EDCs) are present at physiologically functional concentrations, so the dose-response considerations for EDCs are often different than for other environmental chemicals, which are not acting directly on the endocrine system"* (WHO 2002).

These principles were first described in Sheehan 1999.

"Risk assessments for nongenotoxic chemicals assume a threshold below which no adverse outcomes are seen. However, when an endogenous chemical, such as 17 β -estradiol (E2), occurs at a concentration sufficient to cause an effect, the threshold is already exceeded. Under these circumstances, exogenous estradiol is not expected to provide a threshold dose".

"There was no apparent threshold dose for E2. A smaller replication confirmed these results. These results provide a simple biologically based dose-response model and suggest that chemicals which act mechanistically like E2 may also show no threshold dose. If so, even low environmental concentrations of such chemicals may carry risk for sex reversal" (Sheehan et al 1999).

Sheehan *et al.* worked experimentally with sex control in the red-eared slider, a turtle in which sex determination is normally controlled by temperature (via a mechanism in which the hormonal processes involved in sex determination are temperature dependent). They exposed a series of turtle eggs at 28.6°C to a range of doses of 17 β -estradiol. The temperature they chose normally would have resulted in mostly males but some females. They then determined the sex of each egg at hatching. They analyzed the results using a theoretical construct based on the Michaelis-Menten equation, which has been developed in basic chemistry to model enzyme kinetic studies. The data from the large experiment fit the M-M model exceptionally well. The combination of both experimentation and theoretical analysis is very powerful. Their analyses showed that any addition of exogenous estrogen caused a change in the sex ratio of pool of eggs and "that no exogenous estrogen is without risk." This is because in their experimental system, endogenous estrogen is already at a high enough level to exceed the threshold for causing an effect. Endogenous estrogen is already activating the system. A contaminant doesn't have to exceed the threshold because endogenous estrogen already does.

This is an important concept to understand as a risk assessor. Organisms contain substances that put them already past the point of producing effects. The difference from EDCs is that the natural hormones are sending the right messages, in the right order, and of the right magnitude to get the message across and properly into effect. Then they disappear so new messages can be brought forth. An EDC acts on a part of that message stream. It changes the message in a way that makes no sense. Whether or not that message will produce or add to an adverse reaction is dependent on many factors. The fact remains however, that these marauding hormone mimics are causing adverse effects at extreme low doses, often times at all levels tested, with no NOAEL being defined.

Endocrine Effects and a Nonmonotonic Dose Response Curve

Another failing of the risk assessment in the EA and supporting documents is the assumption that a dose

response curve is always linear. This old assumption may be true for many chemicals and for many classic health effects, but it is often times not true for endocrine disrupting chemicals. EDCs have shown the ability to produce effects within a non_monotonic dose response curve.

"A key outcome of the (NTP Low Dose Peer Review) was verification that some endocrine disruptors exhibit dose-response relationships described as nonmonotonic, meaning that within a certain dose range, a chemical's effects on a given end point actually become greater as the dose is reduced. The dose-response curves can be shaped like a U, with a high response at both low and high levels of exposure, or like an inverted U, with the greatest response at intermediate dose levels. According to Frederick vom Saal, a professor in the Division of Biological Sciences at the University of Missouri in Columbia, nonmonotonic curves challenge the EPA's standard assumption of linear or threshold dose responses, which holds that toxic effects always lessen as the dose is reduced toward zero" (NIEHS 2001).

The following is from the Commission on Life Sciences, 2000; "Hormonally Active Agents in the Environment";

"Knowing the shape of the dose-response curve for environmental contaminants is critical for understanding how such contaminants...act on organs and organisms. Understanding the dose-response relationship is also critical for the design of studies to test the effects of contaminants.

If an underlying monotonic dose-response function (i.e., a function where response increases as dose increases or at least does not decrease) and a dose below which there is no effect (a threshold dose) are assumed when designing a toxicologic study, there is a risk of failing to understand or properly test a contaminant that does not display a monotonic dose-response function or a threshold dose.

It is well known that some compounds produce nonlinear and even nonmonotonic dose-response functions in some organisms over certain ranges of dose. Furthermore, some compounds can produce different dose-response functions depending on the target organ and the species exposed" (CLS 2000 p82).

"There are numerous examples of nonmonotonic inverted U-shaped dose-response curves from in vitro studies. These studies involve a variety of natural and anthropogenic estrogens (e.g., estradiol, estriol, nonylphenol, and DES), end points (e.g., cell proliferation, prolactin synthesis, and induction of specific mRNAs), and cell lines (e.g., Jordan et al. 1985; Soto et al. 1991; Bigazzi et al. 1992; Pilat et al. 1993; Truss and Beato 1993; Tzukerman et al. 1994; Olea et al. 1996). Sonnenschein et al. (1989) also observed a nonmonotonic response curve for androgen-induced cell proliferation in LNCAP cells by using a diverse group of steroidal and nonsteroidal compounds". (CLS 2000 p110)

The reasons for the nonmonotonic response curve findings are poorly understood at present. One recognized theory is expressed by Fred vom Saal, the first to document this response in association with EDCs;

"Any endocrinologist will tell you that hormone receptors are up-regulated [stimulated] at low doses and down-regulated at high doses," he says. "In fact, in clinical therapy you can shut down a hormonal system simply by treating with high levels of hormone" (NIEHS 2001).

For risk assessment purposes, it is one more parameter that must be considered. It has been suggested by risk assessors that an inverted U dose response curve actually has two NOAELs and that exposure levels above the curve would have the same effect as those below the threshold. This logic fails on three levels. First, effects are still being generated, they are merely expressing themselves in a different fashion. Second, you reach exposure levels that could produce toxicity through non endocrine disruption

pathways. Third, there is no way to gauge exposure levels in the wild.

Endocrine Effects and Timing of Exposure

Another failing of the EA is that it has not taken into consideration the accepted fact that timing of exposure is often critical to assessment of risk from EDCs. Since the Larson project proposes to apply herbicides and R-11 during the breeding season for most species at risk, it is imperative that this be incorporated into the EA risk assessment.

The importance of understanding timing as a risk assessment parameter is that it once again dispels the risk assessment methodology incorporated in the EA. The concept of acceptable dose levels (those below the threshold NOAEL x 100) are only appropriate if a) the most sensitive time for exposure is the tested exposure period and b) these studies are long term chronic or multi-generational studies to identify "later in life" or trans-generational effects. Since nonylphenol has shown itself to produce effects in the ppb range during the developmental stage of most organisms that have been tested, this would place the risk quotient multiplier in the ppt and low ppb range, which in turn would place all species at serious risk from exposure.

The following are quotes from a wide range of studies that describe the importance of acknowledging timing of exposure as a risk factor for acute, chronic and multi-generational effects.

"Exposure to EDCs during the period when "programming" of the endocrine system is in progress may result in a permanent change of function or sensitivity to stimulatory/inhibitory signals" (WHO 2002).

"(T)he effects 1) may be manifested in an entirely different way, and with permanent consequences, in the early embryo, fetus, and neonate from effects as a result of exposure only in adulthood; 2) can change the course of development and potential offspring, with the outcome depending on the specific developmental period(s) of exposure; and 3) are often delayed and thus may not be fully or obviously expressed until the offspring reaches maturity or even middle age, even though critical exposure occurred during early embryonic, fetal or neonatal life" (Colborn et al 1993).

"Another study presented it as, "There are an infinite number of windows of time during embryonic and the early postnatal period when disruption can take place, each leading to potentially different changes in an individual's course of development and behavior. Response to exposure is unpredictable because the process of development is so delicate and complex," (Colborn et al, 1995).

"Concerning the surfactants used by the FS, Lee et al found that there is a critical period of vulnerability to NP during male reproductive development in the neonatal stage. Changes were found when NPs were given to male pups before 13 d of age, but not when given at > or =13 d of age. NP acts on the male reproductive tissues through the estrogen receptor" (Lee 1998).

"It is apparent from research that the main effects from endocrine disruption usually occurs when exposure happens to species developing in the womb or when newly born. However, effects can also occur from exposure later in life. Permanent changes have occurred to animals exposed in adulthood and the potential exists for chronic low level exposure to also affect adult humans" (vom Saal 1993).

"Normal endocrine function is often dependent on cyclical events, rather than steady-state. Timing is everything, as evidenced by significant differences in adverse outcome as a function of age and stage of development" (USEPA).

"Experts suggest that endocrine disruptors pose the greatest risk during fetal development, which is regulated by hormones at specific levels. Hormonal alterations due to maternal exposure in pregnancy could lead to effects such as reduced cognitive function or cancer that might not be evident for months, even years" (NIEHS 2001).

"In trout exposed to nonylphenol (1 µg/L, 10 µg/L) and to estradiol, the structure of the epidermis was altered: an irregular overall architecture was often accompanied by detached pavement cells, vacuolation of the cytoplasm, and severely deformed cell nuclei. However, the granulation pattern of the mucous cells was influenced exclusively after exposition to nonylphenol. The number of large and irregularly shaped mucosomes depended more on the exposure period than on the concentration of nonylphenol. Furthermore, this alteration has not yet been reported for any other pollutant or stressor and, thus, can be classified as an effect that would strongly indicate exposure to nonylphenol" (Burkhardt-Holm, 2000).

Of special note in recent studies exploring the role of timing are the findings of Nice et al. 2003. The authors provide evidence clearly demonstrating that when larvae are exposed to environmentally relevant concentrations of nonylphenol for a single 48 hour exposure at a key stage in their development, long-term sexual developmental effects are induced. Data provided by this study suggest that exposure to 1 ppb and 100 ppb nonylphenol at days 7 to 8 post-fertilization results in a change in the sex ratio towards females and an increase in the incidence of hermaphroditism (10 mo later, up to 30% of the resulting adults were fully functional hermaphrodites). Gamete viability is also affected, resulting in poor embryonic and larval development (up to 100% mortality) of the subsequent generation (Nice et al 2003). This study is important because it is one of the first to identify serious adverse effects from a single "pulse" exposure of extreme low doses with no NOAEL identified.

The EA puts much weight in the concept that quick degradation of NPE will limit effects, itself a flawed assumption (as discussed in the section "Metabolites and Persistence" below). However, when one low dose of a substance can produce serious long term effects to both individuals and to populations, it matters not how long something persists.

Conclusion

In essence, the "dose-response and threshold" assumptions are the core of any risk assessment. Its use in regulatory science has been a pragmatic step, not something based on theory or on fact. This assumption is a key part of the way that safety standards are set. All risk assessment must first start by identifying a threshold for effects. Then the NOAEL is divided, often by 100. The assumption is that an exposure level calculated in this fashion is safe, and it is used to determine acceptable per day exposure levels.

These fundamental assumptions used to guide current risk assessment are no longer applicable when assessing EDCs. Since the issues surrounding dose response to environmental EDCs are pivotal to exposure risk assessment and consequently to regulatory considerations, numerous research projects are attempting to come to grips with this need for a new risk assessment model. A review of the state of the science of these concerns was recently published in Environmental Health Perspectives. Welshons et al. review the issues associated with the underestimation of true bioactivity when only high doses are used in toxicologic studies. The major points considered include low-dose biological activity not observed by traditional testing, nonlinear dose extrapolation, complex receptor responses, and the effects of exogenous exposure on an already active biological pathway. This was their conclusion;

"Information concerning the fundamental mechanisms of action of both natural and environmental hormones, combined with information concerning endogenous hormone concentrations, reveals how endocrine-disrupting chemicals with estrogenic activity (EEDCs) can be active at concentrations far below those currently being tested in toxicological studies. Using only very high doses in toxicological studies of EEDCs thus can dramatically underestimate bioactivity. Specifically: a) The hormonal action mechanisms and the physiology of delivery of EEDCs predict with accuracy the low-dose ranges of biological activity, which have been missed by traditional toxicological testing. b)

Toxicology assumes that it is valid to extrapolate linearly from high doses over a very wide dose range to predict responses at doses within the physiological range of receptor occupancy for an EEDC; however, because receptor-mediated responses saturate, this assumption is invalid. c) Furthermore, receptor-mediated responses can first increase and then decrease as dose increases, contradicting the assumption that dose-response relationships are monotonic. d) Exogenous estrogens modulate a system that is physiologically active and thus is already above threshold, contradicting the traditional toxicological assumption of thresholds for endocrine responses to EEDCs. These four fundamental issues are problematic for risk assessment methods used by regulatory agencies, because they challenge the traditional use of extrapolation from high-dose testing to predict responses at the much lower environmentally relevant doses. These doses are within the range of current exposures to numerous chemicals in wildlife and humans. These problems are exacerbated by the fact that the type of positive and negative controls appropriate to the study of endocrine responses are not part of traditional toxicological testing and are frequently omitted, or when present, have been misinterpreted" (Welshons et al, 2003).

Further confounding any attempts to use standard risk assessment methodologies for EDCs is the fact that there are so many variables involved.

"A common dose-response relationship for all effects and for all endocrine disruption mechanisms should not be expected. This conclusion is based on the knowledge that there are many different kinds of hormonal actions of chemicals categorized as endocrine disruptors. These activities include estrogenic, antiestrogenic, antiandrogenic, growth factor modulation, cytokine and thyroid modulation, modulation of hormone metabolism, among many others" (WHO 2002).

The EA uses standard dose response methodology, to arrive at meaningless hazard quotients that have no basis in current scientific fact. The entire assessment of risk for endocrine effects from R-11 is flawed beyond repair and needs to be re-analyzed, re-written and then reviewed by independent experts in the field of endocrine toxicology.

Low Dose Endocrine Effects from Nonylphenol

As stated above, current science concerning nonylphenol and endocrine effects has clearly shown that the level of exposure for producing effects, both acute and chronic, is in the low ppb range, contamination levels far beneath those of traditional concern to toxicologists. Since many of these low dose studies have been unable to define a NOAEL, research is now beginning to incorporate the use of dosing levels in the parts per trillion (ppt) range (Christian and Gillies 1999).

The EA incorporates, at best, a cursory analysis of potential adverse endocrine effects to both wildlife and humans, relying upon unrealistic threshold levels and exposure scenarios. Much time could be spent attempting to correct each failing, but in reality the EA is flawed to the point where corrections are not appropriate and, as stated above, a complete re-write is potentially the only salvation.

The following are assessments and quotes from some of the low dose studies currently available for nonylphenol. It is being offered only as an example of data currently available.

"Nonylphenol (NP) and octylphenol (OP) are both acutely toxic to fish (17-3000 µg/L), invertebrates (20-3000 µg/L) and algae (27-2500 µg/L). In chronic toxicity tests no observable effect concentrations (NOEC) are as low as 6 µg/L in fish and 3.7 µg/L in invertebrates. There is an increase in the toxicity of both NPes and OPEs with decreasing EO chain length. NPECs and OPECs are less toxic than corresponding APEs and have acute toxicities similar to APEs with 6-9 EO units. APs and APEs bind to the estrogen receptor resulting in the expression of several responses both in vitro and in vivo, including the induction of vitellogenin. The threshold for vitellogenin induction in fish is 10

1 µg/L for NP and 3 µg/L for OP. APEs also affect the growth of testes, alter normal steroid metabolism, disrupt smoltification and cause intersex (ova-testes) in fish." (Servos (Environment Canada) 1999)

"Among the APs, 4-t-octylphenol and 4-nonylphenol were found to be considerably more potent than any other compound and estrogenic effects were detectable at 1 and 10 µM, respectively. 4-t-Octylphenol and 4-nonylphenol inhibited the binding of E2 to the ER of MCF-7 cells in a competitive ER binding assay." (Kwack SJ, et al. 2002)

"4-n-nonylphenol contamination caused an inverted dose-response curve. At low test concentrations (1.9-30 µg/l) reduced yolk immunoreactivity occurred, while at medium concentrations (120 and 500 µg/l) no significant effects were observable. In the most highly contaminated group (2,000 µg/l) yolk protein immunoreactivity was elevated to 107% of the control. Female yolk protein contents were affected only in the 3,000 µg bisphenol a/l contaminated group, where yolk immunoreactivity was reduced by ca. 10% compared to the control." (Hahn et al. 2002).

"All fish died after 4 days of exposure to 660 µg NP/L. Time-dependent NP bioaccumulation was detected in the tissues of fish exposed to 220 µg NP/L ($P < 0.05$) and histopathological changes were observed in the livers of fish exposed to 220 µg NP/L. Furthermore, an increase in the activity of glutathione-S-transferase (GST) was found in the liver of fish exposed to 220 µg NP/L for 1 week ($P < 0.05$)." "These results indicated that sublethal doses of NP were accumulating in the bodies of the fish and causing histopathological and biochemical changes in the livers of rainbow trout" (Uguz 2002)

"Both chemicals showed a dose-dependent increase in plasma VTG over the entire time course of exposure, with significantly elevated VTG levels by the fifth day of exposure to p-nonylphenol at concentrations of 5.4 µg/L or greater and to methoxychlor at concentrations of 2.5 µg/L or greater. Exposure to 0.64 µg/L p-nonylphenol resulted in highly variable plasma VTG levels of less than 6 mg/ml" (USEPA (Hemmer MJ, et al.) 2002).

"In the chronic study, exposure to NP at 50 µg/L significantly increased total fecundity and neonate deformities" (Zhang L & Baer KN, 2001).

"However, minor kidney histopathology indicated by increased pyknotic nuclei in kidney tubule and interstitial (hematopoietic) cells was detected at lower estrogenic exposures (≥ 10 µg/l NP nominal) than delayed gametogenesis. Considering all histological parameters in the current study, the rank order of potency for pathological effects in 60 dph zebrafish was 10 ng/l EE > 1 ng/l EE = 100 µg/l NP > 30 µg/l NP > 10 µg/l NP10 (nominal concentrations). Zebrafish from the same cohort examined in the current study that had been placed in clean water from 60 to 300 dph had histologically normal testes and no kidney or liver histopathology. However, increased ovarian follicle atresia was detected at 300 dph in zebrafish exposed developmentally to 100 µg/l NP. Therefore, we conclude that functional rather than morphological changes may be more important for future evaluations of developmental exposure to estrogens in fish, and that negative effects in female rather than male gonads may contribute to prolonged breeding impairment" (Weber et al. 2003).

"The percentage of males at 60 dph changed from 45% (9/20) in solvent controls to 0% at 10 ng/l EE and 10% at 100 µg/l NP." "Two fish with ovatestes were observed at 100 µg/l NP, while one was observed at 30 µg/l NP. Western blotting showed induction of Vtg at 30 and 100 µg/l NP." "Breeding trials conducted in adult fish from 120 to 160 dph revealed significant reductions in the percent of viable eggs, hatchability, and swim-up success at 10 ng/l EE and 100 µg/l NP. Our results suggest that functional reproductive capacity (breeding success) may be more sensitive than gross morphological endpoints (condition, ovo-somatic index, sex ratio) in adult zebrafish exposed to xenoestrogens during sexual differentiation and early gametogenesis." (Hill RL Jr, Janz DM. 2003).

"The chronic effect of p-nonylphenol on survival and reproduction for two generations of the freshwater cladoceran Daphnia galeata was examined by life table experiments. The effects on survival and reproduction were used as the intrinsic rate of natural increase, r , with the Euler-Lotka equation and were analyzed with a simple mathematical model (a power function). The population-level EC(50), the concentration of a substance that reduces the intrinsic rate of natural increase by 50%, was estimated as 65.2 microg/L for the first generation and 81.5 microg/L for the second generation. No transgenerational effect that reinforces adverse responses in the offspring generation has been detected. From a 48-h immobility test an acute LC(50) was estimated to be 60.8 microg/L. The acute LC(50) is a good indicator of the chronic population-level effects of this chemical to this species." (Tanaka Y, and Nakanishi J 2002).

"Nonylphenol, an environmental contaminant, has been shown to induce reproductive abnormalities in male rats.... Nonylphenol was administered orally to male rats at 1, 10 and 100 microg/kg body weight per day for 45 days... The weights of the testes and epididymides decreased significantly whereas the weights of seminal vesicles and ventral prostate remained unchanged at all doses of nonylphenol in treated rats.... The results suggest that graded doses of nonylphenol elicit depletion of antioxidant defence system in sperm, indicating nonylphenol-induced oxidative stress in the epididymal sperm of rats". (Chitra KC, et al. 2002)

"The induction of VG and ZRP expression was a more sensitive reaction to the presence of NP than the formation of testis-ova and the reversal of sex. Increased VG expression in trout liver occurred already at 1.05 microg/l NP, whereas VG mRNA levels, quantified by competitive RT-PCR, were not significantly elevated in NP exposed fish. ZRP contents were significantly higher at 10.17 microg/l NP." (Ackermann et al. 2002)

"After exposure to 10 microg NP/l reproduction was impaired as indicated by significantly reduced hatching rates..... The present findings indicate that NP, in an environmentally relevant concentration range, acts as a weak estrogen in directly exposed adult male rainbow trout as indicated by elevated plasma vitellogenin levels. Reproduction success was reduced as indicated by decreased hatching rates. Hormonal imbalances detected in the offspring of exposed fish indicate a transgenerational effect mediated by the endocrine system." (Schwaiger J, et al. 2002)

"Three independent trials were conducted using mortality and burial as endpoints. Amphipod mean lethal concentration to 50% (LC50) was 227 microg/L." (Hecht S, Boese BL. 2002)

"Overall, these results indicate that the lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC) of 4-NP through the life cycle of the F0 medaka were 17.7 and 8.2 microg/L, respectively. In the F1 medaka, no significant effects were observed on hatching success, posthatch mortality, or growth, but sexual differentiation at 60 d posthatch was affected. Induction of testis-ova in the gonads of the F1 fish was observed in both the 8.2- and the 17.7-microg/L concentrations. The results indicate that 4-NP can have significant effects on reproductive potential of medaka at concentrations as low as 17.7 microg/L." (Yokota et al 2001)

"There was also a significant increase in plasma vitellogenin concentration in the fish exposed via the water to 10 microg/l of 4-NP." (Pickford KA, et al. 2003).

"The LOECs of NP and OP for these events were 11.6 and 11.4 microg/L, respectively. These results suggest that NP and OP may have adverse effects at similar concentrations during early life stage in medaka. Additionally, we investigated whether the abnormal sex differentiation induced by these alkylphenols would be permanent or reversible once the medaka were returned to clean water. The appearance of the secondary sex characteristics reverted from female to male when fish were returned to clean water. However, gonadal histology showed that intersex gonads still existed, even after the fish were transferred to clean water for two months. These results suggest that the induced feminization of

secondary sex characteristics in medaka exposed to alkylphenols during the stage of sexual differentiation may not always be permanent, but the gonadal alteration (testis-ova) may continue much longer." (Seki M, et al. 2003).

"In injection and feeding experiments, vitellogenin levels increased significantly after 14 days. After immersion for 14 and 28 days, respectively, significantly higher concentrations of plasma vitellogenin were detected in male carp exposed to 4 microg/L nonylphenol and octylphenol. When transferred to clean water, the elevated plasma vitellogenin levels in carp exposed to 4 microg/L octylphenol for 42 days returned to control levels within 28 days." (Huang RK, Wang CH, 2001).

"Treatments with TBT and 4-NP (1, 10, 100 ppb) had only slight effects on the egg production of the adults and hatching rate of the eggs. However, increased histopathological changes were observed in epithelial tissues of the adult snails, e.g. lung and foot also characterised by extreme inflammatory processes....The observed histopathological effects due to exposure to tributyltin or 4-NP are suggested to lead to long-term adverse reproductive effects mediated by an impairment of the fitness of the snails. In the experiments the steroid-dependant (beta-sitosterol and t-methyltestosterone) degeneration of the albumen gland caused no obvious adverse effects on the fecundity nor fertility of the adults or on F(1)-generation. However, the impact on fertility following a prolonged exposure to high concentrations of the phytoestrogen cannot be predicted." (Czech P, Weber K, Dietrich DR. 2001).

"The observed responses in survival and reproduction were converted to reductions of the intrinsic rate of natural increase r . The population level EC, which is defined as the exposure concentration that reduces r by 50%, was estimated as 16.1 $\mu\text{g/l}$ super(-1)." (Tanaka, Y; Nakanishi, J; 2001).

"Nonylphenol is a biodegradation product of nonionic surfactants and has recently attracted considerable attention due to its estrogenic potential. Sexually mature male rainbow trout were repeatedly exposed (one to four periods of 10 days each) to environmentally relevant concentrations of nonylphenol (1 $\mu\text{g/L}$, 10 $\mu\text{g/L}$) and for comparison, trout were injected with estradiol. Since estrogens are known to induce structural changes within the fish skin, a similar effect of xenobiotics with estrogen-like activity was assumed. Samples of skin were evaluated by means of light and electron microscopy and histochemistry. In trout exposed to nonylphenol (1 $\mu\text{g/L}$, 10 $\mu\text{g/L}$) and to estradiol, the structure of the epidermis was altered: an irregular overall architecture was often accompanied by detached pavement cells, vacuolation of the cytoplasm, and severely deformed cell nuclei. However, the granulation pattern of the mucous cells was influenced exclusively after exposition to nonylphenol. The number of large and irregularly shaped mucosomes depended more on the exposure period than on the concentration of nonylphenol. Furthermore, this alteration has not yet been reported for any other pollutant or stressor and, thus, can be classified as an effect that would strongly indicate exposure to nonylphenol." (Burkhardt-Holm, 2000)

"In this study, rainbow trout eggs were exposed after fertilization to NP concentrations of 1 and 10 $\mu\text{g/l}$. Exposure occurred throughout the embryonic, larval and juvenile period under controlled laboratory conditions. After 12 months, induction of VG mRNA was analyzed in the liver by quantitative RT-PCR, and VG protein using polyclonal antibodies in Western blots. The development of quantitative RT-PCR included primer design, competitive PCR using heterologous standards and titration. Both VG mRNA and protein were induced in NP-exposed rainbow trout in a dose-dependent manner. In male fish, increases in VG mRNA and protein were already observed at 1 $\mu\text{g/l}$ NP. This study shows that chronic exposure of fish early life stages to environmentally realistic concentrations of NP leads to induction of vitellogenin." (Fent, 2000).

It should be evident from the data provided that low dose ppb endocrine effects are occurring across the board to many different species in both acute and chronic toxicity testing. Please use this and all

other available data to do a proper risk assessment for R-11.

Metabolites and Persistence

Another failing of land managers in their risk assessments for R-11 is the repeated claim that only certain metabolites will be present and these will biodegrade rapidly to harmless substances. These assumptions, like much else in the endocrine risk assessment for R-11, is inaccurate.

NP9E's primary degradates are NP1EO, NP2EO, NP1EC, NP2EC, and NP (Environment Canada 2001). All of these substances produce adverse endocrine effects with a potency 100 to 1000 times greater than the parent compound (NP9E), are moderately persistent, and in the case of NP, NP1E and NP2E, have shown a tendency to bioconcentrate (Environment Canada 2001, Coldham 1998, Uguz 2002).

The degradation pathways of NP9E are diverse and not well understood other than identifying the different degradates. Depending on the study and site, these degradates are usually the same, only the amount present differs. Though aerobic conditions will usually aid degradation of NPEs, the path to final degradation remains the same as anaerobic. The more toxic metabolites will be formed and then further break down.

To date, the best analysis of degradation pathway research can be found in Environment Canada's "Priority Substances List Assessment Report, Nonylphenol and its Ethoxylates". This report is listed in USDA (Bakke) 2003 bibliography.

From page 19; "Under aerobic and anaerobic treatment conditions, the biodegradation mechanism involves an initial loss of ethoxy groups, leading to the production of NP1EO and NP2EO and their carboxylate derivatives NP1EC and NP2EC (as well as NPnEC, where $n > 2$, and CAPECs...and CAPEs..."

From page 22; "Degradation in water and sediment. Primary biodegradation of higher-chain NPEs is generally faster than ultimate degradation of more persistent products, such as NP1EO, NP2EO, NP1EC, NP2EC, and NP (Ahel et al 1994b). Microbial acclimation to such chemicals is required for optimal degradation efficiencies (Maquire 1999)". (Environment Canada 2000)

Other research has shown that the lipophilic derivatives of NP9E, which are NP, and NP1E and NP2E, have shown the ability to bioaccumulate. The following studies highlight this;

"Despite rapid metabolism and excretion, a substantial depot of parent compound remained in muscle which will have implications for the maintenance of 4-nonylphenol residues and associated biological activity." (Coldham 1998).

"These results indicated that sublethal doses of NP were accumulating in the bodies of the fish and causing histopathological and biochemical changes in the livers of rainbow trout." (Uguz 2003)

"Few studies on the bioaccumulation of EDs in food have been conducted. Therefore, we evaluated the concentration in food of nonylphenol (NP)...NP concentrations ranged 0.1-0.4 microg/L in the river water, while they ranged 8-130 microg/kg-wet in the periphytons and 8-140 microg/kg-wet in the benthos...Bioaccumulation factors of NP are estimated as 160-650 for the periphytons, and 63-990 for the benthos, respectively.....The results suggest that food may be a more important route for fish exposed to EDs in water environment." (Takahashi A, 2003)

"In order to assess in fish the maternal transfer of alkylphenolic compounds to the progeny, the identification and quantification of the labelled compounds present in oocytes and embryos was conducted after dietary exposure of mature female mosquitofish to 14C-4n-nonylphenol during vitellogenesis and embryogenesis respectively. Radioactivity found in bile and liver extracts accounted for 0.9-0.6 and 0.2-0.1% of ingested radioactivity for females exposed during vitellogenesis and embryogenesis respectively. The amount of extractable radioactivity present in oocytes and embryos was

0.19 and 0.07% of the ingested dose respectively. The radio-HPLC profiles obtained from bile, liver, oocyte and embryo extracts were similar. They showed the presence of 4n-NP-glucuronide as the major metabolite and traces of unchanged 4n-NP. The other metabolites corresponded to 8-hydroxynonylphenol, 9-(4-hydroxyphenyl)-nonanoic acid and para-hydroxybenzoic acid which is the final product of the alkyl chain oxidation. Our results indicate that exposure of ovoviviparous female fish to 4-NP during vitellogenesis and embryogenesis leads to the contamination of the progeny by 4-NP and its metabolites." (Thibaut 2002)

Weakly Estrogenic

This is another common failing of land managers in risk assessments of R-11 in general. The relative strength of any EDC in relation to estradiol (E2) is irrelevant. The only thing that matters is the potential for adverse effects from a given substance at a given dose. E2 has been shown to produce effects with no threshold or in the low ppt range. As shown above, alkylphenol ethoxylates (NP and OP) produce effects in the low ppb range and lower. NP9E produces effects in the high ppb and low ppm range. The other metabolites of NP9E exhibit endocrine toxicity between the ranges of NP and NP9E.

The other point to consider is that, as discussed earlier, NP is a mimic of E2 and therefore is operating under the assumption of additive toxicity values when introduced into a system with E2 already present. This was the central theme of recent research by Rajapakse et al. 2001. Their conclusions were;

"There can be no doubt that weak xenoestrogens such as BPA or o,p'-DDT, when combined with 17 β -estradiol, are able to contribute to estrogenic mixture effects. We show that the impact of xenoestrogens on the actions of the steroid hormone depends on the mixture ratio and on its potency relative to 17 β -estradiol. When combined at approximately equieffective concentrations, substantial modulations of the effects of 17 β -estradiol by the xenoestrogen become discernible."

They go on to state;

"However, human tissues contain many compounds with estrogenic activity. On the basis of our studies, it appears conceivable that a multitude of xenoestrogens, when present in sufficient number and/or concentration, might in principle act together to impact on the actions of steroidal estrogens. Whether such impacts will be physiologically relevant remains to be seen. Definitive answers to this question are currently hampered by our lack of knowledge about the full spectrum of estrogenic agents in human tissues." (Rajapakse et al. 2001)

NP and E2 were shown to exert additive properties in another study by Thorpe, et al, 2001;

*"Experiments were conducted to assess the in vivo potency of binary mixtures of estrogenic chemicals using plasma vitellogenin (VTG) concentrations in juvenile rainbow trout (*Oncorhynchus mykiss*) as the endpoint. The estrogenic potencies of estradiol-17 beta (E2), 4-tert-nonylphenol (NP), and methoxychlor (MXC) were determined following 14day exposures to the individual chemicals and binary mixtures of these chemicals. E2, NP, and MXC all induced concentration dependent increases in plasma VTG, with lowest observed effect concentrations of 4.7 and 7.9 ng L super(-1) for E2, 6.1 and 6.4 mu g L super(-1) for NP.....Mixtures of E2 and NP were additive at the concentrations tested....The data presented illustrate that the model of concentration addition can accurately predict effects on VTG induction, where we know that both chemicals act via the same mechanism in mediating a vitellogenic response."* (Thorpe, KL, et al, 2001)

It is scientifically indefensible to use the "weak estrogen" argument to dismiss concerns about endocrine disruption. This is just one more attempt by the FS to turn a blind eye on a very sensitive and important issue.

EDCs and Potential Effects to Flora

Recent research has raised a new concern that expands the field of potential adverse effects from EDCs considerably. The study Fox 2001 for the first time looked at signalling pathways outside of the endocrine systems of wildlife. Though this study did not include any alkylphenol ethoxylates, it did include EDCs that function the same as NPEs, as estrogen mimics that bind to the estrogen receptor.

Fox et al. show that EDCs interfere with the ability of nitrogen-fixing bacteria to form a symbiotic relationship with their leguminaceous hosts. This symbiosis is the basis for a key ecological process, nitrogen fixation, which is essential for life on earth.

Rhizobial bacteria form symbiotic relationships with legumes, living in nodules within the plant's roots and converting nitrogen from one chemical form to another. The conversion, called nitrogen fixation, is the principal natural process by which nitrogen is made available for use by living organisms.

The plant-bacteria symbiosis is initiated when the bacterium detects a chemical signal exuding naturally from the roots of the legume. The signals belong to a class of compounds, phytoestrogens, which are so described because of their coincidental ability to interact with vertebrate estrogen receptors.

To detect the signal, the bacteria employs receptors analogous to hormone receptors. The phytoestrogen binds to the bacterial receptor and the resulting complex then activates a gene in the bacterium. Activated, the gene initiates an exchange of chemicals between plant and bacteria that stimulates and maintains the nodules in which the bacteria live.

Fox et al. reasoned that if phytoestrogens were able to interact with the estrogen receptor, then synthetic compounds that interact with the estrogen receptor might be capable of binding with the bacterium's phytoestrogen receptor and reducing gene activation.

Fox et al. worked with alfalfa and its symbiotic bacterium *Sinorhizobium meliloti*. The plant exudes a phytoestrogen, the flavonoid, luteolin, which activates the Nod gene in the bacterium.

They created an in vitro testing system in which they could measure Nod gene induction with luteolin alone and then when a series of endocrine disrupting compounds (EDCs) were added to the experiment.

Nod induction by luteolin at 10^{-6} Molar concentration was set as the standard for the experiment, or 100% induction. Adding EDCs separately in different concentrations then allowed Fox et al. to determine the potency of EDCs in suppressing Nod induction.

They performed a second set of experiments with alfalfa roots to determine whether the EDC impact on Nod induction would occur in whole organisms. To do this, they inoculated the roots with a bacterium that turns blue upon exposure to one of the biochemical products of Nod gene activation.

Contaminants with estrogenic activity decreased gene expression by up to 90%. In addition to DDT and bisphenol A, methyl parathion, pentachlorophenol and two plant flavonoids (chrysin and genistein) also interfered with phytoestrogen signaling.

There are two important lessons from this study. The first is that it demonstrates conclusively that the symbiosis between legumes and rhizobial bacteria is vulnerable to signal disruption by synthetic contaminants. How extensively this is occurring in the real world becomes an important question, as this symbiosis is crucial to one of the main biogeochemical cycles that makes life on earth possible, the nitrogen cycle.

The second important lesson from this work is that it reinforces the need to consider endocrine disruption as just one type of chemical impact within a broader framework of signal, or message, disruption. Many of life's crucial processes are controlled by chemical signals. Some of these, hormones, mediate events within and among cells, for example, the activation of specific genes. Fox et al. demonstrate that signal disruption can also take place in chemical message systems controlling

relationships between organisms, in this case the two participants in a symbiotic relationship: legumes and rhizobial bacteria.

At the October 2001 hormone meeting at Tulane University in New Orleans, Jennifer Fox and colleagues presented new data from their studies of the impact of EDCs on symbiosis. In this new set of experiments, they exposed growing plants to EDCs and examined the numbers of nodules formed per plant and the mass of the plants. EDCs suppressed nodule number and plant biomass, as predicted by the study reported in Nature (above). Thus the impact of EDCs on Nod gene activation is likely to have real world effects. Currently Fox et al. are working on two similar projects that should be published soon (J. McLachlan pers. comm.).

The question comes to mind, have EDCs already done damage to non-endocrine signalling systems as this research shows possible; and if so, to what extent. Hopefully their results will encourage researchers to begin to look at other chemically-mediated symbioses for signs of chemical disruption.

For instance, scientists have noticed widespread forest decline involving trees in Europe and North America. These declines are at least in part associated with changes in the abundance of mycorrhizal fungi, which exist symbiotically with tree roots and are essential for nutrient absorption by tree roots. Investigation into possible disruption of the signals that mediate these symbioses might prove very useful to understand the declines.

In Conclusion

The risk assessment for NPE and metabolites in the EA is not a valid attempt at living up to NEPA responsibilities. As previously suggested, what little analysis exists is burdened with inaccurate assumptions and poor judgement. The best advice one could give is to start from the bottom up and actually do a risk assessment.

Endocrine Effects Bibliography

Ackermann et al 2002. Effects of long-term nonylphenol exposure on gonadal development and biomarkers of estrogenicity in juvenile rainbow trout *Oncorhynchus mykiss*. *Aquat Toxicol.* 2002 Oct 30;60(3-4):203-21.

Arnold, Steven F., et al, 1996; Synergistic activation of estrogen receptor with combinations of environmental chemicals. *SCIENCE*, 272:1489-1492 (7 June).

Atienzar et al 2002, 4-n-Nonylphenol and 17-beta estradiol may induce common DNA effects in developing barnacle larvae. *Environ Pollut.* 2002;120(3):735-8.

Birnbaum, LS and SE Fenton. 2003. Cancer And Developmental Exposure to Endocrine Disruptors. *Environmental Health Perspectives* 111:389-394.

Bevan et al 2001, Defects in embryogenesis and growth factor responsiveness induced by exposure to

endocrine disrupting compounds. </cgi-bin/sis/search/r?./temp/u126~8zP9Jr:
@and+@au+@term+Porter+D> Abstr Soc Neurosci 2001;27(Pt 1):658

Chitra KC, et al. 2002; Effect of nonylphenol on the antioxidant system in epididymal sperm of rats. Arch Toxicol. 2002 Sep;76(9):545-51. Epub 2002 Jun 25.

Christian, M and G Gillies. 1999. Developing hypothalamic dopaminergic neurones as potential targets for environmental estrogens. Journal of Endocrinology 160:R1-R6

CLS, 2000; Hormonally Active Agents in the Environment, Commission on Life Sciences (CLS), <http://www.nap.edu/books/0309064198/html/82.html>

Colborn et al, 1993. Developmental Effects of Endocrine-disrupting Chemicals in Wildlife and Humans. Environmental Health Perspectives. Vol 101 No. 5 (No abstract)

Colborn T. 1995. How Research has Succeeded and Failed to Translate Science into Policy: Endocrinological Effects on Wildlife. Environmental Health Perspectives. Vol 103 No. 6 (No abstract)

CSF 2002, Chemical Stakeholders Forum (CSF) Annual Report 2001-2002. Available online at www.defra.gov.uk

Coldham 1998, Biotransformation, tissue distribution, and persistence of 4-nonylphenol residues in juvenile rainbow trout (*Oncorhynchus mykiss*). Drug Metabolism and Disposition Vol. 26, No. 4.

Czech P, Weber K, Dietrich DR. 2001: Effects of endocrine modulating substances on reproduction in the hermaphroditic snail *Lymnaea stagnalis* L. Aquat Toxicol. 2001 Jul;53(2):103-14.

Fox, JE, et al, 2001; Nitrogen fixation: Endocrine disrupters and flavonoid signalling. Nature, 2001, 413 (13 Sept 01):128-129.

Gupta, Chhanda. 2000. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. Proceedings of the Society for Experimental Biology and Medicine 224:61-68.

Hahn T, Schenk K, Schulz R 2002; Environmental chemicals with known endocrine potential affect yolk protein content in the aquatic insect *Chironomus riparius*. Environ Pollut. 2002;120(3):525-8.

Hecht S, Boese BL. 2002; Sensitivity of an infaunal amphipod, *Eohaustorius estuarius*, to acute waterborne exposures of 4-nonylphenol: evidence of a toxic hangover. Environ Toxicol Chem. 2002 Apr;21(4):816-9. (LC50 at 227 ppb)

Thorpe, KL; Hutchinson, TH; Hetheridge, MJ; Scholze, M; Sumpter, JP; Tyler, CR, 2001, Assessing the Biological Potency of Binary Mixtures of Environmental Estrogens using Vitellogenin Induction in Juvenile Rainbow Trout (*Oncorhynchus mykiss*). Environmental Science & Technology [Environ. Sci. Technol.], vol. 35, no. 12, pp. 2476-2481, 15 Jun 2001, ISSN 0013-936X

USDA (Bakke) 2003, Human and Ecological Risk Assessment of Nonylphenol Polyethoxylate-based (NPE) Surfactants in Forest Service Herbicide Applications”, Pacific Southwest Region (Region 5), Pesticide Use Specialist, May 2003

USEPA (Hemmer MJ, et al.) 2002; Vitellogenin mRNA regulation and plasma clearance in male sheepshead minnows, (*Cyprinodon variegatus*) after cessation of exposure to 17 beta-estradiol and p-nonylphenol. Aquat Toxicol. 2002 Jul;58(1-2):99-112.

Uguz C, Iscan M, Erguven A, Isgor B, Togan I. 2003; The bioaccumulation of nonylphenol and its adverse effect on the liver of rainbow trout (*Onchorynchus mykiss*). Environ Res. 2003 Jul;92(3):262-70.

Weber LP, Hill RL Jr, Janz DM. 2003; Developmental estrogenic exposure in zebrafish (*Danio rerio*): II. Histological evaluation of gametogenesis and organ toxicity. Aquat Toxicol. 2003 May 29;63(4):431-46.

Welshons, WV, KA Thayer, BM Judy, JA Taylor, EM Curran and FS vom Saal. 2003. Large effects from small exposures. I. Mechanisms for endocrine disrupting chemicals with estrogenic activity. Environmental Health Perspectives doi:10.1289

WHO, 2002; Global Assessment of the State-of-the-Science of Endocrine Disruptors. International Programme On Chemical Safety. (Damstra, T, S Barlow, A Bergman, R Kavlock and G Van Der Kraak (editors)). http://www.who.int/pcs/emerg_site/edc/global_edc_TOC.htm

Yokota et al 2001 Life-cycle toxicity of 4-nonylphenol to medaka (*Oryzias latipes*). Environ Toxicol Chem. 2001 Nov;20(11):2552-60.

Zhang L & Baer KN, 2001; The effects of 4-nonylphenol on reproduction and embryo development in *Daphnia magna*. Toxicologist 2001 Mar;60(1):163

Zhang 2003 The effects of 4-nonylphenol and ethanol on acute toxicity, embryo development, and reproduction in *Daphnia magna*. Ecotoxicol Environ Saf. 2003 Jul;55(3):330-7.

For the reasons outlined above, the EA must be retooled to incorporate further analysis, provide a full range of alternatives, develop an alternative that will achieve the desired goal and provide mitigations to

protect health and the environment. We look forward to reviewing the completed document.

Sincerely,
Dan Zimmerman
Environmental Investigator