The Journal of International Advanced Otology

Int Adv Otol 2014; 10(1): 76-9 • DOI:10.5152/iao.2014.017

Original Article

The Effects of Nonylphenol on Hearing in Rats

Fatih Yücedağ, Erdoğan Okur, Cevdet Uğuz, Fahriye Zemheri, Selçuk Kuzu, Abdullah Ayçiçek, Orhan Kemal Kahveci

Department of Otorhinolaryngology, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey (FY, EO, SK, AA, OKK)
Department of Medical Biology and Genetics, Afyon Kocatepe University Faculty of Veterinary Medicine, Afyonkarahisar, Turkey (CU, FZ)

OBJECTIVE: Nonylphenol is a neurotoxic substance widely present in the environment. Although its neurotoxic effects are well-known, to our knowledge, the ototoxic effects of nonylphenol on hearing have not been published in the literature yet. We aimed to investigate the effect of nonylphenol on hearing function in rats.

MATERIALS and METHODS: Fifty rats were randomly divided into five Groups each containing 10 animals. Group 1 was a control Group and Group 2 was a solvent control containing ethanol alone, whereas Groups 3, 4, and 5 were treatment Groups exposed to the different concentrations of nonylphenol dissolved in ethanol for six- weeks. Distortion product otoacoustic emission measurements were evaluated at the end of exposure.

RESULTS: In the distortion product otoacoustic emission measurement, signal-to-noise ratio values did not show any statistically significant differences between the control and ethanol Groups ($p>0.05$). But, we found significant differences between signal-to-noise ratio values of control and nonylphenol Groups at 4000 and 6000 Hz frequencies ($p<0.05$). Also, we found statistically significant difference between signal-to-noise ratio values of ethanol and nonylphenol Groups at 4000 and 6000 Hz frequencies ($p<0.05$). There was no statistically significant difference for signal-to-noise ratio values among nonylphenol Groups (Groups 3-5) ($p>0.05$).

CONCLUSION: Our study showed that nonylphenol has negative effects on hearing function in rats but the effects do not seem to be dose-dependent. Further studies are needed to find whether nonylphenol has an effect on hearing loss in rats as well as hearing in human beings.

KEY WORDS: Nonylphenol, hearing loss, ototoxicity, otoacoustic emission

INTRODUCTION

Alkyphenol polyethoxylates (APEs), called estrogenic environmental disrupters, are widely used in detergents, herbicides and pesticides, paints, cosmetics, plastic materials as non-ionic surfactants, or as antioxidants [1]. The sum of the world's annual production of APEs is more than 500,000 metric tons, and it has been shown that more than 60% of this amount accumulated in water masses, including streams, rivers, lakes, and seas. The APEs in water undergo degradation process to give short-side-chain derivatives of APEs, such as nonylphenol (NP), octylphenol (OP), and butylphenol (BP). These derivatives are called alkylphenols (APs), which have estrogenic features and more to degradation than APEs [1-3].

Humans are mainly exposed to APEs and their derivatives, such as NP-consuming fishery products and ingesting contaminated drinking water. Human exposure to NP may also occur either through the skin during the usage of shampoos, detergents, and cosmetics, through the mucosal membrane during the usage of spermicidal lubricants for birth control, or through inhalation during airborne pesticide application for the eradication of insects. NP is the most widely used AP, and many estrogenic, toxic, and carcinogenic side effects are seen [4, 5].

The aim of this study is to evaluate whether NP has any adverse effects on hearing among rats and to ensure that necessary measures are taken for people living in industrial areas, particularly for those working for the production of these substances.

MATERIALS and METHODS

Fifty healthy Sprague-Dawley rats with an average weight of 250 grams (200-300 grams) were employed in the study. The study was approved by Afyon Kocatepe University Local Ethics Committee for Animal Experiments (B.30.2.AKU.0.9Z.00.00/68). Rats were accommodated in an environment where the noise level is less than 50 dB and exposed to 12 hours of light and dark cycles at a temperature of 25°C, where the rats could reach water and food freely. During the study, attention was paid that the environment was kept quiet. The experimental design was outlined in Table 1. Briefly, 50 rats were divided into five Groups containing 10 animals in each Group. Group 1 was control, and no chemical materials are applied on this Group; Group 2 was solvent control containing eth-

This study was presented as a poster presentation at the 34th Turkish National Otorhinolaryngology and Head - Neck Surgery Congress, 12 October 2012, Antalya, Turkey.

Corresponding Address:
Fatih Yücedağ, Clinic of Otolaryngology ANS Training and Practice Hospital Afyon Kocatepe University Faculty of Medicine, 03200, Afyonkarahisar, Turkey
Phone: +90 506 466 03 82; Fax: +90 272 246 33 22; E-mail: drfatihyucedag@gmail.com
Submitted: 05.10.2013 **Revision Received:** 29.12.2013 **Accepted:** 05.01.2014

anol alone; and Groups 3, 4, and 5 were treatment Groups exposed to the different concentrations of NP in ethanol. Both ears of rats in all Groups (total of 20 ears) were assessed in this study. Standard rat food and water were given to all Groups. Rats in Groups 2, 3, 4, and 5 were exposed to chemicals for six weeks. Ethanol alone was given to Group 2, and NP dissolved in ethanol in different concentrations was given to Groups 3, 4, and 5. Application of ethanol alone or NP dissolved in ethanol was pulverized into the cages containing rats. Following pulverization, cages were covered with cloth, and rats were allowed to inhale pulverized ethanol alone or ethanol containing different concentrations of NP for 30 minutes. Pulverization was performed once a day, and rats were allowed to breathe aerosolized particles for 30 minutes. The amount of ethanol pulverized in cages in both ethanol alone and ethanol containing different concentrations of NP was 1 mL for each rat, which means that 10 animals received a 10-mL aerosolized solution by pulverization. Pulverization was performed once daily. As mentioned above, different concentrations of NP dissolved in ethanol were prepared as 0.1 µg/mL, 10 µg/mL, and 1000 µg/mL and given to Groups 3, 4, and 5, respectively. Distortion product otoacoustic emission (DPOAE) measurements were obtained from rats at the end of the sixth week of exposure by anesthetizing them with intramuscular ketamine hydrochloride (Ketalar; Pfiser Ltd., Vienna, Austria) 45 mg/kg and xylazine (Rompun; Bayer Ltd., Leverkusen, Germany) 5 mg/kg injection.

In this study, DPOAE was used to evaluate the emissions. Distortion product emissions were measured by the "ILO 288 Echoport USB" and "EZ-screen 2" (Otodynamics Ltd Clinical OAE System, USA) software device. Measurements were made with the smallest size of the tympanometry rubber probe attached to the device. DPOAE (2f1-f2 cubic distortion product components) was measured in General Diagnostic mode. The ratio between the frequencies f2 and f1 (f2/f1) was found to be 1:22. Stimulus intensity was taken to the frequency of f1 as L1 and to the frequency of f2 as L2; the difference between the levels of L1-L2 was kept as 10 dB SPL (L1=65 dB SPL, L2=55dB SPL). DPOAEs were measured with a microphone in the external ear canal at 2f1-f2 frequency and the signal-to-noise ratio (SNR) at 1001, 1501, 2002, 3003, 4004, 6006, and 7996 Hz frequency bands of the geometric mean of f1 and f2 was based on. To assess the DPOAE responses, SNR is more reliable when compared to DPOAE amplitudes [6]. We carried out the evaluation process, based on SNR at 1000-8000 Hz frequencies.

Statistical Analysis

SPSS (Statistical Program for Social Sciences) statistical software SPSS for Windows version 17.0, Inc. Chicago, IL, USA) was used. One-sample Kolmogrov-Simirnov test was used to determine normal distribution data concordance. Mann-Whitney U test was used to analyze normal distribution of discordant data; independent sample t-test was used to analyze normal distribution of concordant data. Measured data were expressed as the mean±standard error (SE). $p<0.05$ was considered significant.

RESULTS

Results are shown in Table 2 and Figure 1. In the DPOAE measurement, SNR values did not show any statistically significant difference between the control and ethanol Group ($p>0.05$). SNR values at fre-

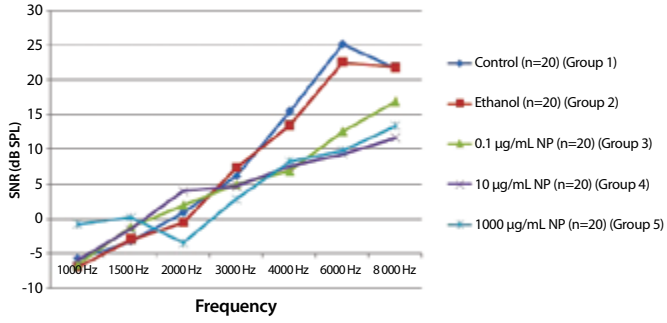

Figure 1. Comparison of mean SNR values between control Group and other Groups

NP: nonylpheno; SNR: signal to noise ratio; dB: decibel; Hz: hertz

Table 1. Characteristics of the study Groups

Groups	N	Drug dosages	Applications
Group 1	10 rats	Control Group	No chemical materials.
Group 2	10 rats	Solvent Control (Ethanol)	1 mL/day ethanol in aerosol form for 6 weeks.
Group 3	10 rats	0.1 µg/mL NP Group	1 mL, 0.1 µg/mL/day NP in aerosol form for 6 weeks.
Group 4	10 rats	10 µg/mL NP Group	1 mL, 10 µg/mL/day NP in aerosol form for 6 weeks.
Group 5	10 rats	1000 µg/mL NP Group	1 mL, 1000 µg/mL/day NP in aerosol form for 6 weeks.

NP: nonylphenol

Table 2. Mean±Standart Error (SE) SNR Values

	1000 Hz	1500 Hz	2000 Hz	3000 Hz	4000 Hz	6000 Hz	8000 Hz
Control Group	-5.77±0.89	-3.24±1.57	0.88±1.68	6.01±2.52	15.42±1.99	25.12±3.16	21.76±4.44
Solvent Control (Ethanol)	-6.94±1.53	-2.97±1.84	-0.56±2.67	7.35±2.27	13.41±2.05	22.50±3.50	21.78±4.06
0.1 µg/mL NP Group	-6.59±1.37	-1.23±1.39	1.97±1.50	4.97±2.15	6.90±2.20	12.58±4.15	16.91±4.86
10 µg/mL NP Group	-5.99±2.23	-1.53±3.65	3.95±3.45	4.65±2.59	7.50±2.88	9.30±4.50	11.61±4.09
1000 µg/mL NP Group	-0.74±1.85	0.23±2.21	-3.43±2.45	2.83±2.01	8.30±2.33	9.89±4.16	13.37±4.37

NP: nonylphenol; Hz: Hertz; SE: standart error; SNR: signal-to-noise ratio

quencies of 1000, 1500, 2000, 3000, and 8000 Hz were not statistically significant between control and NP Groups ($p>0.05$). However, SNR values at 4000 and 6000 Hz were statistically significant in control and NP Groups ($p<0.05$). Also, we found statistically significant differences between SNR values in the ethanol and NP Groups at 4000 and 6000 Hz ($p<0.05$). There was no statistically significant difference for SNR values among NP Groups (Group 3, 4, 5) ($p>0.05$).

DISCUSSION

Nonylphenol has been shown to be widely present in the environment as well as daily consumable goods and to have estrogenic, toxic, and carcinogenic effects on breast [7-8] and on testis or ovary [9]. In this study, we sought to determine whether NP has ototoxic effects on rats. It is well known that NP accumulates in aquatic organisms. Through the food chain, it can reach humans directly by consuming fishery products or indirectly using fish flour as animal feed [10]. The presence of NP is 32 ng/mL in breast milk of mothers consuming fish three times a week [11].

Arges et al. [12] have reported that alkylphenol has toxic effects to cell membranes in animals, plants, and micro-organisms due to the hydrophobic alkyl residuum. It is also reported that alkylphenol disrupts the energy production in mitochondrial membranes [12]. Uguz et al. [13] reported that NP has an adverse effect on mitochondrial membrane potential. Furthermore, Karadeniz et al. [14] have shown that NP causes oxidation of guanine base. This may suggest that NP can cause mutations in mitochondrial DNA (mtDNA), which lacks DNA repair mechanisms. It has been reported that mtDNA mutations can cause hearing loss in both syndromic and non-syndromic conditions. In our study, ototoxicity has occurred in rats exposed to NP. Further studies on a molecular level are needed to determine whether the ototoxicity caused by NP is associated with pathological disorders of mtDNA or mitochondrial membrane.

Hughes et al. [15] have proven that alkylphenol causes testicular cell death due to inhibition of endoplasmic reticulum Ca^{2+} pumps. Also, it has been shown that alkylphenols containing NP cause cell death in skeletal muscle due to inhibition of sarcoplasmic reticulum Ca^{2+} pumps by similar mechanisms. Michelangeli et al. [16] and Bragadin et al. [17] have reported that NP reduces ATP synthesis and increases mitochondrial membrane permeability of potassium. Ototoxicity caused by NP, as in our study, may occur as a result of apoptosis due to mitochondria-mediated cell death. Indeed, it has been shown that NP triggers mitochondria mediates cell death 'apoptosis' in liver cells via cytochrome c, caspase 9, caspase 3, bcl-2, and bax [18]. NP may possibly have similar effects on brain cells, since NP is a lipophilic substance and brain is one of the most important lipoid organs in the body. Furthermore, whole organs in the nervous system, including brain, cerebellum, and spinal cord, share common lipoid characteristics. Therefore, NP may pose potential neurotoxic effects on the whole nervous system. Zhang et al. [19] reported that inducible nitric oxide synthase and cyclooxygenase-2 enzymes, which cause inflammation, are increased in mice brains due to chronic NP exposure. They stated that this situation may be associated with chronic inflammation and neurotoxicity [19]. Uncontrolled chronic inflammation of the central nervous system can lead to neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases [20-22].

The purpose of aerosol exposure of NP to rats was to demonstrate that the aerosolized NP exposure may cause more damage in the tissues, especially in the brain, than that taken orally. This may be due to the degradation of NP in the liver when it is taken orally. In contrast to oral exposure, NP may not go through a degradation process directly when it is taken through breathing and may directly go into the bloodstream and thus go into the tissues and exert its adverse effects in the tissues. The only question was that some aerosolized NP-containing solution fell onto the ground of the cages and/or on to the skin of rats. Thus, rats could be exposed orally to a certain degree by licking the cages or the skin of themselves or each other. However, we believe that this could be negligible. We believe that most exposures occur through inhalation, although some rats inhale more than others. However, there was no large variation in terms of SNR values between rats in the same treatments.

In the study, ethanol was used as the solvent and NP was dissolved in ethanol. To determine the effect of ethanol on DPOAE, if any, only ethanol was given to Group 2. We found that there were no statistically significant differences between DPOAE values in control and ethanol Groups. This shows that ethanol may not have any negative effects on hearing.

In the present study, there was a statistically significant difference between SNR values of the control and NP Groups, especially at 4000 and 6000 Hz frequencies. This ototoxic effect on rats could be attributed to NP exposure. This effect could occur because of the destructive effect of NP on the structure of outer hair cells in the cochlea or diminishing the production of ATP in cells due to the effect of NP. Similarly, we also found a statistically significant difference between SNR values of the ethanol and NP Groups, especially at 4000 and 6000 Hz frequencies. Therefore, we believe that the ototoxic effect in rats could be due to the NP exposure. On the other hand, SNR values at frequencies of 1000, 1500, 2000, 3000, and 8000 Hz were not statistically significant in any Group ($p>0.05$). These findings suggest that low frequencies may be particularly more resistive to nonylphenol exposure and that the ototoxic effect of NP is more evident at frequencies of 4000 Hz and 6000 Hz. There was no significant difference for SNR values among NP Groups in which NP was administered as 0.1 µg/mL, 10 µg mL, and 1000 µg/mL, respectively. These findings suggest that the ototoxic effect of NP may not be dose-dependent. However, we only administered certain concentrations of NP to rats, which is the limitation of this study. Further studies should be performed in which different doses of NP are used to reveal if there is any dose-related effect of NP.

In conclusion, we found that NP has adverse effects on hearing in rats. People who live in industrial areas, especially workers employed in production of these substances, could be at greater risk for loss of hearing. Further studies need to be conducted if NP has an effect on hearing loss among in rats as well as human beings.

Ethics Committe Approval: Ethics committee approval was received for this study from the Local ethics committee of for Animal Experiments of Afyon Kocatepe University (Date:03.08.2011.30.2.AKU.0.9Z.00.00/68).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - F.Y., C.U., E.O.; Design - C.U., F.Y., O.K.K.; Supervision -O.K.K., A.A.; Funding - C.U., A.A.; Materials - F.Y., E.O., C.U.; Data Collection and/or Processing - C.U., F.Z., F.Y.; Analysis and/or Interpretation - E.O., S.K., F.Y.; Literature Review - F.Y., S.K.; Writing - E.O., C.U., S.K.; Critical Review - E.O., C.U.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Nimrod AC, Benson WH. Environmental estrogenic effects of alkylphenol ethoxylates. Crit Rev Toxicol 1996; 26: 335-64. [CrossRef]
2. Ahel M, Giger W, Koch M. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-1. Occurrence and transformation in sewage treatment. Water Res 1994;28:1131-42. [CrossRef]
3. Hawrelak M, Bennett E, Metcalfe C. The environmental fate of the primary degradation products of alkylphenol ethoxylate surfactants in recycled paper sludge. Chemosphere 1999; 39: 745-52. [CrossRef]
4. Uguz C, Iscan M, Ergüven A, Isgor B, Togan I. The bioaccumulation of nonyphenol and its adverse effect on the liver of rainbow trout (Onchorynchus mykiss). Environ Res 2003; 92: 262-70. [CrossRef]
5. Cakmak G, Togan I, Uğuz C, Severcan F. FT-IR spectroscopic analysis of rainbow trout liver exposed to nonylphenol. Appl Spectrosc 2003; 57: 835-41. [CrossRef]
6. Lamm K, Lamm H, Arnold W. Effect of hyperbaric oxygen therapy in comparison to conventional or placebo therapy or no treatment in idiopathic sudden hearing loss, acoustic trauma, noise-induced hearing loss and tinnitus. A literature survey. Adv Otorhinolaryngol 1998; 54: 86-99. [CrossRef]
7. Blom A, Ekman E, Johannisson A, Norrgren L, Pesonen M. Effects of xenoestrogenic environmental pollutants on the proliferation of a human breast cancer cell line (MCF-7). Arch Environ Contam Toxicol 1998; 34: 306-10. [CrossRef]
8. Legler J, van den Brink CE, Brouwer A, Murk AJ, van der Saag PT, Vethaak AD, et al. Development of a stably transfected estrogen receptor-mediated luciferase reporter gene assay in the human T47D breast cancer cell line. Toxicol Sci 1999; 48: 55-66. [CrossRef]
9. Skakkebaek NE, Rajpert-De Meyts E, Jørgensen N, Carlsen E, Petersen PM, Giwercman A, et al. Germ cell cancer and disorders of spermatogenesis: an environmental connection? APMIS 1998; 06: 3-11. [CrossRef]
10. Muncke J. Exposure to endocrine disrupting compounds via the food chain: Is packaging a relevant source? Sci Total Environ 2009; 407: 4549-59. [CrossRef]
11. Ademollo N, Ferrara F, Delise M, Fabietti F, Funari E. Nonylphenol and octylphenol in human breast milk. Environ Int 2008: 34: 984-7. [CrossRef]
12. Argese E, Marcomini A, Miana P, Bettiol C, Perin G. Submitochondrial particle response to linear alkylbenzen sulfonates, nonylphenol polyethoxylates and their biodegradation derivatives. Environ Toxicol Chem 1994; 13: 737-42. [CrossRef]
13. Uguz C, Varisli O, Agca C, Agca Y. Effects of nonylphenol on motility and subcellular elements of epididymal rat sperm. Reproductive Toxicology 2009; 28: 542-9. [CrossRef]
14. Karadeniz H, Caliskan A, Uguz C. Electrochemical monitoring of the interaction between 4-nonylphenol and DNA by graphite and carbon nanotube modified graphite electrodes. Anal Sci 2010; 26: 1065-9. [CrossRef]
15. Hughes PJ, McLellan H, Lowes DA, Kahn SZ, Bilmen JG, Tovey SC, et al. Estrogenic alkylphenols induce cell death by inhibiting testis endoplasmic reticulum Ca (2+) pumps. Biochem Biophys Res Commun 2000; 277: 568-74. [CrossRef]
16. Michelangeli F, Orlowski S, Champeil P, East JM, Lee AG. Mechanism of inhibition of the (Ca2(+)-Mg2+)-ATPase by nonylphenol. Biochemistry 1990; 29: 3091-101. [CrossRef]
17. Bragadin M, Perin G, Iero A, Manente S, Rizzoli V, Scutari G. An in vitro study on the toxic effects of nonylphenols (NP) in mitochondria. Chemosphere 1999; 38: 1997-2001. [CrossRef]
18. Jubendradass R, D'Cruz SC, Rani SJ, Mathur PP. Nonylphenol induces apoptosis via mitochondria- and Fas-L-mediated pathways in the liver of adult male rat. Regul Toxicol Pharmacol 2012; 62: 405-11. [CrossRef]
19. Zhang YQ, Mao Z, Zheng YL, Han BP, Chen LT, Li J, et al. Elevation of inducible nitric oxide synthase and cyclooxygenase-2 expression in the mouse brain after chronic nonylphenol exposure. Int J Mol Sci 2008; 9: 1977-88. [CrossRef]
20. Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease. J Neuroimmunol 2007;184: 69-91. [CrossRef]
21. Tansey MG, McCoy MK, Frank-Cannon TC. Neuroinflammatory mechanisms in Parkinson's disease: potential environmental triggers, pathways, and targets for early therapeutic intervention. Exp Neurol 2007; 208: 1-25. [CrossRef]
22. Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O. Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci USA 2003; 100: 13632-7. [CrossRef]