

ALKYLPHENOLS & ETHOXYLATES RESEARCH COUNCIL

COMMENTS ON THE JAPAN ENVIRONMENTAL HEALTH DEPARTMENT, MINISTRY OF THE ENVIRONMENT REPORT ON THE TEST RESULTS OF ENDOCRINE DISRUPTING EFFECTS OF NONYLPHENOL ON FISH (DRAFT)

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INTRODUCTION AND SUMMARY

The Alkylphenols & Ethoxylates Research Council (APERC), which represents manufacturers, processors, users and raw material suppliers of alkylphenols and alkylphenol derivatives¹ submits the following comments to the Organization for Economic Co-operation and Development on the Japan Ministry of Environment shared assessment report “Test Results of Endocrine Disrupting Effects of Nonylphenol on Fish” [1].

1. The risk assessment should rely on reasonable, statistically derived, predicted environmental concentrations (PECs). The PEC for maximum exposure concentrations should be 0.59 µg/L, which is the upper 95th percentile value.

The Japan Ministry of the Environment (MoE) conducted an ambient water survey for nonylphenol (NP). This valuable dataset provides a representative description of exposure of NP in the surface waters of Japan. NP was detected in the water column at 617 sites out of a total of 1,574, in two years (detection ratio 39%) and the concentration range was non-detectable (ND) (< 0.03 – 0.1) – 21 µg L⁻¹. The arithmetic mean concentration was 0.17 µg L⁻¹ (calculated assuming ND equaled 0 µg L⁻¹) and the median, 75th, 90th and 95th percentile concentrations of the distribution equaled ND, 0.10, 0.30 and 0.59 µg L⁻¹, respectively. The arithmetic mean concentrations equaled 0.19 and

¹ Members of APERC include: Dover Chemical Corporation; GE Plastics; Mitsubishi Chemical Corporation; Rhodia Inc.; Rohm and Haas Company; Schenectady International, Inc.; Stepan Canada; Sunoco, Inc.; and, The Dow Chemical Company.

0.22 $\mu\text{g L}^{-1}$ assuming calculation based on the assumption that NDs are one half the detection limit and equal to the detection limit, respectively.

The use of the 95th percentile of the survey distribution represents the reasonable maximum exposure estimate and is entirely consistent with typical approaches used for risk assessment purposes. The 95th percentile represents the reasonable worst-case scenario, *i.e.*, a one in twenty recurrence probability. Additionally, the median or 50th percentile value of the measured distribution should be used to represent a more typical anticipated concentration, *i.e.*, a one in two recurrence probability.

2. The baseline risk assessment should be based on ecologically relevant endpoints related to survival, growth and reproduction.

As detailed by Campbell and Hutchinson [2], the assessment of the effects of endocrine active substances on populations of organisms should be conducted by measuring ecologically relevant endpoints related primarily to reproductive fitness (*e.g.*, egg production, egg hatchability, larval survival). The predicted no effect concentration (PNEC) component of the MoE risk determination was based on two key studies, a medaka partial life cycle study and a medaka fish full life cycle (FFLC) study [3,4]. Both studies examined ecologically relevant endpoints, which provide a basis for risk assessment. However, the MoE risk document relies on endpoints of uncertain ecological relevance, such as histological findings, at an NP concentration of 6.08 $\mu\text{g L}^{-1}$.

As discussed further below, in order to calculate a PNEC, the MoE should rely on ecological endpoints that are meaningful, such as survival, growth and reproduction. Using this approach, a PNEC can be calculated from the lowest no observable effect concentration (NOEC), which was 8.2 $\mu\text{g L}^{-1}$ and was based on survival. Dividing the NOEC by the MoE safety factor of 10, a PNEC of 0.82 $\mu\text{g L}^{-1}$ can be derived.

Medaka Partial Life Cycle Study - A partial life cycle study using medaka fish (*Oryzias latipes*) was performed to assess the effects of nonylphenol (mixture) and 4-tert-octylphenol (4-t-OP) on sex differentiation of the medaka. Medaka (60 eggs/treatment)

were exposed to five test concentrations of each substance (NP: 3.30, 6.08, 11.6, 23.5 and 44.7 $\mu\text{g L}^{-1}$) (4-t-OP: 6.94, 11.4, 23.7, 48.1 and 94.0 $\mu\text{g L}^{-1}$) as mean measured concentrations under flow through conditions for 60 days post-hatch.

No effects on survival, hatching of fertilized eggs or post-hatch mortality were observed at any concentration tested. As for growth of fish, at 60 day post-hatch in the NP treated group, significant decreases were observed in total length and body weight in the 44.7 $\mu\text{g L}^{-1}$ treatment, the highest NP dose tested, and body weight reductions in the 23.5 $\mu\text{g L}^{-1}$. In the 4-t-OP test, no growth reduction was observed at the concentrations tested.

The sex ratios estimated from the appearance of secondary sex characteristics of the surviving fish at 60 days post-hatch were significantly skewed toward female at ≥ 23.5 $\mu\text{g L}^{-1}$ in the NP test and ≥ 48.1 $\mu\text{g L}^{-1}$ in the 4-t-OP test. Based on ecologically relevant survival, growth and reproductive endpoints, the lowest observable effect concentration (LOEC) and NOEC for the study with NP were 23.5 and 11.6 $\mu\text{g L}^{-1}$ and the LOEC and NOEC for the study with OP were 48.1 and 23.7 $\mu\text{g L}^{-1}$.

Medaka Full Life Cycle Study - A medaka full life cycle toxicity study was also conducted. Medaka eggs (60/treatment) were exposed to mean measured NP concentrations of 4.2, 8.2, 17.7, 51.5 and 183 $\mu\text{g L}^{-1}$ under flow through conditions from fertilized egg to 104 days post-hatch.

The 183 $\mu\text{g L}^{-1}$ treatment significantly reduced embryo survival and swim-up success of the F0 fish. The cumulative mortality of the F0 fish from swim up to 60 days post-hatch was significantly increased in the 17.7 and 51.5 $\mu\text{g L}^{-1}$ treatments. No concentration related effect was observed on the growth of the fish at 60 days post-hatch. However, the sex ratio estimated from the appearance of secondary sex characteristics was skewed toward female in the 51.5 $\mu\text{g L}^{-1}$ treatment. Because the sex ratio of the F0 fish in the 51.5 $\mu\text{g L}^{-1}$ treatment group was skewed toward female, mating pairs from ≤ 17.7 $\mu\text{g L}^{-1}$ were selected at post-hatch day 70 and their fecundity and fertility were observed daily until 103 days post-hatch. No statistically significant effects on fecundity or fertility

were observed at any of the treatments examined ($\leq 17.7 \mu\text{g L}^{-1}$). In the F0 generation the LOEC and NOEC based on mortality were 17.7 and $8.2 \mu\text{g L}^{-1}$, respectively. In the progeny generation (F1) no significant effects were observed on hatching, post-hatch mortality or growth at the concentrations tested, *i.e.*, 4.2 , 8.2 and $17.7 \mu\text{g L}^{-1}$.

Taken together, the studies can be used to establish a PNEC. Based on the two studies, growth and larval survival were the most sensitive endpoints, posing effects as low as $17.7 \mu\text{g L}^{-1}$. The lowest NOECs were 8.2 and $11.6 \mu\text{g L}^{-1}$ for the full life cycle and partial life cycle studies, respectively. The PNEC can be calculated from the lowest NOEC, which was $8.2 \mu\text{g L}^{-1}$ and was based on survival. Dividing the NOEC by the MoE safety factor of 10, a PNEC of $0.82 \mu\text{g L}^{-1}$ results and can be used in a baseline risk assessment.

3. The ecological risk assessment of NP in the surface waters of Japan should not be based on supplemental experimental observations at the serum and tissue level.

The use of plasma or liver vitellogenin levels has been properly excluded by the MoE as an appropriate indication of nonylphenol exposure and secondarily endocrine/reproductive disruption because of the lack of a direct mechanistic link to reproductive dysfunction and elevated vitellogenin levels. Also noted is the fact that the protein has been measured at appreciable levels in male fish not exposed to nonylphenol. If induction of vitellogenin is indeed a harbinger of reproductive dysfunction in fish, the question remains at what levels does induction of vitellogenin induce reproductive impairment.

Tissue level effects in gonadal tissue should not be used for the risk assessment if they do not directly correlate with ecologically relevant measures of reproductive success. The MoE developed its PNEC based on the induction of the histological finding in the medaka partial life cycle study of testis-ova in the NP $11.6 \mu\text{g L}^{-1}$ treatment group. At issue however is the relevance of this endpoint with regard to reproductive performance and ecological significance. As noted, no effects on hatching of fertilized eggs or post-hatch mortality were noted for NP. In addition, the partial life cycle study reported

"active spermatogenesis" in all testis with testis-ova at the lower three concentrations, plus the control (0, 6.08, 11.6 and 23.5 $\mu\text{g L}^{-1}$ NP). No testis-ova were reported in the control or the 6.08 $\mu\text{g L}^{-1}$ concentrations and were found in only 4 of 20 fish at 11.6 $\mu\text{g L}^{-1}$, in 9 of 20 fish at 23.5 $\mu\text{g L}^{-1}$ and in only 4 of 20 at 44.7 $\mu\text{g L}^{-1}$. In addition, no other reproductive endpoints were affected at concentrations below 44.7 $\mu\text{g L}^{-1}$, including hatchability, time to hatch or sex organ weights. The assessment reports that sex ratios were skewed in the 23.5 and 44.7 $\mu\text{g L}^{-1}$ groups, but this is false, as the researchers appear to have combined all females and males with one or more testis-ova as females. Since the fish with testis-ova had active spermatogenesis, they should not be considered female. Thus, the tissue level effect of testis-ova is not correlated with any measure of reproductive success. Therefore, the LOEC related to ecologically relevant reproductive endpoints appears to be 44.7 $\mu\text{g L}^{-1}$, showing that relevant measures of reproductive success is not as sensitive as survival or growth endpoints.

4. Refined predicted no effect concentrations can be calculated that are protective of the structure and function of communities and ecosystems and make full use of the toxicity database.

For the baseline risk assessment, the MoE's use of the PEC/PNEC application factor of 10 is appropriate given the nature and extent of the toxicity dataset. However, a refined PNEC can be calculated using alternative methodologies, such as probabilistic risk assessment. One such example is the probabilistic approach after the methods of Parkhurst *et al.* [3] or the joint USEPA/Agricultural Chemical Industry ECOFRAM (<http://www.epa.gov/oppefed1/ecorisk/index.htm>) efforts. Additionally, a population based approach after the methods of the Dutch RIVM [4] in which a Hazard Concentration 5% (HC5) is calculated could be employed. The HC5 represents the ecological hazard concentration for protection of 95% of the population in a community. Both of these approaches may be advantageous when compared to the factor of 10 approach advocated by the MoE because each take into account the scope of the toxicity database and allow for the approximation of a chemical concentration that would be protective of 95% of the exposed community, which is considered sufficient to ensure the continued growth and development of the community.

5. The data in the MoE report support a calculated PEC/PNEC ratio that is less than 1 and therefore is indicative of adequate margins of safety for exposed fish species.

The MoE has appropriately established the PEC of the PEC/PNEC ratio, at $0.59 \mu\text{g L}^{-1}$ utilizing the 95th percentile of the MoE ambient water survey distribution. Taking the conservative approach, the MoE has selected the NOEC of $6.08 \mu\text{g L}^{-1}$ derived from the medaka partial life study. The NOEC reflects the dose concentration at which no testis-ova were exhibited in the exposed medaka. Assuming an assessment factor (AF) of 10, the calculated PEC/PNEC ratio equals 0.97 ($0.59/0.608 = 0.97$) less than 1, with 1 or below indicative of an adequate margin of safety. The MoE has however at least partially discounted this indication of a low potential for adverse risk by noting that 71 of the 1,574 measured ambient water samples (4.5%) exceeded the PNEC value and thus was indicative of significant risk. This is not consistent with typical approaches given that the AF of 10 is designed to provide, where toxicity datasets are sufficiently robust, a safety margin with sufficient “flexibility” as to account for uncertainties associated with exposure and effects distributions.

In the medaka partial life cycle study, there were no observed effects on hatching of fertilized eggs or post-hatch mortality at any NP dose tested. In the medaka FFLC, reduced embryo survival and swim-up success were noted at the highest concentration tested. The cumulative mortality of the F0 fish from swim up to 60 days post-hatch was significantly increased in the 17.7 and $51.5 \mu\text{g L}^{-1}$ treatments. As an alternate approach to that promulgated by the MoE, one could assume that the critical toxicity endpoint was not the formation of testis-ova but was mortality or growth in exposed fish species.

Utilizing the NOEC (mortality) of $8.2 \mu\text{g L}^{-1}$ from the F0 generation on the medaka FFLC study or the NOEC (growth) of $11.6 \mu\text{g L}^{-1}$ derived in the medaka partial life cycle study in conjunction with the PEC of $0.59 \mu\text{g L}^{-1}$ and the AF of 10, the calculated PEC/PNEC ratio is less than 1 in both cases and indicative of adequate margins of safety for exposed fish species. Because the link between testis-ova and reproductive performance is not causal, the appropriate toxicity endpoints are mortality, growth or reproductive

performance indices. Since reproductive performance was affected at only the highest NP concentrations tested, the use of mortality or growth as toxicity endpoints is comparatively conservative and would ensure the health of the exposed aquatic communities.

Utilizing the PEC/PNEC ratio of 0.97 ($0.59/0.608 = 0.97$) based on the 95th percentile of ambient water survey, the medaka NOEC with regard to testis-ova formation and the AF of 10, it can be concluded that adequate margins of safety exist regarding exposures of fish populations to nonylphenol. Furthermore, if NOEC values reflecting mortality or growth are employed the anticipated margins of safety are increased. It can be concluded therefore that even when utilizing the lowest NOEC based on histological findings not causally linked to reproductive impairment that adequate safety margins exists for nonylphenol in the environment. Utilization of toxicity endpoints that are more directly related to population dynamics increases the estimated safety margins for potentially exposed fish species.

6. APERC promotes the use of responsible environmental management practices among users of nonylphenol and its derivatives in order to maintain acceptable environmental levels of these substances.

The need for risk management efforts to reduce environmental levels of NP in Japan does not seem to be widespread considering that the data in the MoE baseline assessment report supports the conclusion that adequate margins of safety for exposed fish species exist. In cases where environmental levels of NP do not fall within adequate margins of safety, APERC supports the use of responsible environmental management practices, such as recycling and adequate wastewater treatment, to achieve acceptable environmental levels of NP whereas a reduction of use mandate should only be recommended in the absence of other more effective approaches. Moreover, as noted in the MoE report, substitution can lead to serious environmental and human health effects and APERC recommends a comprehensive health and environmental assessment of substitutes.

References

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