Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Bilici E, Uğuz C, Altunbaş K (2025).** Examining the impact of 4-nonylphenol on G-361 malignant melanoma cell proliferation and apoptosis activation. *Van Vet J*, 36 (2), 110-114.
DOI: https://doi.org/10.36483/vanvetj.1654843

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Examining the Impact of 4-Nonylphenol on G-361 Malignant Melanoma Cell Proliferation and Apoptosis Activation

Esra BİLİCİ[1,*] Cevdet UĞUZ[2] Korhan ALTUNBAŞ[3]

[1] *Uşak University, Eşme Vocational School, Laboratory and Veterinary Health Program, 64600, Uşak, Türkiye*
[2] *Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Medical Biology and Genetics, Afyon, Türkiye*
[3] *Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Histology and Embriyology, Afyon, Türkiye*

Received: 10.03.2025 Accepted: 19.06.2025

ABSTRACT Nonylphenol (NP) is among the most prevalent endocrine-disrupting compounds found in the environment. Although the carcinogenic properties of endocrine disruptors have been well investigated, their possible anticancer effects remain comparatively underexplored. This study investigated the cytotoxic and pro-apoptotic effects of NP on G-361 human malignant melanoma cell line. The major objective of the stuyd was to assess anticancer effects of the endocrine-disrupting compound NP on G-361 melanoma cells. The MTT assay was used to determine cell viability, and quantitative real-time PCR was performed to analyze the expression levels of apoptosis-associated genes, including Caspase-3, Bcl-2, Bax, P53, P21, and β-actin. The results indicated a significant decrease in cell viability following treatment with 100 µg/ml NP. Notably, NP exerted a dose-dependent cytotoxic effect, suggesting its possible role in the modulation of apoptotic pathways in melanoma cells. These results require further investigation of different pathways by which NP exerts its effects to gain a deeper insight into its molecular mechanism.

Keywords: *Melanoma, Nonylphenol, Cell Proliferation, P53.*

ÖZ

G-361 Malignant Melanoma Hücrelerinde 4-Nonilfenolün Hücre Proliferasyonu ve Apoptoz Aktivasyonu Üzerindeki Etkisinin Araştırılması

Nonilfenol (NP), çevrede bulunan en yaygın endokrin bozucu bileşikler arasındadır. Endokrin bozucuların kanserojen özellikleri iyi araştırılmış olsa da olası antikanser etkileri nispeten yeterince araştırılmamıştır. Bu çalışma, NP'nin G-361 insan malign melanom hücre hattı üzerindeki sitotoksik ve proapoptotik etkilerini araştırmıştır. Çalışmanın temel amacı, endokrin bozucu bileşik NP'nin G-361 melanom hücreleri üzerindeki antikanser etkilerini değerlendirmekti. Hücre canlılığını belirlemek için MTT testi kullanıldı ve Caspase-3, Bcl-2, Bax, P53, P21 ve β-actin dahil olmak üzere apoptozla ilişkili genlerin ifade düzeylerini analiz etmek için kantitatif gerçek zamanlı PCR gerçekleştirildi. Sonuçlar, 100 µg/ml NP ile tedaviden sonra hücre canlılığında önemli bir azalma olduğunu gösterdi. Özellikle, NP doz bağımlı bir sitotoksik etki gösterdi ve bu da melanom hücrelerinde apoptotik yolların modülasyonunda olası bir rolü olduğunu düşündürdü. Bu sonuçlar, NP'nin moleküler mekanizmasına daha derin bir bakış açısı kazanmak için etkilerini gösterdiği farklı yolların daha fazla araştırılmasını gerektiriyor.

Anahtar Kelimeler: *Melanoma, Nonilfenol, Hücre Proliferasyonu, P53.*

INTRODUCTION

Melanoma is an aggressive cancer arising from melanocytes, which presents a formidable clinical challenge due to its high metastatic potential and increasing global incidence (Cummins et al. 2016; Galluzzi et al. 2018). A comprehensive understanding of the molecular mechanisms underlying melanoma progression is essential for the formulation of targeted therapeutic interventions (Shi et al. 2024). Nonylphenol (NP) is a commonly used alkylphenol in industrial applications and often found in diverse environmental matrices, including surface water, groundwater, and food items such as meat, milk, and vegetables (Park et al. 2020; Gałązka and Jankiewicz 2022). It has been detected in almost every aquatic matrix present in the environment, including both drinking water and groundwater. Moreover, contaminated products like meat, milk, and vegetables facilitate their entry into the food chain (Cherry et al. 2001). Consequently, there has been a growing body of toxicological research focused on the adverse effects of NP in recent years. The NP exposure has been implicated in hormonal imbalances, induction of apoptosis, and

✉ *Corresponding author: esra.bilici@usak.edu.tr

carcinogenesis (Inkaya et al. 2025). Besides mimicking estrogenic activity, NP can also interfere with cellular processes related to survival and apoptosis, resulting in negative consequences (Phillips & Tanphaichitr, 2008).

Both in vitro and in vivo studies have demonstrated the estrogenic activity of NP in various organs, tissues, and cell types (Wróblewska-Łuczka et al. 2023). The NP exhibits estrogenic activity by binding to estrogen receptors, therefore disrupting endocrine function (Soto et al. 1991; White et al. 1994; de Weert et al. 2008). Studies on melanoma development during pregnancy and the investigation of sex-related differences in melanoma progression have resulted in the idea that estrogens may significantly influence the pathophysiology of melanoma (Behari and Kesari 2006).

Considering the potential carcinogenicity ans possible transgenerational effects of NP, this study assessed its effects on cell proliferation and gene expression. The study specifically investigated the mRNA expression levels of the Caspase-3, Bcl-2, Bax, P53, P21, and β-actin genes. Furthermore, cell viability of G-361 melanoma cells exposed to environmentally relevant concentrations of NP was assessed through MTT assay.

MATERIAL AND METHODS

The study protocol received ethical approval from the Afyon Kocatepe University Animal Experiments Local Ethics Committee (approval number: 49533702/79, dated 01/07/2019).

Cell Culture and Treatment Protocol

The G-361 human melanoma cell line was obtained from ATCC (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were cultured at 37°C in a humidified incubator with 5% CO2. NP was first solubilized in DMSO to provide a 1 M stock solution, which was then diluted to the required concentrations in the culture medium.

MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] Assay for Cell Viability

The MTT assay was used to determine cell viability and the half-maximal inhibitory concentration (IC50) of NP in G-361 cells following manufacturer's instructions. The G-361 cells were plated in 96-well plates at a density of 3×10^4 cells per well and incubated for 48 hours. Cells were then treated with NP at doses between 0.1 and 100 µg/ml for 24 hours. Following treatment, the MTT reagent was added, and absorbance was quantified at 570 nm using a spectrophotometer. Cell viability was expressed as a percentage relative to untreated control cells.

RNA Isolation Quantitative Real-Time PCR

Total RNA was isolated from NP-treated G-361 cells using a commercial isolation kit (Abt, 102-01-10), followed by on-column DNase treatment. Complementary DNA (cDNA) was produced with Sybr Green PCR Master Mix (Abm, 204454) following the manufacturer's instructions. Primer sequences (Table.1) were constructed with the Primer3 Plus program. Quantitative PCR was performed with 1 pM of each primer, using GAPDH as the internal control. The thermal cycling protocol included an initial denaturation and polymerase activation at 94°C for 1 minute, followed by 40 cycles of 94°C for 10 seconds, 57°C for 10 seconds, and 72°C for 20 seconds.

Table 1: Primers used in the study.

PRİMER	F	R
CDNK1A	CCGTGAGCGATGGAACTTCGC	TGGGAAGGTAGAGCTTGGGCA
Bax	CGCCTCACTCACCATCTGGAA	CCTCAAGACCACTCTTCCCCA
Bcl-2	GAGGGGCTACGAGTGGGATGC	GGAGGAGAAGATGCCCGGTGC
Casp3	GGAAGCGAATCAATGGACTCTGGA	CCTGAGGTTTGCTGCATCGAC
P53	CTACAGTACTCCCCTGCCCT	ACCATCGCTATCTGAGCAGC
ß-actin	CACCCCAGCCATGTACGTTGC	CCGGAGTCCATCACGATGCCA

Statistical Analysis

Data analysis was conducted using SPSS version 22.0. The Shapiro-Wilk test was used to assess the normality of data distribution. Parametric tests were used for normally distributed data, while non-parametric techniques, including the Mann-Whitney U test with Bonferroni correction and Kruskal-Wallis test, were used for non-normally distributed datasets (Conover 1980). The results are presented as mean±standard error, with p-values < 0.05 regarded statistically significant.

RESULTS

The apoptotic effects caused by endocrine-disrupting phenols have been previously reported (Ruberto et al., 2022). Based on these findings, G-361 human melanoma cell line was used to investigate the in vitro effects of nonylphenol (NP). The MTT assay was used to evaluate cell viability and assess the cytotoxic effects of NP on G-361 cell growth. Cells were treated with NP at doses ranging from 0.1 to 100 µg/mL (Figure 2). They were dissolved in 0.1% DMSO for 48 h. A significant decrease in cell viability was observed across this concentration range compared with the control group. Treatment with 100 µg/mL NP significantly reduced viability (Figure 1). The measured half-maximal inhibitory concentration (IC_{50}) was 64.82 µg/mL.

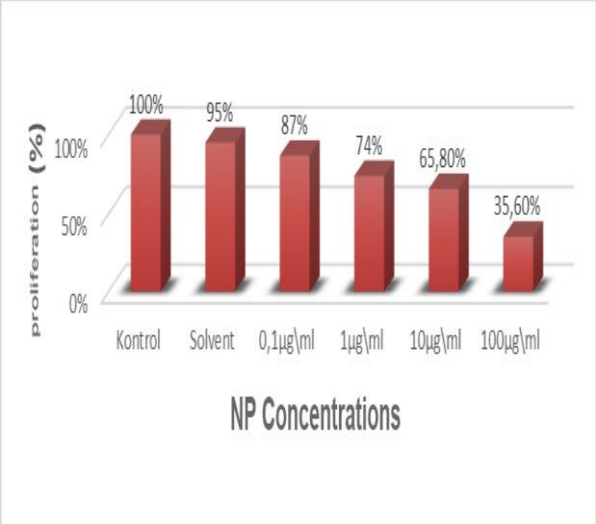

Figure 1: Cell viability levels obtained from various concentrations of NP.

Following 48 hours of incubation, statistical analysis revealed non-significant changes in cell viability among the control, solvent, and NP-treated groups at doses of 0.1 and 1 µg/mL ($p>0.05$). Cell viability was significantly

decreased at NP concentrations of 10 and 100 µg/mL compared with the control group ($p<0.001$).

Quantitative analysis of Caspase3, P21, Bax, Bcl2, P53 protein expression levels in G-361 melanoma cells after 48 hours of treatment with Control, Solvent, 0.1, 1, 10 and 100 µM NP was performed Figure 3 (A-E). Graphs show each protein band's relative intensity normalized to β-actin. In comparison to controls, the data are shown as the mean ± SD of three separate studies (n=3; *$p<0.05$ and **$p<0.01$). (A) According to the results obtained, it was determined that the expression level was statistically significantly higher in the groups treated with other NP concentrations compared to the control group. (B) When compared with the control group, it was determined that the expression level was statistically significantly higher in the groups applied with 1 µg/ml ($p<0.01$), 10 µg/ml ($p<0.01$) and 100 µg/ml ($p<0.001$) NP. When compared with the control group, a statistically significant decrease was observed in the Solvent group ($p<0.05$). (C) Compared to the control group, Bax expression level was shown to be statistically significantly higher ($p<0.01$) in the NP applied groups except the solvent group. Although there was an increase in the solvent group compared to the control group, it was not statistically significant. (D) According to the statistical analysis of the obtained data, a statistical decrease was observed in the solvent group ($p<0.01$), 10 µg/ml ($p<0.05$) and 100 µg/ml NP applied groups compared to the control group. Although a decrease was observed in the 0.1 and 1 µg/ml NP applied groups compared to the control group, it was not statistically significant. (E) When compared with the control group, a statistically significant increase ($p<0.01$) was detected in all NP-applied groups.

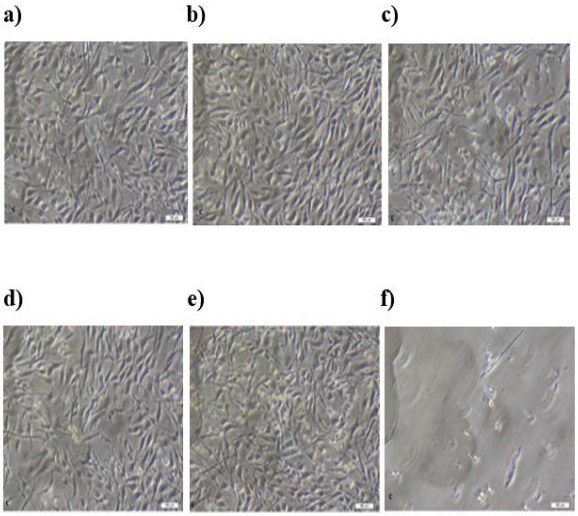

Figure 2: Cell density in NP-treated G-361 melanoma cells decreases in a dose-dependent manner (a-f). Scale bar=100 µm.

Cellular Morphological Alterations Induced by Nonylphenol at Varying Concentrations After 48 Hours of Exposure

Morphological characteristics of G-361 melanoma cells after a 48-hour exposure to increasing doses of NP are given in Figure 1. Cells frequently adhered to the culture substrate, forming a monolayer. At lower NP concentrations (e.g., 0.1 µg/mL; panel a), a slight decrease in cell growth was observed. Conversely, exposure to higher concentrations of NP (e.g., 100 µg/mL; panel f) caused significant cytotoxic effects, characterized by a significant reduction in cell density and breakdown of monolayer integrity.

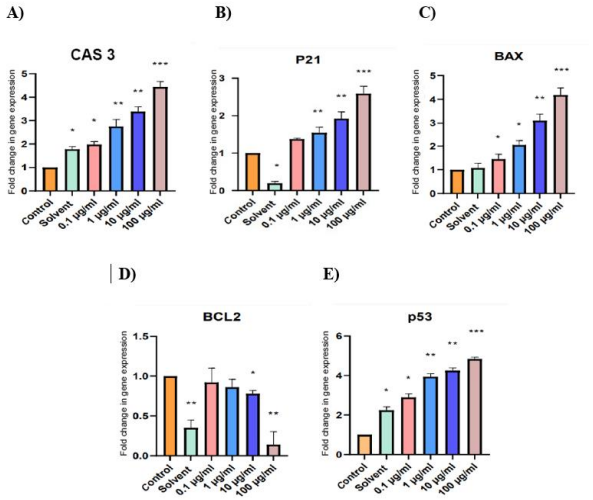

Figure 3: Effects of NP on apoptotic protein expression in G-361 melanoma cells.

DISCUSSION AND CONCLUSION

Endocrine-disrupting chemicals (EDCs) are known for their ability to disrupt hormonal homeostasis in mammals, resulting in negative impacts on immunological, neurological, reproductive, and developmental systems (Yang et al. 2016). Increasing evidence demonstrates that EDCs adversely affect human health, leading to reduced fertility and initiation or progression of diabetes, obesity, and endometriosis, and many hormone-associated cancers (Ariemma et al. 2016; Jambor et al. 2016; Kim et al. 2016). The EDCs may mistakenly bind to hormone receptors, disrupting normal endocrine functions and facilitating the onset and progression of hormone-sensitive cancers, such as breast, ovarian, and prostate malignancies (Park and Choi 2014; In et al. 2015). The NP has been associated with the development of many tumor types (Xie et al. 2019).

Previous studies investigated the effects of NP on colon cancer using COLO205 and SW480 colon cancer cell lines (Qiu et al. 2002). The effect of NP on apoptosis and cell growth was examined by MTT assays and flow cytometry. It was shown that NP reduced the growth of COLO205 and SW480 colon cancer cells in a dose-dependent manner (Qiu et al. 2002). Duan et al. (2016) conducted a separate cell culture study and found that dissolving NP in dimethyl sulfoxide (DMSO) at a concentration of 0.1% (v/v) did not cause cytotoxicity. The current study used ethanol and DMSO as solvents, exhibiting similar cell survival at a 0.1% concentration, demonstrating no solvent-induced cytotoxicity. Likewise, Kim et al. (2016) reported a concentration-dependent reduction in cell viability following NP exposure in prostate cancer cells. The MTT assay in the current study indicated that100 µg/mL NP significantly decreased cell viability relative to the control group ($p<0.05$), exhibiting a concentration-dependent decrease across the investigated range.

Yang et al. (2020) investigated the impact of NP on several cancer cell lines and reported that colon cancer cells (COLO205) had increased viability and proliferation when exposed to NP at a concentration of 10^{-6} mol/L. Forte et al. (2016) similarly demonstrated that exposure to 10^{-6} mol/L NP significantly impacted cell viability more than 10^{-12} mol/L in non-tumorigenic human prostate cell lines. Bistakowa et al. (2016) used the H295R human adrenocarcinoma cell line to examine the

concentration-dependent effects of NP at 0.04, 0.2, 1, 2.5, and 5 µg/mL, indicating varying degrees of cytotoxicity. Bistakowa et al. (2016) indicated that treatment with 5 µg/mL NP led to a slight decrease in cell viability relative to the control group. Jambor et al. (2017) found that all NP-treated groups, at doses of 0.04, 0.2, 1, 2.5, and 5 µg/mL, significantly affected the viability of Leydig cells. The results of current study align with previous cytotoxicity assessments performed on several cell lines exposed to NP and its derivatives, indicating that higher NP concentrations are consistently linked to decreased cell viability.

Malignant melanomas have varying expression levels of pro-apoptotic and anti-apoptotic proteins, which contribute to their resistance to programmed cell death (Shariat et al., 2003; Shariat et al., 2004). Bcl-2 is a crucial regulator that inhibits apoptosis and influences apoptotic pathways. It has been extensively investigated as a prognostic marker in melanoma (Nakopoulou et al. 1998). Increased Bcl-2 expression has been linked to poorer clinical results in several cancer types, such as lymphoma, leukemia, neuroblastoma, and prostate cancer (Kong et al. 1998). The decreased Bcl-2 gene expression in all NP-treated groups of the current study indicate decreased cellular function and the initiation of apoptosis. Bcl-2 is an anti-apoptotic protein, essential in preventing programmed cell death. Numerous studies have investigated the impact of different chemicals on cancer cells by analyzing the expression of Bcl-2 and the pro-apoptotic gene Bax, highlighting their pivotal roles in apoptosis regulation (Zeestraten et al. 2013; Zhu et al. 2013). Evidence suggests that both Bax and Bcl-2 are essential for the regulation of apoptosis and cell growth (Thomas et al. 2013). The NP has been reported to affect the Bcl-2 protein family, which are key regulators of apoptotic pathways. Low et al. (2011) reported that NP exposure led to the downregulation of the anti-apoptotic gene Bcl-2 and the overexpression of the pro-apoptotic gene Bax.

Understanding the molecular basis of apoptosis is an important goal in cancer research. Numerous in vitro studies have examined this issue (Ogur 2014; Lee et al. 2017; Zhang et al. 2022). The present study examined whether NP stimulates the intrinsic (mitochondrial) apoptotic pathway in G-361 melanoma cells. The mRNA expression levels of p53, Bax, and Bcl-2 (critical regulators of the intrinsic apoptotic pathway) were measured 48 h after exposure to different doses of NP. Figure 3 shows fold change expression data indicating NP-induced apoptotic signaling in melanoma cells. The current study demonstrated that exposure to NP at doses of 10 µg/mL and 100 µg/mL resulted in fourfold increase in P53 gene expression relative to the control group. The upregulation was statistically significant in comparison to the solvent control and the 0.1 µg/mL and 1 µg/mL NP-treated groups ($p<0.001$). The observed increase in P53 expression is probably due to NP-induced DNA damage in melanoma cells. Moreover, this increase in P53 is associated with a significant change in the Bcl-2/Bax ratio, promoting pro-apoptotic signaling. These results correspond with previous study (Fridman and Lowe 2003), indicating that NP induces apoptosis in G-361 cells via P53-mediated mechanisms.

Bax is a pro-apoptotic member of the Bcl-2 family, which is essential in modulating apoptosis and inhibiting tumorigenesis and progression. The present study revealed a dose-dependent increase in Bax gene expression with increasing NP concentrations, indicating enhanced pro-apoptotic signaling. The group exposed to 100 µg/mL NP had the greatest levels of Bax expression. Caspase-3 is a crucial executor in the apoptotic pathway, and it showed a significant overexpression under increased NP dose in the current study. The data indicate that increased NP exposure enhances apoptotic activation, as seen by increased expression of both Bax and Caspase-3.

The results demonstrated that NP induces apoptosis through the upregulation of Caspase-3 expression. Lopez and Tait (2015) indicated that tumor cells often exhibit greater susceptibility to the extrinsic apoptotic pathway. P21 is a cell cycle inhibitor regulated by P53-dependent and independent pathways, and it showed increased expression at all tested NP doses (0.1–100 µg/mL) after 48 hours. These results align with El-Deiry (2016), who highlighted the growing significance of P21 in cancer biology. This study used an in vitro melanoma model to evaluate the pro-apoptotic effects of NP, revealing that NP alters the expression of key apoptotic and anti-apoptotic genes. In contrast to most studies using in vivo models, this study offers conclusive evidence of NP's molecular impact on melanoma cells.

It is concluded that NP induces damage to melanoma cells, and the gene expressions observed in this study confirm earlier results. The analysis of Caspase-3 gene expression revealed that expression levels increased in direct correlation with the NP dose, indicating a dose-dependent activation of apoptotic pathways. Correspondingly, evaluation of the anti-apoptotic gene Bcl-2 indicated that NP decreased its expression, with a higher reduction under increased concentration. The evaluation of P53 gene expression revealed increased expression levels in all NP-treated groups. These results indicate that future research is necessary to investigate the impact of NP on other molecular pathways to get a deeper understanding of its apoptotic mechanisms in melanoma cells.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

This research was supported by Afyon Kocatepe University Scientific Research Projects Coordination Office with the project number "19. SAĞ.BİL.13".

This study is derived from publicly defended PhD thesis of the Esra BİLİCİ named author.

AUTHOR CONTRIBUTIONS

Idea / Concept: EB, CU, KA
Audit / Consultancy: CU, KA
Data Collection and/or Processing: EB
Analysis and/or Interpretation: EB, CU, KA
Article Written by: EB, CU, KA
Critical Review: CU, KA

REFERENCES

Ariemma F, D'Esposito V, Liguoro D et al. (2016). Low-dose bisphenol-a impairs adipogenesis and generates dysfunctional 3t3-l1 adipocytes. *PloS one,* 11 (3), e0150762.

Behari J, Kesari KK (2006). Effects of microwave radiations on reproductive system of male rats. *Embryo Talk*, 1, 81-85.

Bistakowa J, Forgacs Z, Bartos, Zsuzsa et al. (2016). Effects of 4-nonylphenol on the steroidogenesis of human adrenocarcinoma cell line (NCI-H295R). *J Environ Sci Heal A, 52* (3), 221–227.

Conover WJ (1980). Practical nonparametric statistics. *2nd Edition John Wiley & Sons*, 229-239.

Cummins DL, Cummins JM, Pantle H et al. (2016). Cutaneous malignant melanoma. *Mayo Clin Proc,* 81 (4), 500–507.

Duan P, Hu C, Butler HJ et al. (2016). Effects of 4-nonylphenol on spermatogenesis and induction of testicular apoptosis through oxidative stress-related pathways. *Reprod Toxicol*, 62, 27–38.

El-Deiry WS (2016). p21(WAF1) Mediates cell-cycle inhibition, relevant to cancer suppression and therapy. *Cancer Res,* 76 (18), 5189-91.

Forte M, Lorenzoa, MD, Carrizzoc A et al. (2016). Nonylphenol effects on human prostate non tumorigenic cells. *Toxicology,* 16, 21-32.

Fridman JS, Lowe SW (2003). Control of apoptosis by p53. *Oncogene*, 22, 9030–9040.

Gałązka A, Jankiewicz U (2022). Endocrine disrupting compounds (nonylphenol and bisphenol a)-sources, harmfulness and laccase-assisted degradation in the aquatic environment. *Microorganisms,* 10 (11), 2236.

Galluzzi L, Chan TA, Kroemer G et al. (2018). The hallmarks of successful anticancer immunotherapy. *Sci Transl Med,* 10 (459), eaat7807.

In SJ, Kim SH, Go RE et al. (2015). Benzophenone-1 and nonylphenol stimulated MCF-7 breast cancer growth by regulating cell cycle and metastasis-related genes via an estrogen receptor α-dependent pathway. *J Toxicol Environ Health A*, 78 (8), 492–505.

Inkaya EN, Tokgöz E, Barlas N. (2025). In vivo investigation of the effects of nonylphenol on the pituitary-adrenal axis and pineal gland in male rats. *Basic Clin Pharmacol Toxicol,* 136 (3), e70003.

Jambor T, Lukáčová J, Tvrdá E et al. (2016). The impact of 4-nonylphenol on the viability and hormone production of mouse leydig cells. *Folia Biol (Praha),* 62 (1), 34-39.

Jambor T, Tvrda E, Tusimova E et al. (2017). In vitro effect of 4-nonylphenol on human chorionic gonadotropin (hCG) stimulated hormone secretion, cell viability and reactive oxygen species generation in mice Leydig cells. *Environmental Pollution*, 222, 219-225.

Kim SH, Nam KH, Hwang KA et al. (2016). Influence of hexabromocyclododecane and 4-nonylphenol on the regulation of cell growth, apoptosis and migration in prostatic cancer cells. *Toxicol In Vitro*, 32, 240–247.

Kong G, Shin KY, Lee JJ (1998). Bcl-2 and p53 expressions in invasive bladder cancers. *Acta Oncol*, 37, 715-20.

Lee JW, Han HK, Park S et al. (2017). Nonylphenol increases tumor formation and growth by suppressing gender-independent lymphocyte proliferation and macrophage activation. *Environ Toxicol,* 32, 1679-87.

Lopez J, Tait SWG (2015). Mitochondrial apoptosis: Killing cancer using the enemy within. *Br J Cancer*, 112 (6), 957–962.

Low IC, Kang J, Pervaız S (2011). Bcl-2: A prime regulator of mitochondrial redox metabolism in cancer cells. *Antioxid Redox Signal,* 15 (12), 2975-2987.

Nakopoulou L, Vourlakou C, Zervas A (1998). The prevalence of bcl-2, p53, and Ki-67 immunoreactivity in transitional cell bladder, carcinomas and their clinicopathologic correlates. *Hum Pathol,* 29 (2), 146-154.

Ogur R (2014). Studies with myrtus communis L.: anticancer properties. *J Intercult Ethnopharmacol*, 3 (4), 135-137.

Park HJ, Zhang M, Lee WY et al. (2020). Toxic effects of nonylphenol on neonatal testicular development in mouse organ culture. *Int J Mol Sci*, 21 (10), 3491.

Park MA, Choi KC (2014). Effects of 4-nonylphenol and bisphenol A on stimulation of cell growth via disruption of the transforming growth factor-β signaling pathway in ovarian cancer models. *Chem Res Toxicol*, 27 (1), 119–128.

Phillips KP, Tanphaichitr N (2008). Human exposure to endocrine disrupters and semen quality. *J Toxicol Environ Health B Crit Rev*, 11 (3-4), 188–220.

Qiu Y, Waters CE, Lewis AE et al. (2002). Oestrogen-induced apoptosis in colonocytes expressing oestrogen receptor beta. *J Endocrinol*, 174 (3), 369-377.

Ruberto S, Santovito A, Simula ER et al. (2022). Bisphenols induce human genomic damage and modulate HERVs/env expression. *Environ Mol Mutagen*, 63 (6), 275-285.

Shariat SF, Kim J, Raptidis G (2003). Association of p53 and p21 expression with clinical outcome in patients with carcinoma in situ of the urinary bladder. *Urology,* 61 (6), 1140-1145.

Shariat SF, Tokunaga H, Zhou J (2004). p53, p21, pRB and p16 expression predict clinical outcome in cystectomy with bladder cancer. J *Clin Oncol*, 22 (6), 1014-1024.

Shi F, Li T, Shi H, Wei Y, Wang J, Liu C, Liang R (2024). Identification of potential therapeutic targets for skin cutaneous melanoma on the basic of transcriptomics. *Skin Res Technol,* 30 (8), e13916.

Soto AM, Justicia H, Wray JW, Sonnenschein C (1991). p-Nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. *Environ Health Perspect*, 92, 167–173.

Thomas S, Quınn BA, Das SK (2013). Targeting the bcl-2 family for cancer therapy. *Expert Opin Ther Targets*, 17 (1), 61-75.

Weert E, Hoekstra-Weebers JE, May AM, Korstjens I, Ros WJ, van der Schans CP (2008). The development of an evidence-based physical self-management rehabilitation programme for cancer survivors. *Patient Educ Couns*, 71 (2), 169-190.

White K, Grether ME, Abrams JM, Young L, Farrell K, Steller H (1994). Genetic control of programmed cell death in Drosophila. *Science,* 264 (5159), 677-683.

Wróblewska-Łuczka P, Góralczyk A, Łuszczki JJ (2023). Synergy, additivity and antagonism between esculetin and six commonly used chemotherapeutics in various malignant melanoma cell lines-an ısobolographic analysis. *Molecules*, 28 (9), 3889.

Xie M, Liang JL, Huang HD et al. (2019). Low doses of nonylphenol promote growth of colon cancer cells through activation of erk1/2 via g protein–coupled receptor 30. *Cancer Res Treat,* 51 (4), 1620-1631.

Yang J, Huang Q, Liu H et al. (2020). 4-Nonylphenol and 4-tert-octylphenol induce anxiety-related behaviors through alternation of 5-HT receptors and transporters in the prefrontal cortex. *Comp Biochem Physiol C Toxicol Pharmacol,* 230, 108701.

Yang L, Lin L, Wenga S, et al. (2016). Sexually disrupting effects of nonylphenol and diethylstilbestrol on male silver carp (Carassius auratus) in aquatic microcosms. *Ecotoxicol Environ Saf*, 71 (2), 400-411.

Zeestraten EC, Benard A, Reimers MS et al. (2013). The prognostic value of the apoptosis pathway in colorectal cancer: a review of the literature on biomarkers identified by ımmunohistochemistry. *Biomark Cancer,* 4 (5), 13-29.

Zhang NJ, Zhang Y, Yin S et al. (2022). Nonylphenol promoted epithelial-mesenchymal transition in colorectal cancer cells by upregulating the expression of regulator of cell cycle. *Chem Res Toxicol*, 35 (9), 1533-1540.

Zhu M, Hong D, Bao Y, Wang C, Pan W (2013). Oridonin induces the apoptosis of metastatic hepatocellular carcinoma cells via a mitochondrial pathway. *Oncology Letters,* 6 (5), 1502-1506.