EJAR

Developmental toxicity of nonylphenol in zebrafish (*Danio rerio*) embryos

Tamer El-Sayed Ali

Oceanography Department, Faculty of Science, Alexandria University, Egypt.
E-mail: tameraly@yahoo.com

Received 5th June 2010, Accepted 27th July 2010

Abstract

Nonylphenol (NP) is a widely used industrial organic compound that enters the environment as a microbial degradation product of nonylphenol polyethoxylates (NPEs), being ubiquitous environmental contaminant especially in aquatic ecosystems. Evidence of the severe effects of NPs on different fish species exists in a number of axes including endocrine and non-endocrine as well. In this study, we report the effect of NP on zebrafish (*Danio rerio*) embryogenesis. Fish eggs (1 hour post fertilization, hpf) were exposed to graded concentrations of NP (0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µM), ranging from high lethal levels to lower, more environmentally relevant levels. The embryos/larvae were checked at 24, 48 and 72 hpf to study the development and survival calculation. Images were depicted at all treatment levels to complete the picture of the morphological abnormalities in different organs. Embryogenesis was highly affected by NP in a dose-dependent pattern. Through a comparative approach of the treated embryos with the control group, it could be revealed that 0.01, 0.03, 0.1 and 0.3 µM have no side effect on the embryos, meanwhile 1 and 3 µM caused severe oedema and less development 24 hpf, but 10 µM is lethal from the beginning. The results of the current study show that more attention should be given to assess the risk of these compounds in the aquatic environment.

Keywords: Nonylphenol, Zebrafish, Toxicity, Embryogenesis, Developmental abnormalities.

1. Introduction

Nonylphenol (NP) is a widely used industrial organic compound that enters the environment as a microbial degradation product of nonylphenol polyethoxylates (NPEs). It is present in sewage sludge (Lee *et al*., 2004), surface waters (Isobe and Takada, 2004) and sediments (Stachel *et al*., 2005), being ubiquitous environmental contaminant. Evidence of the severe effects of NPs on different fish species exists in a number of axes including endocrine and non-endocrine. The majority of research has concentrated on the estrogenic effects of NPs as endocrine disrupting chemicals on several fish species, where plasma vitellogenine (VTG) gene expression has been used as a biomarker for fish exposure to oestrogens (Sumpter and Jobling, 1995). Kinetics of hepatic VTG mRNA expression, plasma VTG accumulation and VTG clearance have been determined after exposure to NP in zebrafish, *Danio rerio*, (Yang *et al*., 2006; Holth *et al*., 2008), rainbow trout, *Onchorynchus mykiss*, (Uguz *et al*., 2003; Vetillard and Bailhache, 2006), Atlantic salmon, *Salmo salar*, (Yadetie and Male, 2002), Japanese medaka, *Oryzias latipes*, (Balch and Metcalfe, 2006), Fathead minnows, *Pimephales promelas*, (Barber *et al*., 2007), common carp, *Cyprinus carpio*, (Barse *et al*., 2006). However, for our knowledge, reporting the non-estrogenic action of such compounds are poorly studied. NP can cause developmental toxicity in aquatic organisms and it was demonstrated in killifish, *Fundulus heteroclitus*, and zebrafish, *Danio rerio*, causing both lethal and sublethal developmental abnormalities after 96 h and 48 h of exposure, respectively (Kelly and Di Giulio 2000; Kammann *et al*., 2009, respectively).

Embryos of zebrafish have often been used in toxicity studies of environmentally relevant substances (Kammann *et al*., 2006; Kosmehl *et al*., 2006; Scholz *et al*., 2008, Kammann *et al*., 2009). Zebrafish is a suitable experimental organism in toxicological research due to its short spawning intervals, easily manipulation and adaptation to the laboratory setting, rapid development and generation time, transparent eggs and can be maintained in a relatively small space, (Nagel, 2002; Braunbeck *et al*., 2005) facilitating visual identification of morphogenetic movements and organogenesis with a standard dissecting microscope. Several endpoints can be detected in parallel, thereby enhancing the toxicity spectrum covered by the assay (Kammann *et al*., 2009).

The present study aimed to determine concentration-dependent effects of graded series of NP on the development of zebrafish embryos according to

gross morphology and to examine the extent to which the magnitude of the effects is dependent on the concentration of NP with which the embryos are treated.

1. Materials and methods

1.1. Compounds

Seven stock solutions (0.1, 0.3, 1, 3, 10, 30 and 100 mM) of NP (a mixture of isomers, CAS Number: 84852-15-3, Sigma-Aldrich, Netherlands) were dissolved in dimethyl sulfoxide (DMSO, 0.01%) immediately prior to use and then directly diluted 10000 times in Dutch standard water (nominal concentrations: 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µm). Solvent (DMSO, 0.01%) and negative controls were incorporated in the experiment.

1.2. Fish maintenance

Zebrafish were raised and kept under standard laboratory conditions at about 28°C and a photoperiod of 14:10 h. light: dark (Brand *et al*., 2002). Fish were fed with dry fish feed, Tetra-Pro Flakes (Tetra GmbH, Germany) in the morning and hatched brine shrimp (Artemia cysts from INVE, Grantsvillle, UT, USA) in the afternoon. The fish were acclimated in glass aquaria containing copper free water. Typically, the eggs were spawned synchronously at dawn of the next morning. One hour later, eggs quality has been checked under the microscope (Leica MZ 75), being sure to select the healthy, fertilized eggs for the experiment. Fish breeding and embryo manipulation were conducted according to Westerfield *et al*. (1997).

1.3. Zebrafish embryos test

Selected eggs (one hour post fertilization, hpf) were placed in 24-well cell culture sterilized plates (one embryo/well). Embryos were exposed to the different concentrations of NP at the 4:8 – cell stage (1:1.25 hour post fertilization, hpf). Ten embryos/concentration were used and incubated at 28°C. Embryos/larvae were screened daily - till 72 hours - and scored for survival, alterations in morphology, developmental abnormalities and endpoints of toxicity (Nagel, 2002). Toxic/lethal end points (coagulation, missing heart beat, missing somites, missing tail detachment, missing spontaneous movement) and non-lethal malformations (pericardial or yolk sac oedema, bent notochord, fin malformation, no pigmentation, incomplete head and eye development) were separately reported. Developed embryos/larvae were examined and daily photographed by a stereo microscope. Paintshop Program 8 image analysis software was utilized to control a Roper digital camera on the microscope. Images were depicted at all treatment levels to complete the picture of the morphological abnormalities in different organs.

1.4. Calculation of LC_{50} and EC_{50}

The LC_{50} and EC_{50} were calculated at 72 hours post fertilization (hpf) from the concentration-percentage lethality and concentration-percentage effect curves, respectively for all end points separately as well as for the sum of lethal affected embryos. Logistic curves with binomially distributed errors were used to describe the relationships. From these, LC_{50} and EC_{50} values and their 95% confidence intervals were calculated using Graph Pad Prism 5.01.

2. Results

To gain more insight in the embryotoxic effects of NP, zebrafish embryos were exposed from one hour post fertilization (hpf) for the 72 hpf of development to follow up the developmental alterations caused by graded levels of NP (Figures 1-3).

For the groups treated with 0.01, 0.03 and 0.1 µM NP, no effect was observed during all the experimental period. Otherwise, higher concentrations of NP leads to lethal and non-lethal malformations in embryos varied according to the concentration and duration of exposure. Respecting to 1 and 3 µM, the NP started its toxic non-lethal action 24 hpf with simple oedema which increased stepwise leading to severe head, yolk sac and heart oedemas after 72 hours of exposure at the first concentration level and death at the second one. Concentration 10 µM was toxic within the first three hours of exposure, all embryos stopped their development in the epiboly stage while at 0.1 µM, 20% of the exposed embryos died (Figure 4).The developmental effects of NP were dose dependent with an EC_{50} value of 0.8 µM for all endpoints (Figure 5).

3.1. 24 hours post fertilization

The concentrations of 0.01, 0.03, 0.1 and 0.3 µm have not caused morphological alterations in embryos compared with those of the control group during the first 24 h of development. Well developed healthy embryos with somites, yolk sac, tail, head, eyes, prominently sculptured brain and few pigment cells were present along the axis dorsal to the yolk extension and on the dorsal part of the yolk ball, similar to the control ones. While, embryos exposed to 1 and 3 µM showed yolk sac oedema and extended mal-formed yolk sac in the second only case (Figure 1).

3.2. 48 hours post fertilization

Embryos exposed to concentrations of 0.01, 0.03, 0.1 and 0.3 µM showing embryos with well-developed notochord, otolith, caudal fin, head, eyes and pigment extends the whole length of the body, similar to the control group embryos. The 1 µM treated-group showed bigger oedema and a slightly unstraight notochord. Embryos exposed to 3 µM group seems

more affected with the time, showing line oedema and blood clotting around yolk sac accompanied with growth retardation (small head and eyes) and mal formed tail (curved, short, no tail fin). However, blood was circulated through a closed set of channels and clear heart beats were measured and ranged between 119-120 beats/ min., as all other groups (Figure 2).

3.3. 72 hours post fertilization

Hatched larvae with quite elongated pectoral fin buds and vigorous heart beats were observed in the control group and those treated with 0.01, 0.03, 0.1 and 0.3 μM of NP. Also, it was shown that the yolk sac started to be shrunk making the pericardial cavity more conspicuous. For the embryos treated with 1 and 3 μM, severe oedemas all around the body accompanied by growth retardation and curved notochord were shown. The embryos of these treatments still looked like those that were 48 h old and approximately no hatching was recorded in these groups except 20% of those of the 1 μM treated group. Focusing on the 3 μM treated group, malformed-coiled tail, necrosis in brain and other body tissues, blood clotting in yolk sac and reduction in heart beats number (80 beats/min.) were also detected (Figure 3).

3. Discussion

Chronic and acute toxicities of NP on aquatic organism have been recently reviewed by Staples *et al*. (2004). The degree of toxicity of NP varies according to the dose and exposure period. Additionally, the nature of its effects on the zebrafish embryo differs according to the embryonic/larval phase. According to Kammann *et al*. (2009), acute toxicity test with zebrafish embryo can only be a first step for the assessment of the environmental risk of NPs. In the present study, NP caused abnormal development at nominal concentration of 1 μM at the beginning of the test, reached to severe oedemas after 72 hours of exposure, whereas higher concentrations led to full development arrest and mortality. The action of lethality varied according to the concentration, meaning that, for the highest nominal concentration of 10 μM, the experiment was terminated at 24 h of examination, whereas for 3 μM the beginning of lethality was shown at 72 h with a delayed-hatched larvae. This explained the acute immediate toxicity of the first concentration and the non-lethal action (endpoints are inhibition of the embryonal development and oedemas) of the second one during the first 72 hpf. This study indicated that the developmental effect of NP is dose dependent with a LC_{50} value of 1 μM. Recently, Kammann *et al*. (2009) demonstrated that NP caused lethal as well as non-lethal malformation during zebrafish embryo development, presenting EC_{50} for lethal endpoints of 6.7 mg/L, after 48 hours of exposure.

The recorded abnormalities, lethal and non-lethal malformations occurred at different concentrations levels may be due to the ability of NP to be metabolized in the fish causing numerous direct and indirect effects ranging from changes in gene expression (Arukwe *et al*., 2002; Larkin *et al*., 2003) through induction of estrogen responsive genes (Andreassen *et al*., 2005) and protein (Arukwe *et al*., 2001). Kazeto *et al*. (2004) demonstrated that exposure of zebrafish juveniles of 17dpf to 0.01-1 μM NP, strongly enhanced the expression of CYP19A2 gene in dose-dependent manner. Effect of NP on brain muscarinic receptors (Jones *et al*., 1998) of increase apoptosis (Weber *et al*., 2002), expression of acute phase protein (Baldwinm *et al*., 2005) and changes in phase II electrophilic detoxication (Hughes and Gallagher, 2004) was also reported.

The present study confirms the action of NP as a toxic compound causing internal and morphological malformation as well as mortality in zebrafish at dose rates approximately equal to the LC_{50} (1 μM) at 72 hour post fertilized larvae level. The range of responses of NP shows that exceeding a thresholds concentration of 0.3 μM would put the embryos in risk.

This study could be considered a report for the non-estrogenic action of NP on zebrafish embryos. The next step for further investigations must be applied on post-hatched larvae and adults of zebrafish, thus to provide valuable information on NP toxicity on such phases and also metabolites of NP should be tested.

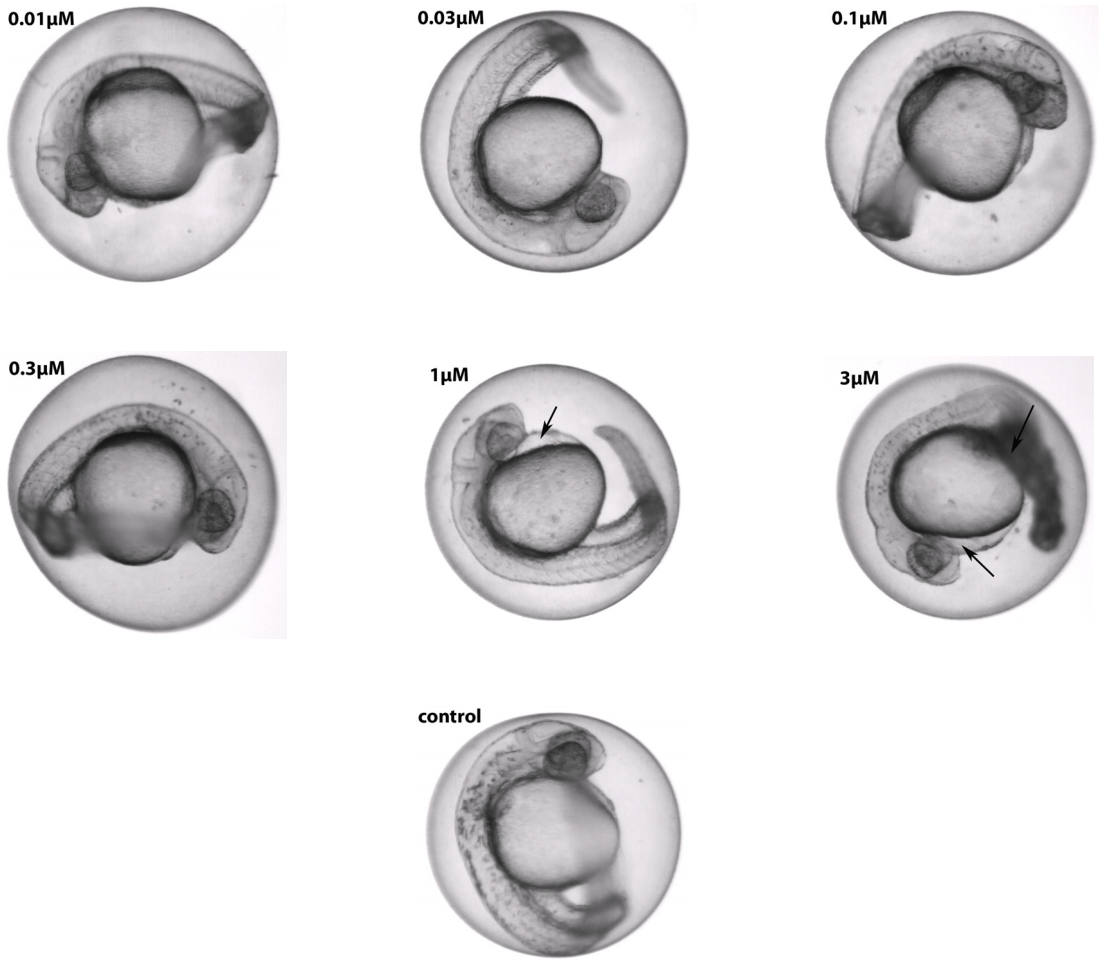

Figure 1. Morphological changes in zebrafish embryos exposed to different concentrations of NP and were photographed live in lateral orientation through a stereomicroscope at 24 h post fertilization (hpf). Embryos exposed to concentrations of 0.01, 0.03, 0.1, 0.3 µM, showing well developed embryo with yolk sac, tail, head, eyes and pigmentation similar to the control group embryos. Embryos exposed to 1 µM showing yolk sac oedema (arrow). Embryos exposed to 3 µM, showing extended malformed yolk sac accompanied with oedema (×4).

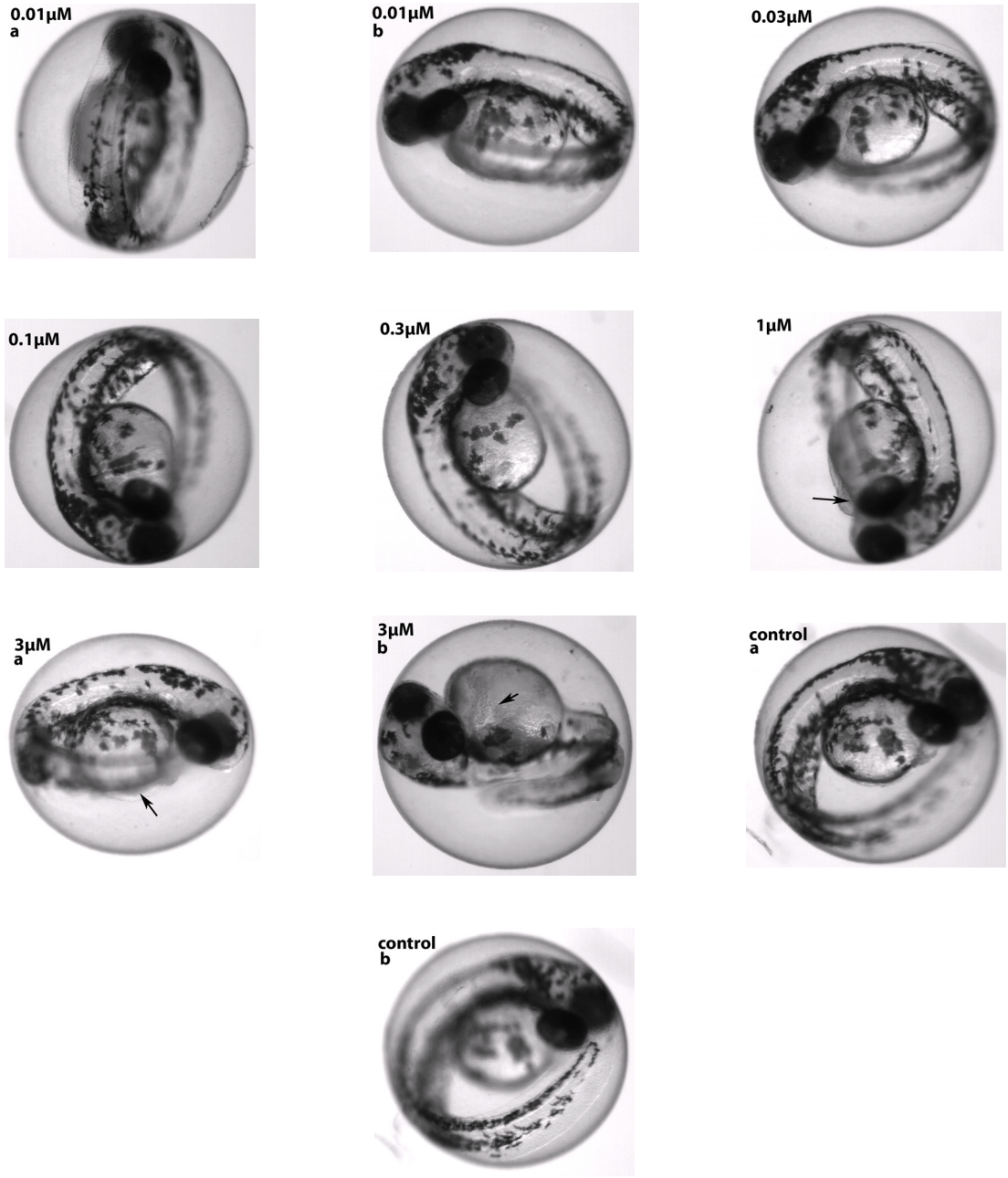

Figure 2: Morphological changes in zebrafish embryos exposed to different concentrations of NP and were photographed live through a stereomicroscope at 48 h post fertilization (hpf). Embryos exposed to concentrations of 0.01, 0.03, 0.1, 0.3 µM, showing embryos with well developed notochord with muscles, otolith, caudal fin, head, eyes and pigmentation similar to the control group embryos. 1µM group, showing bigger oedema and a slightly unstraight notochord. 3 µM group, (a) line oedema around yolk sac (arrow), growth retardation (small head and eyes), (b) mal formed tail (curved, short, no tail fin), blood clotting around yolk sac (arrow, visualized as tissue discoloration), simple scoliosis was shown (×4).

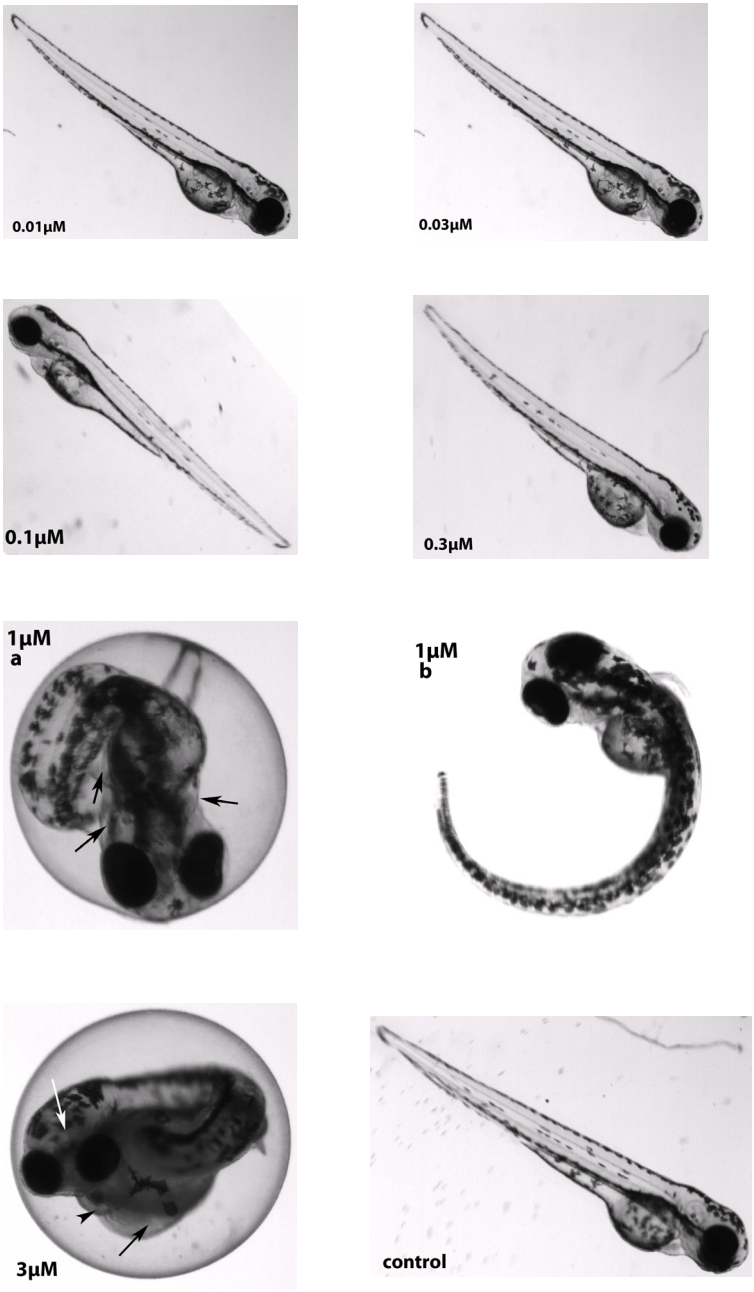

Figure 3: Morphological changes in zebrafish embryos exposed to different concentrations of NP and were photographed live through a stereomicroscope at 72 h post fertilization (hpf). Embryos exposed to concentrations of 0.01, 0.03, 0.1, 0.3 µM, showing well developed hatched larvae similar to the control group larvae (×4). 1µM trated group, showing delayed growth "un hatched" with oedema all around the body "eye, pericardial and yolk sac oedema" (a, arrows, ×4), or hatched with a curved notochord (b, ×2). 3 µM group, necrosis in body tissue and brain (white arrow, detected by condensed spots of pigments), oedema with blood clotting in yolk (arrow) and heart (head arrow), coiled tail, but the heart still beating. (×4).

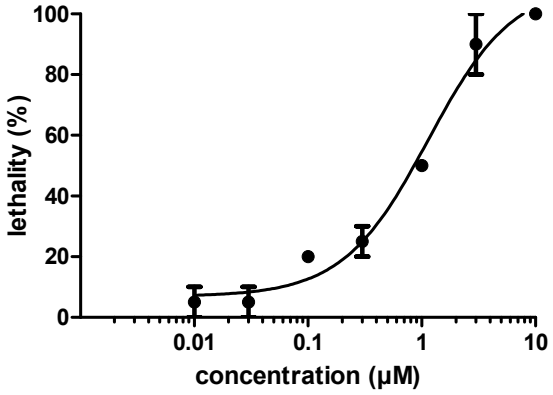

Figure 4: Dose-effect curve of lethal malformation (% relative to control) of zebrafish embryos caused by different concentrations of NP.

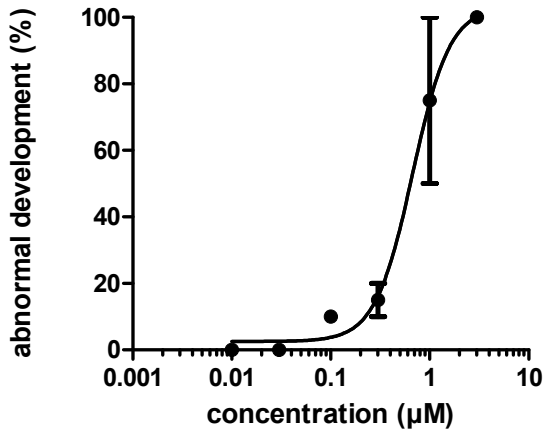

Figure 5: Abnormal development (% relative to control) of zebrafish embryos caused by different concentrations of NP at 72 hour post fertilization.

Acknowledgments

The author would like to express thanks to Professor Juliette Legler, Institute of Environmental Studies, Vrije University, Amsterdam, The Netherlands for giving the opportunity to realize this work by using the institute facilities.

References

Andreassen, T.K.; Skjoedt, K. and Korsgaard, B.: 2005, Upregulation of estrogen receptor alpha and vitellogenin in eelpout (*Zoarces viviparus*) by waterborne exposure to 4-tert-octylphenol and 17beta-estradiol. *Comparative Biochemistry and Physiology* C, 140: 340-346.

Arukwe, A.; Kullman, S.W. and Hinton, D.E.: 2001, Differential biomarker gene and protein expressions in nonylphenol and estradiol-17beta treated juvenile rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology* C, 129: 1-10.

Arukwe, A.; Kullman, S.W.; Berg, K.; Goksoyr, A.; Hinton, D.E.: 2002, Molecular cloning of rainbow trout (*Oncorhynchus mykiss*) eggshell zona radiata protein complementary DNA: mRNA expression in 17beta-estradioland nonylphenol-treated fish. *Comparative Biochemistry and Physiology* B, 132: 315-326.

Balch, G. and Metcalfe, C.: 2006, Developmental effects in Japanese medaka (*Oryzias latipes*) exposed to nonylphenol ethoxylates and their degradation products. *Chemosphere,* 62: 1214-1223.

Baldwinm, W.S.; Roling, J.A.; Peterson, S. and Chapman, L.M.: 2005, Effects of nonylphenol on hepatic testosterone metabolism and the expression of acute phase proteins in winter flounder (*Pleuronectes americanus*): comparison to the effects of Saint John's Wort. *Comparative Biochemistry and Physiology* C, 140: 87-96.

Barber, L.B.; Lee, K.E.; Swackhamer, D.L.; Heiko, L. and Schoenfuss, H.L.: 2007, Reproductive responses of male fathead minnows exposed to wastewater treatment plant effluent, effluent treated with XAD8 resin, and an environmentally relevant mixture of alkylphenol compounds. *Aquatic Toxicology,* 82: 36-46.

Barse, A.V.; Chakrabarti, T.; Ghosh, T.K.; Pal, A.K. and Jadhao, S.B.: 2006, One-tenth dose of LC50 of 4-*tert*-butylphenol causes endocrine disruption and metabolic changes in *Cyprinus carpio*. *Pesticide, Biochemistry and Physiology*, 86: 172-179.

Brand, M.; Granato, M.; Nüsslein-Volhard, C.: 2002, 'Keeping and raising zebrafish', in: C. Nüsslein-Volhard and R. Dahm (eds.), Zebrafish: A Practical Approach. IRL Press, Oxford, pp. 261.

Braunbeck, T.; Böttcher, M.; Hollert, H.; Kosmehl, T.; Lammer, E.; Leist, E.; Rudolf, M. and Seitz, N.: 2005, Towards an alternative for the acute fish LC50 test in chemical assessment: the fish embryo toxicity test goes multi-species-an update. *Altex-Altern Tierexp,* 22: 87-102.

Holth, T.F.; Nourizadeh-Lillabadi, R.; Blaesbjerg, M.; Grung, M.; Holbech, H.; Petersen, G.I.; Aleström, P. and Hylland, K.: 2008, Differential gene expression and biomarkers in zebrafish (*Danio rerio*) following exposure to produced water components. *Aquatic Toxicology,* 90(4): 277-291.

Hughes, E.M. and Gallagher, E.P.: 2004, Effects of 17-beta estradiol and 4-nonylphenol on phase II electrophilic detoxification pathways in largemouth bass (*Micropterus salmoides*) liver. *Comparative Biochemistry and Physiology* C, 137: 237-247.

Isobe. T. and Takada, H.: 2004, Determination of degradation products of alkylphenol

polyethoxylates in municipal wastewaters and rivers in Tokyo, Japan. *Environmental Toxicology and Chemistry,* 23: 599-605.

Jones, S.B.; King, L.B.; Sappington, L.C.; Dwyer, F.J.; Ellersieck, M. and Buckler, D.R.: 1998, Effects of carbaryl, permethrin, 4-nonylphenol, and copper on muscarinic cholinergic receptors in brain of surrogate and listed fish species. *Comparative Biochemistry and Physiology* C, 120: 405-414.

Kammann, U., Vobach, M. and Wosniok, W., 2006, Toxic effects of brominated indoles and phenols to zebrafish embryos' *Archives of Environmental Contamination and Toxicology,* 51(1): 97-102.

Kammann, U.; Vobach, M.; Wosniok, W.; Schäffer, A., Telscher, A.: 2009, Acute toxicity of 353-nonylphenol and its metabolites for zebrafish embryos. *Environmental Science and Pollution Research*, 16: 227-231.

Kazeto Y.; Place, A.R. and Trant, J.M.: 2004, Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (*Danio rerio*) juveniles. *Aquatic Toxicology,* 69: 25-34.

Kelly, S.A. and Di Giulio, R.T.: 2000, Developmental toxicity of estrogenic alkylphenols in killifish (*Fundulus heteroclitus*). *Environmental Toxicology and Chemistry,* 19: 2564-2570.

Kosmehl, T.; Hallare, A.V.; Reifferscheid, G.; Manz, W.; Braunbeck, T. and Hollert, H.: 2006, A novel contact assay for testing genotoxicity of chemicals and whole sediments in zebrafish embryos. *Environmental Toxicology and Chemistry,* 25: 2097–2106.

Larkin, P.; Knoebl, I. and Denslow, N.D.: 2003, Differential gene expression analysis in fish exposed to endocrine disrupting compounds. *Comparative Biochemistry and Physiology* B, 136: 149-161.

Lee, H.; Peart, T.E.; Chan, J. and Gris, G.: 2004, Occurrence of endocrine disrupting chemicals in sewage and sludge samples in Toronto, Canada. *Water Quality Research Journal of Canada,* 39: 57-63.

Nagel, R.: 2002, DarT: the embryo test with the zebrafish *Danio rerio*-a general model in ecotoxicology and toxicology. *Altex-Altern Tierexp*, 19 (Suppl 1): 38–48.

Scholz, S.; Fischer, S.; Gündel, U.; Küster, E.; Luckenbach, T. and Voelker, D.: 2008, The zebrafish embryo model in environmental risk assessment—applications beyond acute toxicity testing. *Environmental Science and Pollution Research*, 15: 394-404.

Stachel, B.; Jantzen, E.; Knoth, W.; Kruger, F.; Lepom, P.; Oetken, M.; Reincke, H.; Sawal, G.; Schwartz, R. and Uhlig, S.: 2005, The Elbe flood in August 2002—organic contaminants in sediment samples taken after the flood event. *Journal of Environmental Science and Health* A, 40: 265-287.

Staples, C.; Mihaich, E.; Carbone, J.; Woodbrun, K. and Klecka, G.: 2004, A weight of evidence analysis of the chronic ecotoxicity of nonylphenol ethoxylates, nonylphenol ether carboxylates, and nonylphenol. *Human and Ecological Risk Assessment,* 10: 999-1017.

Sumpter, J. P. and Jobling, S.: 1995, Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environmental Health Perspectives,* 103: 173-178.

Uguz, C.; Iscan, M.; Erguven, A.; Isgor, B. and Togan, I., 2003, The bioaccumulation of nonyphenol and its adverse effect on the liver of rainbowtrout (*Onchorynchus mykiss*). *Environmental Research*, 92: 262-270.

Vetillard, A. and Bailhache, T.: 2006, Effects of 4-*n*-Nonylphenol and Tamoxifen on Salmon Gonadotropin-Releasing Hormone, Estrogen Receptor, and Vitellogenin Gene Expression in Juvenile Rainbow Trout. *Toxicological Sciences,* 92(2): 537-544.

Weber, L.P.; Kiparissis, Y.; Hwang, G.S.; Niimi, A.J. and Jantz, D.M.: 2002, Increased cellular apoptosis after chronic aqueous exposure to nonylphenol and quercetin in adult medaka (*Oryzias latipes*). *Comparative Biochemistry and* Physiology, 131: 51-59.

Westerfield, M.; Doerry, E.; Kirkpatrick, A. E.; Driever, W. and Douglas, S. A.: 1997, An on-line database for zebrafish development and genetics research. *Semin Cell Developmental Biol*ogy, 8: 477-488.

Yadetie, F. and Male, R.: 2002, Effects of 4-nonylphenol on gene expression of pituitary hormones in juvenile Atlantic salmon (*Salmo salar*). *Aquatic Toxicology*, 58: 113-129.

Yang, F.X.; Xu, Y. and Hui, Y.: 2006, Reproductive effects of prenatal exposure to nonylphenol on zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology*, 142: 77-84.

التاثيرات السامة لمركب النانيل فينول علي نمو أجنة أسماك الزيبرا

تامر السيد على

قسم علوم البحار ـ جامعة الإسكندرية

يعتبر النانيل فينول مركب عضوى أيضي وسطي ينتج عن التحولات الميكروبية لمركبات الأيثوكسلات والتى تدخل فى العديد من الصناعات وخاصة المنظفات الصناعية , لذا فهو يتواجد في البيئات المائية و يعتبر أحد الملوثات الخطرة على البيئة. لقد ركزت هذة الدراسة علي دراسة تاثيرات مركب النانيل فينول علي نمو أجنة أسماك الزيبرا. ولذا فقد تم تعريض بيض هذا النوع من الأسماك بعد ساعه واحده من تلقيحه الى تركيزات مختلفة ومتدرجة لمركب النانيل فينول لمدة 72 ساعة لمعرفة مدى تأثيره على نمو و معدلات الحياه لأجنه هذ النوع من الأسماك. ولقد أوضحت الدراسة أن إستجابة هذة الأجنة يتدرج مع درجة تركيزات الجرعة المستخدمة. كما أوضحت هذة الدراسة أيضا أن التعرض المبكر لهذا النوع من المركبات لة تاثير مباشر علي موت هذة الأجنة و قد يسبب تشوهات عند تعرضها لجرعات اكبر من μ0,3 مول بينما الجرعات التي تسبب تشوهات ل 50% من الأجنة هي μ0,8 مول بعد 72 ساعة من التعرض.