REFERENCES

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K (eds) (1999) Short Protocols in Molecular Biology. Wiley, New York.

Barnes S and Peterson TG (1995) Biochemical targets of the isoflavone genistein in tumor cell lines. *Proc Soc Exp Biol Med* 208:103-108.

Bragadin M, Perin G, Iero A, Manente S, Rizzoli V and Scutari G (1999) An *in vitro* study on the toxic effects of nonylphenols (NP) in mitochondria. *Chemosphere* 38 (9):1997-2001.

Chao M (1992) Growth factors signaling: where is the specificity? *Cell* 68:995-997.

Chen D, Fong HW and Davis JS (2001) Induction of c-fos and c-jun messenger ribonucleic acid expression by prostaglandin F2α is mediated by a protein kinase C-dependent extracellular signal-regulated kinase mitogen-activated protein kinase pathway in bovine luteal cells. *Endocrinology* 142:887-895.

Colborn T, Dumanoski D and Myers JP (1996) Our Stolen Future. Dutton, New York.

Colborn T, vom Saal FS, and Soto AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 101(5):378-384.

Committee on Hormonally Active Agents in the Environment (1999) Hormonally Active Agents in the Environment. National Academy Press, Washington, D.C. 430 pp..

Crain DA, Rooney AA, Orlando EF and Guillette Jr LJ (2000) Endocrine-disrupting contaminants and hormone dynamics: Lessons from wildlife. *In:*

Environmental Endocrine Disruptors: An Evolutionary Perspective. Taylor and Francis, NY, USA. pp. 1-15.

Cummings AM (1997) Methoxychlor as a model for environmental estrogens. *Crit Rev Toxicol* 15(3):571-579.

Daly HB (1991) Reward reductions found more aversive by rats fed environmentally contaminated salmon. *Neurotixicol Teratol* 13(4):449-453.

Daly HB, Hertzler DR and Sargent DM (1989) Ingestion of environmentally contaminated Lake Ontario salmon by laboratory rats increases avoidance of unpredictable aversive nonreward and mild electric shock. *Behav Neurosci* 103(6):1356-1365.

Decker T and Lohmann-Matthes M (1988) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *J Immunol Methods* 15:61-69.

De Guise S, Lagace A and Beland P (1994) Tumors in St. Lawrence beluga whales. *Vet Pathol* 31(4):444-449.

De Guise S, Martineau D, Beland P and Fournier M (1995) Possible mechanisms of action of environmental contaminants on St. Lawrence beluga whales (Delphinapterus leucas). *Environ Health Perspect* 103(Suppl. 4):73-77.

de Swart RL, Ross PS, Vedder LJ, Timmerman HH, Heisterkamp S, Van Loveren H, Vos J, Rijnders PJH, and Osterhaus ADME (1994) Impairment of immune function in harbor seals (*Phoca vitulina*) feeding on fish from polluted waters. *Ambio* 23(2):155-159.

de Swart RL, Ross PS, Vos JG and Osterhaus ADME (1996) Impaired immunity in harbour seals (*Phoca vitulina*) exposed to bioaccumulated environmental contaminants: Review of a long-term feeding study. *Environ Health Perspect* 104(Suppl.4):823-828.

Dichter MA, Tischler AS and Greene LA (1977) Nerve growth factor-induced change in electrical excitability and acetylcholine sensitivity of a rat pheochromocytoma cell line. *Nature* 2368:501-504.

Donger C, Krejci E, Serradell AP, Eymard B, Bon S, Nicole S, Chateau D, Gary F, Fardeau M, Massoulie J and Guicheney P (1998) Mutation in the human acetylcholinesterase-associated collagen gene, COLQ, is responsible for congenital myasthenic syndrome with end-plate acetylcholinesterase deficiency (Type Ic). *Am J Human Genetics* 63:967-975.

Facemire CF, Gross TS and Guillette Jr. LJ (1995) Reproductive impairment in the Florida panther: Nature or nurture. *Environ Health Perspect* 103(Suppl. 4):79-86.

Fox GA (1992) Epidemiological and pathobiological evidence of contaminant-induced alterations in sexual development in free-living wildlife. Pp.147-158 in Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. eds. Princeton: Princeton Scientific Publ Co.

Fujimiya Y, Kobori H, Oshiman K, Soda R, and Ebina T (1998) Tumoricidal activity of high molecular weight polysaccharides derived from Agaricus blazei via oral administration in the mouse tumor model. *J Jap Soc Food Sci Technol* 45:246-252.

Fujimiya Y, Suzuki Y, Katakura R and Ebina T (1999) Tumor-specific cytocidal and immunopotentiating effects of relatively low molecular weight products derived from the basidiomycete Agaricus blazei Murill. *Anticancer Res* 19: 113-118.

Fujimiya Y, Suzuki Y, Oshiman K, Kobori H, Moriguchi K, Nakashima H, Matumoto Y, Takahara S, Ebina T, and Katakura R (1998) Selective tumoricidal effect of soluble proteoglucan extracted from the basidiomycete, Agaricus blazei Murill, mediated via natural killer cell activation and apoptosis. *Cancer Immunol Immunother* 46:147-159.

Grassman A, Fox G, Scanlon P and Ludwig J (1996) Organochlorine-associated immunosuppression in pre-fledgling Caspian terns and herring gulls from the Great Lakes: An ecoepidemiological study. *Environ Health Perspect* 104(Suppl. 4):829-842.

Gray Jr. LE, Ostby JS, Kelce WR (1994) Developmental effects of an environmental antiandrogen: The fungicide vinclozolin alters sex differentiation of the male rat. *Toxicol Appl Pharmacol* 129(1):46-52.

Gray MA, Teather KL and Metcalfe CD (1999) Reproductive success and behavior of Japanese medaka (Oryzias latipes) exposed to 4-tert-octylphenol. *Env Toxicol Chem* 18 (11):2587-2594.

Greene LA (1978) Nerve growth factor prevents the death and stimulates the neuronal differentiation of clonal PC12 pheochromocytoma cells in serum-free medium. *J Cell Biol* 78:747-755.

Greene LA and Rein G (1977) Release of (^{3}H)-norephinephrine from a clonal line of pheochromocytoma cells (PC12) by nicotinic cholinergic stimulation. *Brain Res* 138:521-528.

Greene LA and Rukenstein A (1981) Regulation of acetylcholinesterase activity by nerve growth factor: Role of transcription and dissociation from effects on proliferation and neurite outgrowth. *J Biol Chem* 256:6363-6367.

Greene LA and Tischler AS (1976) Establishment of a noradrenergic clonal cell line of rat pheochromocytoma cells which resond to nerve growth factor. *Proc Natl Acad Sci USA* 73:2424-2428.

Greenspan RJ, Finn Jr. JA and Hall JC (1980) Acetylcholinesterase mutants in Drosophila and their effects on the structure and function of the central nervous system. *J Comp Neurology* 189:741-774.

Guillette Jr LJ and Crain DA (1996) Endocrine-disrupting contaminants and reproductive abnormalities in reptiles. *Comments on Toxicology* 5(4-5):381-399.

Guillette Jr LJ, Gross TS, Masson GR, Matter JM, Percival HF and Woodward AR (1994) Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ Health Perspect* 102(8):680-688.

Guillette Jr LJ, Gross TS, Gross DA, Rooney AA and Percival HF (1995) Gonadal steroidogenesis *in vitro* from juvenile alligators obtained from contaminated and control lakes. *Environ Health Perspect* 103(Suppl. 4):31-36.

Guillette Jr LJ, Pickford DB, Crain DA, Rooney AA and Percival HF (1996) Reduction in penis size and plasma testosterone concentrations in juvenile

alligators living in a contaminated environment. *Gen Comp Endocrinol* 101(1):32-42.

Guo YL, Lambert GH and Hsu CC (1995) Growth abnormalities in the population exposed *in utero* and early postnatally to polychlorinated biphenyls and dibenzofurans. *Environ Health Perspect* 103 (Suppl. 6):117-122.

Guo YL, Lin CJ, Yao, WJ, Ryan JJ and Hsu CC (1994) Musculoskeletal changes in children prenatally exposed to polychlorinated biphenyls and related compounds (Yu-Cheng children). *J Toxicol Environ Health* 41:83-93.

Gygi SP, Rochon Y, Franza BR and Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol* 19:1720-1730.

Hammond B, Katzenellenbogen BS, Krauthammer N and McConnell J (1979) Estrogenic activity of the insecticide chlordecone (Kepone) and interaction with uterine estrogen receptor. *Proc Natl Acad Sci USA* 76:6641-6645.

Honma Y, Okabe KJ, Kasukabe T, Hozumi M, Kajigaya S, Suda T, and Miura Y (1991) Induction by some protein kinase inhibitors of differentiation of a mouse megakaryoblastic cell line established by coinjection with Abelson murine leukemia virus and recombinant SV40 retrovirus. *Cancer Res* 51:4649-4655.

Hsu SC, Ma S, Hsu S, Wu N, Hsu C, Yeh C and Wu S. (1985) Discovery and epidemiology of PCB poisoning in Taiwan: A four-year follow-up. *Environ Health Perspect* 59:5-10

Ito H, Shimura K, Itoh H, and Kawabe M (1997) Antitumor effects of a new polysaccharide-protein complex (ATOM) prepared from Agaricus blazei

(Iwade Strain 101) "Himematsutake" and its mechanism in tumor-bearing mice. *Anticancer Res* 17:277-284.

Itoh H, Ito H, Amano H, and Noda H (1994) Inhibitory action of a (1-6)-β-D-glucan-protein complex (F III-2-b) isolated from Agaricus blazei Murill ("Himematsutake") on Meth A fribrosarcoma-bearing mice and its antitumor mechanism. *Jap J Pharmacol* 66:265-271.

Jacobson JL and Jacobson SW (1996) Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N Engl J Med* 335(11):783-789.

Jacobson JL, Jacobson SW and Humphrey HE (1990) Effects of exposure to PCBs and related compounds on growth and activity in children. *Neurotox Teratol* 12:319-326.

Jacobson JL, Jacobson SW, Padgett RJ, Brumitt GA and Billings RL (1992) Effects of prenatal PCB exposure on cognitive processing efficiency and sustained attention. *Develop Psy* 23(2):297-306.

Jobling S, Nolan M, Tyler CR, Brighty G, and Sumpter JP (1998) Widespread sexual disruption in wild fish. *Environ Sci Technol* 32(17):2498-2506.

Jones PA, Baker VA, Irwin AJE and Earl LK (1998) Interpretation of the *in vitro* proliferation response of MCF-7 cells to potential oestrogens and non-oestrogenic substances. *Toxicology in Vitro* 12:373-382.

Karin M (1991) The AP-1 complex and its role in transcriptional control by protein kinase C. In The hormonal control regulation of gene transcription (Cohen, P., and Foulkes, J.G., Eds.), Elsevier Science Publishers, Amsterdam, pp. 235-253.

Kim UH, Fink DJ, Kim HS, Park DJ, Contreras ML, Guroff G and Rhee SG (1991) Nerve growth factor stimulates phosphorylation of phospholipase C-γ in PC12 cells. *J Biol Chem* 266:1359-1362.

Klein R, Jing S, Nanduri V, O'Rourke E and Barbacid M (1991) The trk proto-oncogene encodes a receptor for nerve growth factor. *Cell* 65:189-197.

Korzeniewski C and Callewaert DM (1983) An enzyme-release assay for natural cytotoxicity. *J Immunol Methods* 64:313-320.

Levi-Montalcini R and Angeletti PU (1968) The nerve growth factor. *Physiol Rev* 48:534-569.

Lilienthal H, Neuf M, Munoz C and Winneke G (1990) Behavioral effects of pre- and postnatal exposure to a mixture of low chlorinated PCBs in rats. *Fundam Appl Toxicol* 15:457-467.

Lilienthal H and Winneke G (1991) Sensitive periods for behavioral toxicity of polychlorinated biphenyls: Determination of cross-fostering in rats. *Fundam Appl Toxicol* 17:368-375.

Lorenc JF, Lambeth G and Scheffer W (1992) Alkylphenols. In: Kirk-Othmer Encyclopedia of Chemical Technology (Kioschwitz JI and Howe-Grant M, eds.), pp. 113-143. Wiley, New York.

Lucas CA, Czlonkowska A and Kreutzberg GW (1980) Regulation of acetylcholinesterase by nerve growth factor in the pheochromocytoma PC12 cell line. *Neuroscience Lett* 18:333-337.

Lucas CA and Kreutzberg GW (1985) Regulation of acetylcholinesterase secretion from neuronal cell cultures. -- 1. Actions of nerve growth factor, cytoskeletal inhibitors and tunicamycin. *Neuroscience* 14:349-360.

Maher P (1988) Nerve growth factor induces protein-tyrosine phosphorylation. *Proc Natl Acad Sci USA* 85:6788-6791.

Mäkelä S, Poutanen M, Lehtimäki J, Kostian ML, Santti R, and Vihko R (1995) Estrogen-specific 17β-Hydroxysteroid oxidoreductase type 1 (E.C. 1.1.1.62) as a possible target for the action of phytoestrogens. *Proc Soc Exp Biol Med* 208:51-59.

Malby TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A and Peterson RE (1992c) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 3. Effects on spermatogenesis and reproductive capability. *Toxicol Appl Pharmacol* 114(1):118-126.

Malby TA, Moore RW, Goy RW and Peterson RE (1992b) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. *Toxicol Appl Pharmacol* 114(1):108-117.

Malby TA, Moore RW and Peterson RE (1992a) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 1. Effects on androgenic status. *Toxicol Appl Pharmacol* 114(1):97-107.

Martin PM, Horwitz KB, Ryan DS, and McGuire WL (1978) Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinology* 103:1860-1867.

Miksicek RJ (1993) Commonly occurring plant flavonoids have estrogenic activity. *Mol Pharmacol* 44:37-43.

Minic J, Molgo J, Karlsson, E and Krejci E (2002) Regulation of acetylcholine release by muscarinic receptors at the mouse neuromuscular junction depends on the activity of acetylcholinesterase. *Eur J Neuroscience* 15:439-448.

Morrisey RE, George JD, Price CJ, Tyl RW, Marr MC and Kimmel CA (1987) The developmental toxicity of bisphenol A in rats and mice. *Fundam Appl Toxicol* 8(4):571-582.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 65:55-63.

Nair-Menon JU, Campbell GT and Blake CA (1996) Toxic effects of octylphenol on cultured rat and murine splenocytes. *Toxicol Appl Pharmacol* 139:437-444.

Nobuo K, Lin TS, Fukudome T, Kimura J, Sakamoto T, Kaji R and Shibasaki H. (2002) Pathophysiology of weakness in a patient with congenital end-plate acetylcholinesterase deficiency. *Muscle & Nerve* 25:585-592.

Okafuji K, Kaku K, Seguchi M, Tanaka H, Azuno Y, and Kaneko T (1995) Effects of activin A/erythroid differentiation factor on erythroid and megakaryocytic differentiations of mouse erythroleukemia (friend) cells: Evidence for two distinct modes of cell response. *Experimental Hematol* 23: 210-216.

Ooi VEC and Liu F (2000) Immunomodulation and anti-cancer activity of polysaccharide-protein complexes. *Cur Med Chem* 7:715-729.

Osaki Y, Kato T, Yamamoto K, Okubo J, and Miyazaki T (1994) Antimutagenic and bactericidal substances in the fruit body of a basidiomycete Agaricus blazei, Jun-17. *Yakugaku Zasshi* 114:342-350.

Paech K, Webb P, Kuiper GJM, Nilsson S, Gustafsson J, Kushner PJ, and Scanlan TS (1997) Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. *Science* 277: 1508-1510.

Philips A, Chalbos D and Rochefort H (1993) Estradiol increases and anti-estrogens antagonize the growth factor-inducted activator protein-1 activity in MCF7 breast cancer cells without affecting c-fos and c-jun synthesis. *J Biol Chem* 268:14103-14108.

Pradines A, Magazin M, Schiltz P, LeFur G, Caput, D. and Ferrara, P (1995) Evidence for nerve growth factor-potentiating activities of the nonpeptidic compound SR57746 in PC12 cells. *J Neurochem* 64: 1954-1964.

Quantin B and Breathnach R (1989) Epidermal growth factor stimulates transcription of c-jun proto-oncogene in rat fibroblasts. *Nature* 334:538-539.

Raychoudhury SS, Blake CA and Millette CF (1999) Toxic effects of octylphenol on cultured rat spermatogenic cells and sertoli cells. *Toxicol Appl Pharmacol* 157:192-202.

Rieger F, Shelanski ML and Greene LA (1980) The effects of nerve growth factor on acetylcholinesterase and its multiple forms in cultures of rat PC12 pheochromocytoma cells: Increased total specific activity and appearance of the 16S molecular form. *Devl Biol* 76:238-243.

Rocch P, Ferreri AM, Simone G, Magrini E, Cavallazzi L, and Paolucci G (1995) Inhibitors of protein kinases induce differentiation in human neuroblastoma cell lines. *Anticancer Res* 15:1381-1385.

Rogan WJ, Gladen BC, Hung K, Koong S, Shih L, Taylor JS, Wu D, Yang NB, Ragan NB and Hsu C (1988) Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science* 241 (4863): 334-336.

Ryder K and Nathans D (1998) Induction of proto-oncogene c-jun by serum growth factors. *Proc. Natl. Acad. Sci. USA* 85:8464-8467.

Schantz SL, Levin ED, Bowman RE, Heironimus MP and Laughlin NK (1989) Effects of perinatal PCB exposure on discrimination-reversal learning in monkeys. *Neurotoxicol Teratol* 11(3):243-250.

Schmidt CW (1997) Amphibian deformities continue to puzzle researchers. *Environ Sci Technol* 31(7):324A-326A.

Schwartzman RA and Cidlowski JA (1993) Apoptosis: the biochemistry and molecular biology of programmed cell death. *Endocrine Rev* 14:133-151.

Schweitzer ES (1993) Regulated and constitutive secretion of distinct molecular forms of acetylcholinesterase from PC12 cells. *J Cell Sci* 106:731-740.

Seegal RF (1996) Epidemiological and laboratory evidence of PCB-induced neurotoxicity. *Crit Rev Toxicol* 26(6):709-737.

Sharpe RM, Fisher JS, Millar MM, Jobling S and Sumpter JP (1995) Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. *Environ Health Perspect* 103(12):1136-1143.

Shepard TH, Pyne GE, Kirschvink JF, and McLean M (1960) Soybean goiter: Report of three cases. *N Engl J Med* 262:1099-1103.

Sohrabji F, Greene LA, Miranda RC, Toran-Allerand CD (1994) Reciprocal regulation of estrogen and NGF receptors by their ligands in PC12 cells. *J Neurobiol* 25:974-988.

Soltoff SP, Rabin SL, Cantley LC and Kaplan DR (1992) Nerve growth factor promotes the activation of phosphatidylinositol 3-kinase and its association with the trk tyrosine kinase. *J Biol Chem* 267:17472-17477.

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

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N and Serrano FO (1995) The E-Screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ Health Perspect* 103 (Suppl7):113-122.

Takaku T, Kimura Y, and Okuda H (2001) Isolation of an antitumor compound from Agaricus blazei Murill and its mechanism of action. *J Nutr* 131:1409-1413.

Tham DM, Gardner CD, and Haskell WL (1998) Potential health benefits of dietary phytoestrogens: A review of the clinical, epidemiological, and mechanistic evidence. *J Clin Endocrinol Metab* 83:2223-2235.

Timmons SR, Nwankwo JO and Domann FE (2002) Acetaldehyde activates Jun/AP-1 expression and DNA binding activity in human oral keratinocytes. *Oral Oncology* 38:281-290.

Verdeal K and Ryan DS (1979) Naturally-occurring estrogens in plant foodstuffs – A review. *J Food Protection* 42:577-583.

Villalobos M, Olea N, Brotons JA, Olea-Serrano MF, de Almodovar JMR and Pedraza V (1995) The E-SCREEN Assay: A comparasion of different MCF7 cell stocks. *Env Health Perspect* 103:844-850.

Vistica DT, Skehan P, Scudiero D, Monks A, Pittman A, and Boyd MR (1991) Tetrazolium-based assays for cellular viability: A critical examination of selected parameters affecting formazan production. *Cancer Res* 51:2514-2520.

vom Saal FS (1995) Environmental estrogenic chemicals: Their impact on embryonic development. *Human Ecol Risk Assess* 1(2):3-15.

Weeks JA, Adams WJ, Guiney PD, Hall JF and Naylor CG (1996) Risk assessment of nonylphenol and its ethoxylates in U.S. river water and sediment. *Royal Soc Chem* 189:276-291.

White R, Jobling S, Hoare SA, Sumpter JP and Parker MG (1994) Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* 135:175-182.

Wu B, Fodor EJB, Edwards RH, and Rutter WJ (1989) Nerve growth factor induces the proto-oncogene c-jun in PC-12 cells. *J Biol Chem* 264:9000-9003.

Yamashita F and Hayashi M (1985) Fetal PCB syndrome: Clinical features, intrauterine growth retardation and possible alteration in calcium metabolism. *Environ Health Perspect* 59:41-45.

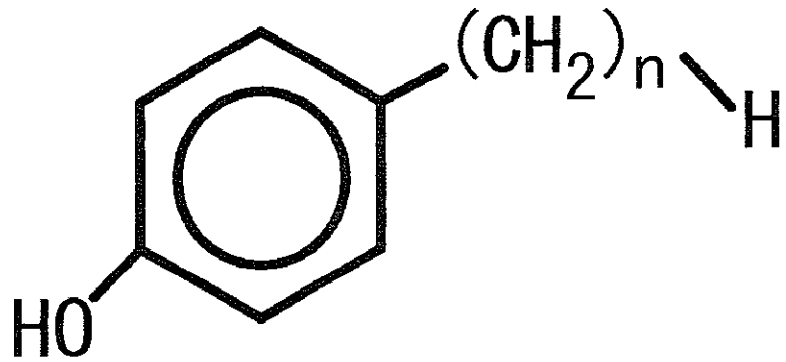

Figure 1. Chemical structure of *p*-alkylphenols. n = 5 (pentyl), 6 (hexyl), 7 (heptyl), 8 (octyl), 9 (nonyl).

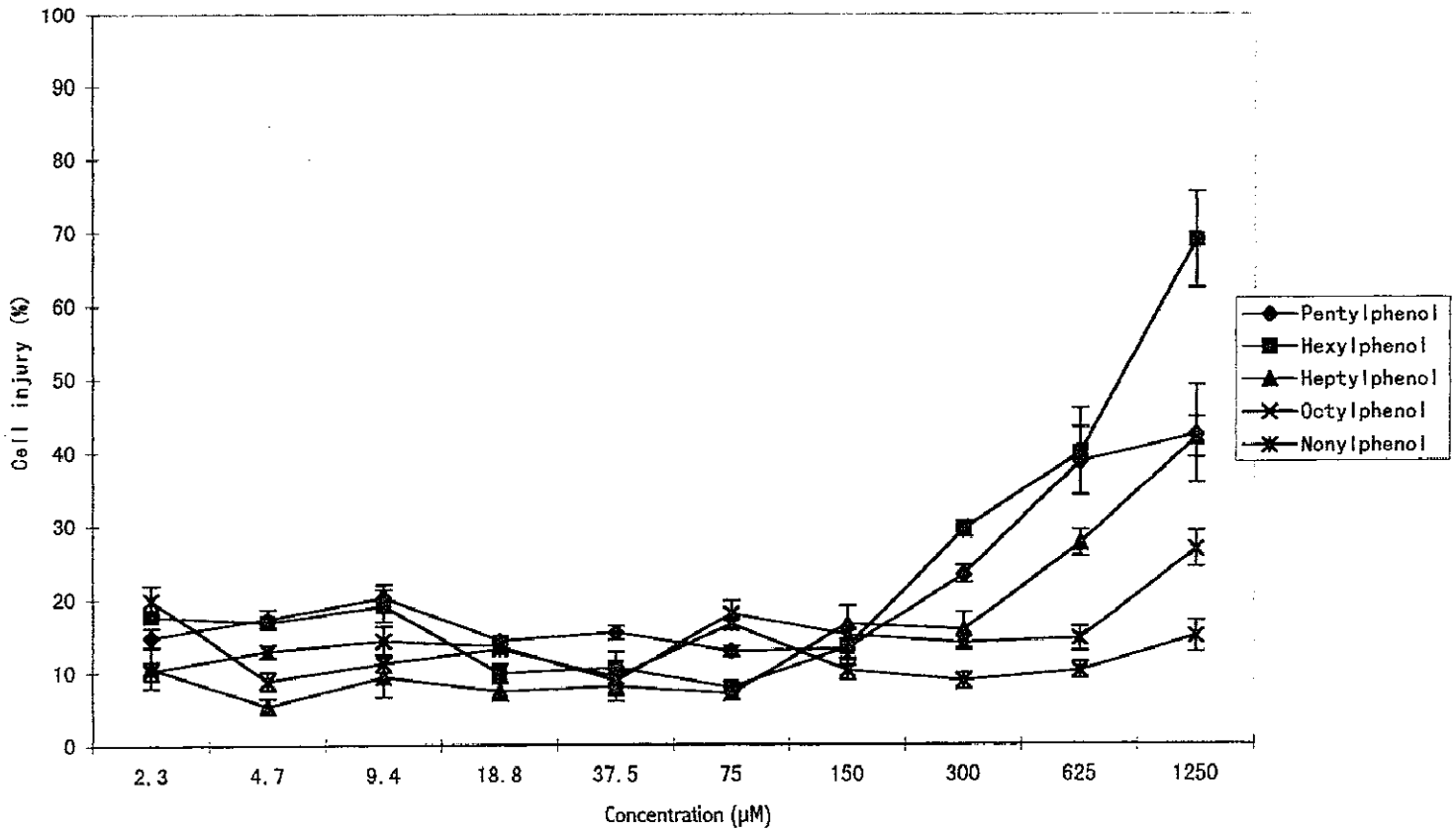

Figure 2. Lactate dehydrogenase activity (expressed as cell injury rate) of PC12 cells after 15 min incubation with varying concentrations of different alkylphenols. Results based from three independent replicates are expressed as percent cell injury ± SEM.

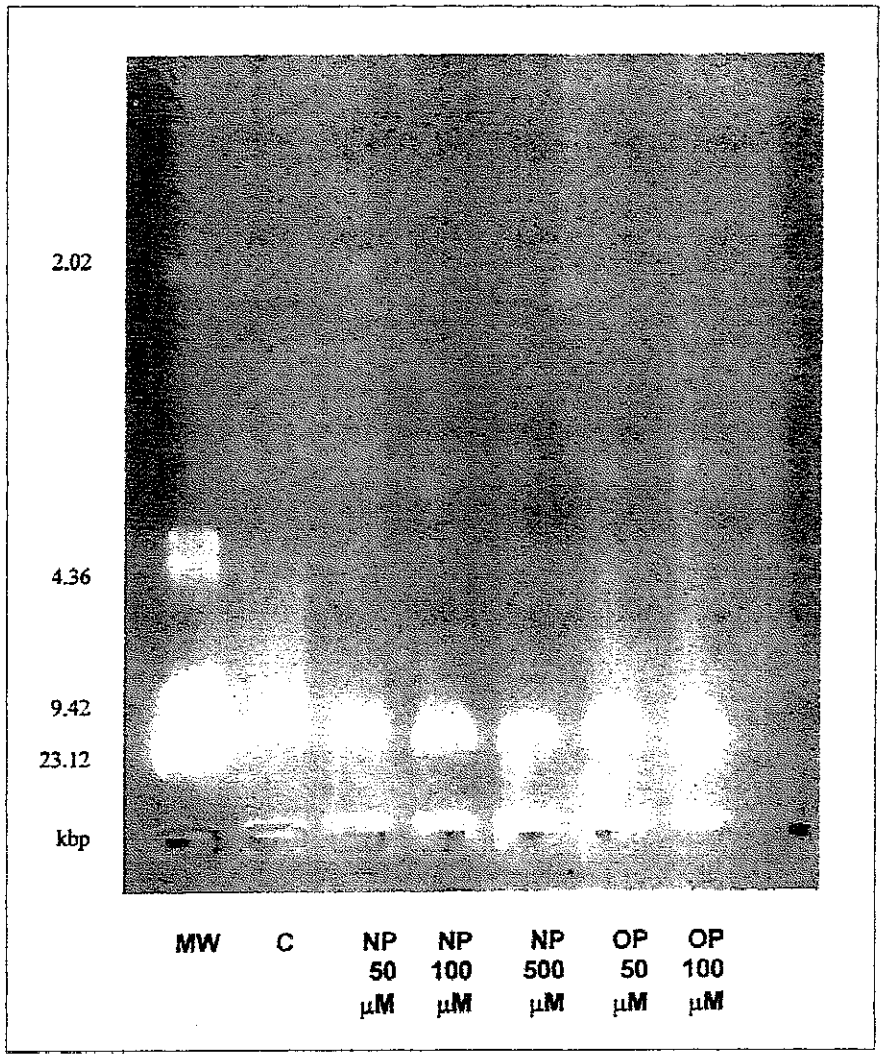

Figure 3. Ethidium-bromide-stained agarose gel showing genomic DNA from the rat pheochromocytoma cell line PC 12. Results show no fragmentation of DNA after 4 h treatment with the indicated concentrations of *p*-nonylphenol (NP) and *p*-octylphenol (OP). PC12 DNA was extracted using the Sodium Iodide Method and electrophoresed on a 1.5% agarose gel. C, control; MW, molecular weight marker. Results represent three independent replicates.

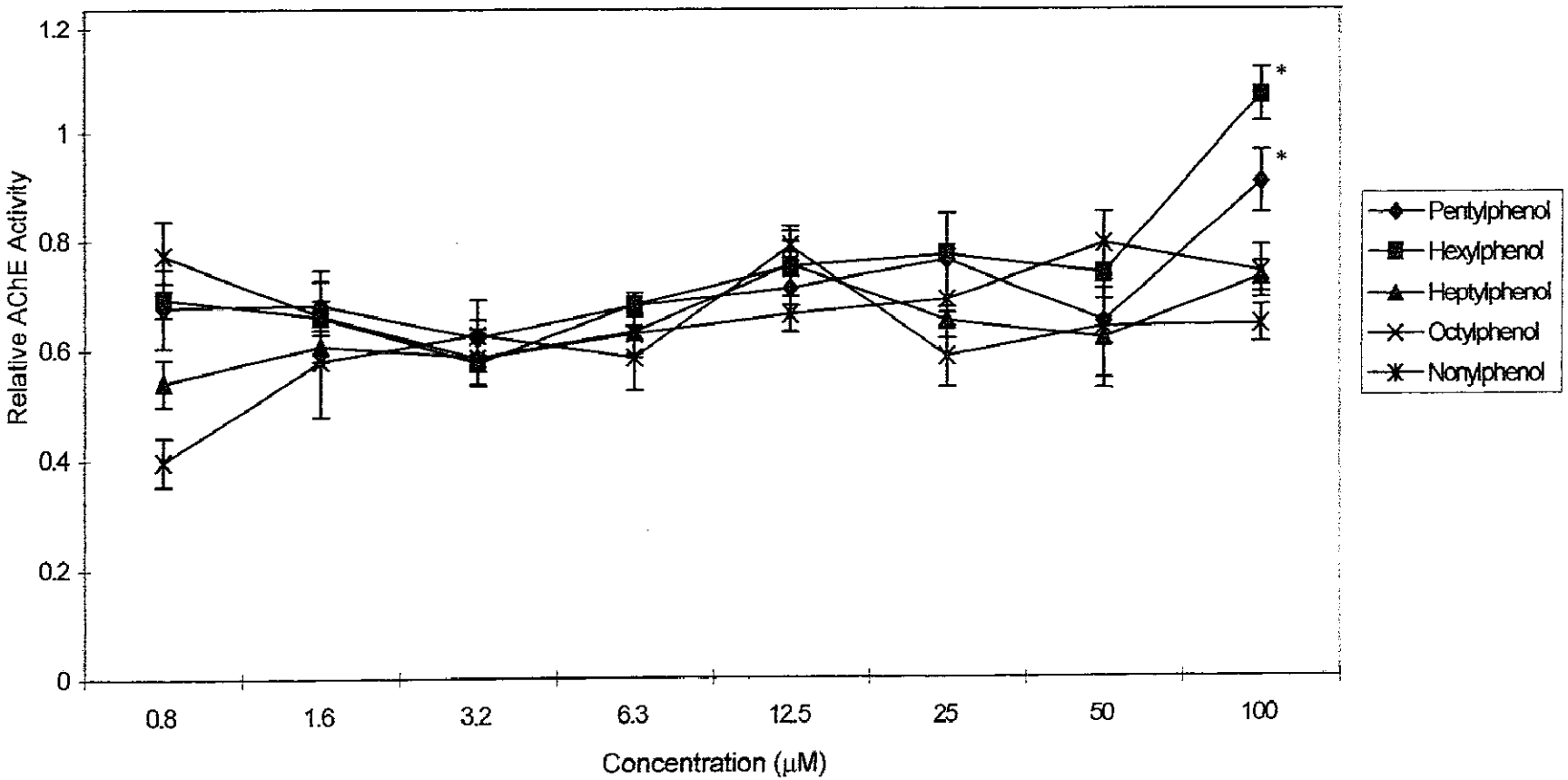

Figure 4. Acetylcholinesterase (AChE) activity of PC12 cells after 24 h incubation with 40 ng ml^{-1} nerve growth factor (NGF), which stimulates AChE release, and varying concentrations of different alkylphenols. Results based from at least three independent replicates are expressed as relative acetylcholinesterase activity, which is obtained by dividing the mean AChE activity of cells incubated with alkylphenolic compounds and NGF by the mean AChE activity of cells incubated with NGF alone (positive control), ± SEM. Relative AChE activity of positive control is therefore 1. Results show significant decreases compared to control ($p<0.05$, t-test), except for those with asterisk (*).

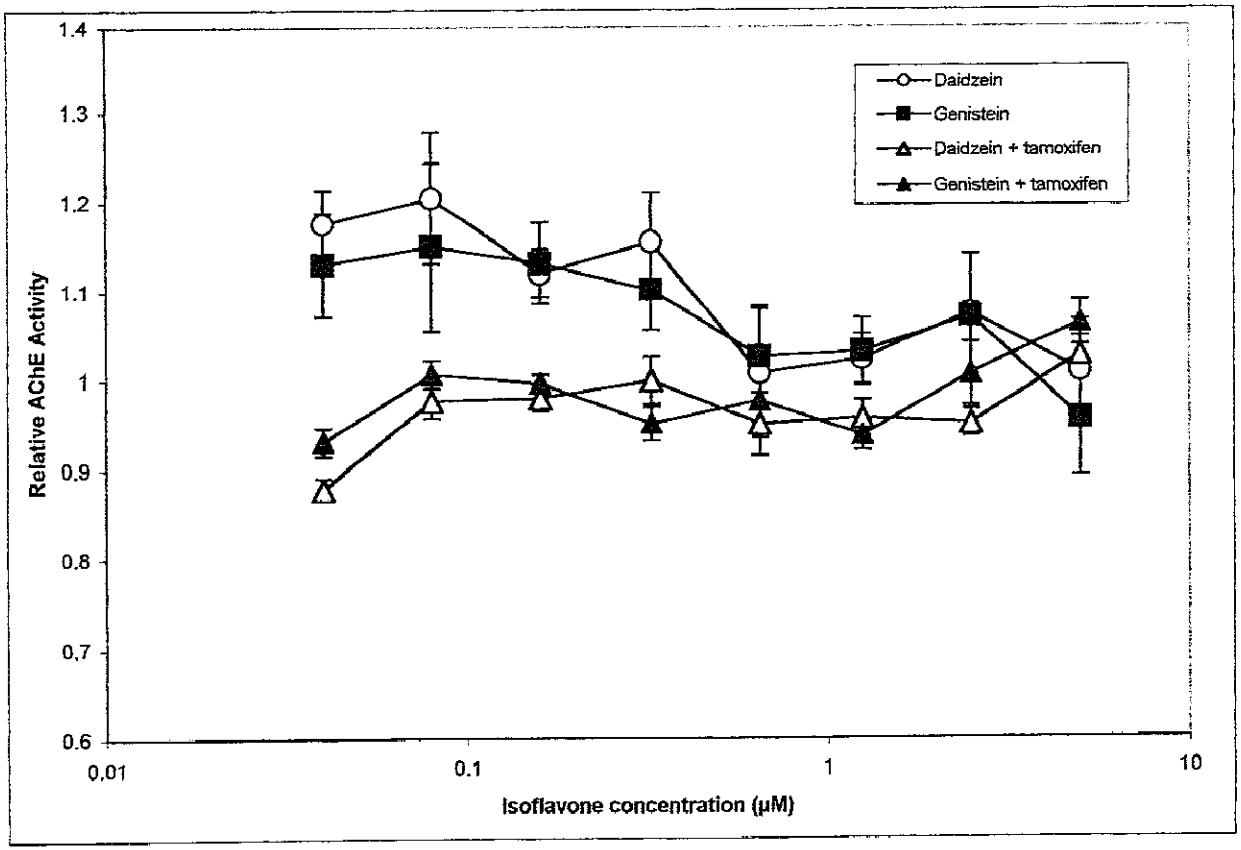

Figure 5. Acetylcholinesterase (AChE) activity of PC12 cells after 24 h incubation with 40 ng ml^{-1} nerve growth factor (NGF), which stimulates AChE release, and varying concentrations of the phytoestrogens genistein and daidzien, with or without 1.5 µM tamoxifen, a known estrogen receptor (ER) antagonist. Results based from three independent replicates are expressed as relative acetylcholinesterase activity, which is obtained by dividing the mean AChE activity of cells incubated with the test compounds and NGF by the mean AChE activity of cells incubated with NGF alone (positive control), ± SEM. Relative AChE activity of positive control is therefore 1. Results show significant differences ($p<0.05$, t-test) in the AChE activities of cells in the presence or absence of tamoxifen at phytoestrogen concentrations of up to 0.325 µM, indicating involvement of the ER in AChE induction.

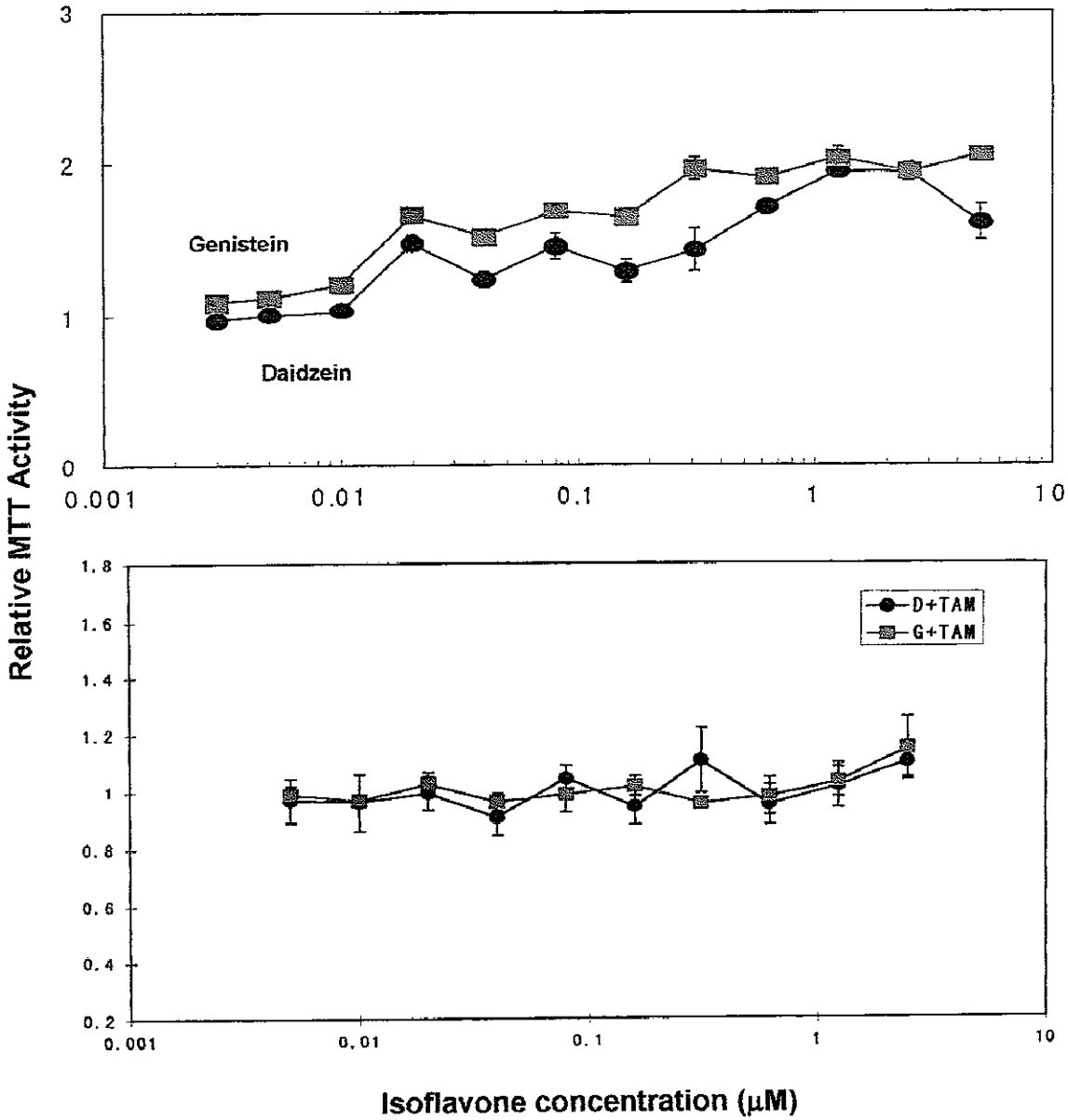

Figure 6. Proliferative activity of MCF7 cells after 6 days incubation with the phytoestrogens genistein and daidzein in the absence *(Fig. 6a, top)* and presence *(Fig. 6b, bottom)* of 1.5 μM tamoxifen, a known estrogen receptor antagonist. Results based from three independent replicates are expressed as relative estrogenic (MTT) activity, which is obtained by dividing the mean proliferative activity of cells incubated with the test compounds with or without tamoxifen by the mean proliferative activity of the control (no additions), ± SD. Relative estrogenic activity of control is therefore 1. Results show increased proliferation of MCF7 cells with increasing phytoestrogen concentration *(Fig. 6a)*, which was effectively blocked by tamoxifen *(Fig. 6b)*. Data analyzed using one-way ANOVA showed significant differences ($p<0.05$) starting at 0.02 μM isoflavone concentration.

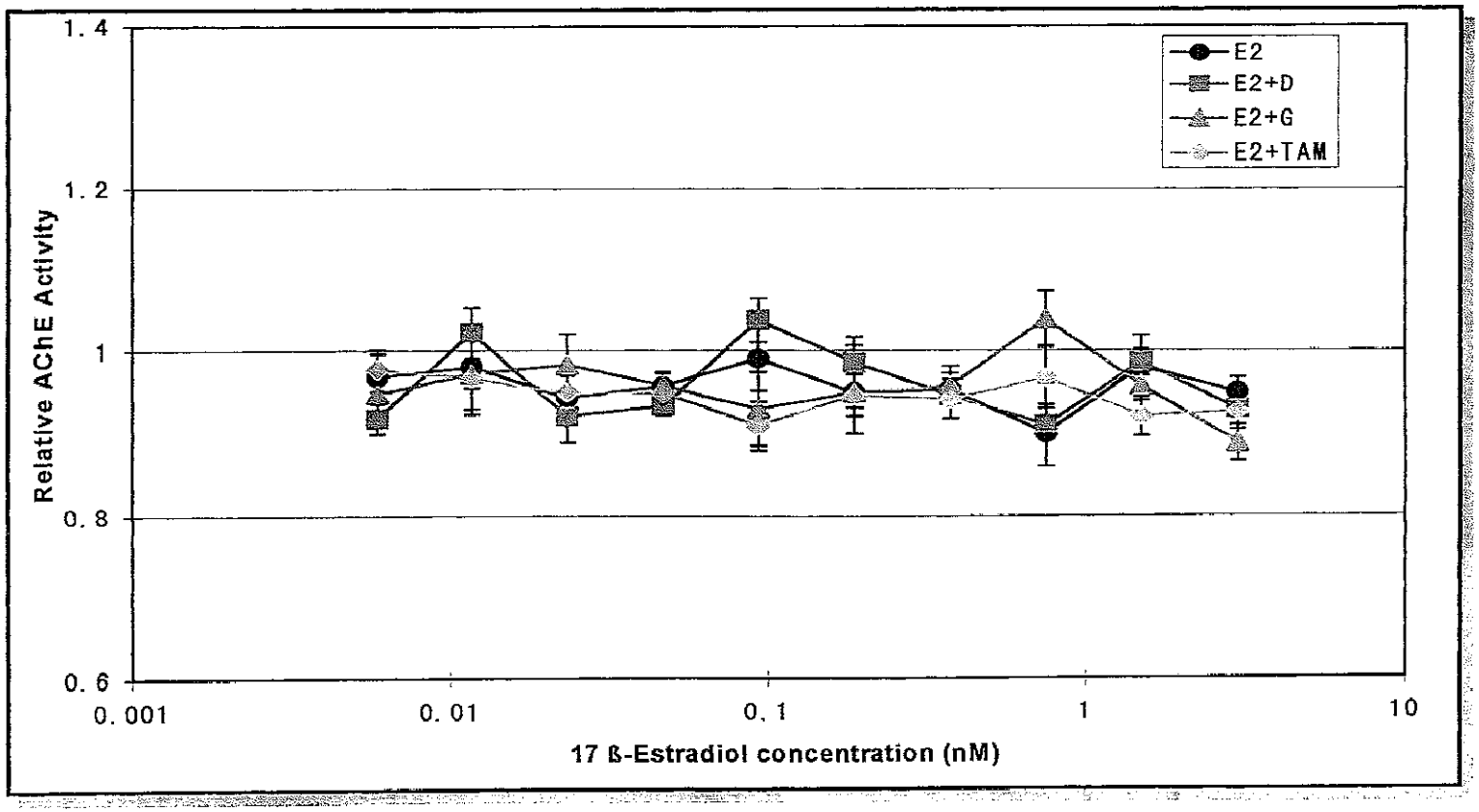

Figure 7. Acetylcholinesterase (AChE) activity of PC12 cells after 24 h incubation with 40 ng ml^{-1} nerve growth factor (NGF), which stimulates AChE release, and varying concentrations of 17 β-estradiol (E2) and E2 plus 0.08 μM genistein (G) and 0.08 μM daidzein (D) or 1.5 μM tamoxifen (TAM). Results based from three independent replicates are expressed as relative acetylcholinesterase activity, which is obtained by dividing the mean AChE activity of cells incubated with the test compounds and NGF by the mean AChE activity of cells incubated with NGF alone (positive control), ± SEM. Relative AChE activity of positive control is therefore 1. Results show no significant differences ($p > 0.05$, t-test) in the AChE activities of cells incubated with E2 in the presence or absence D, G, or TAM, suggesting that the presence of an estrogenic compound alone, in this case, E2, does not necessarily lead to AChE induction.

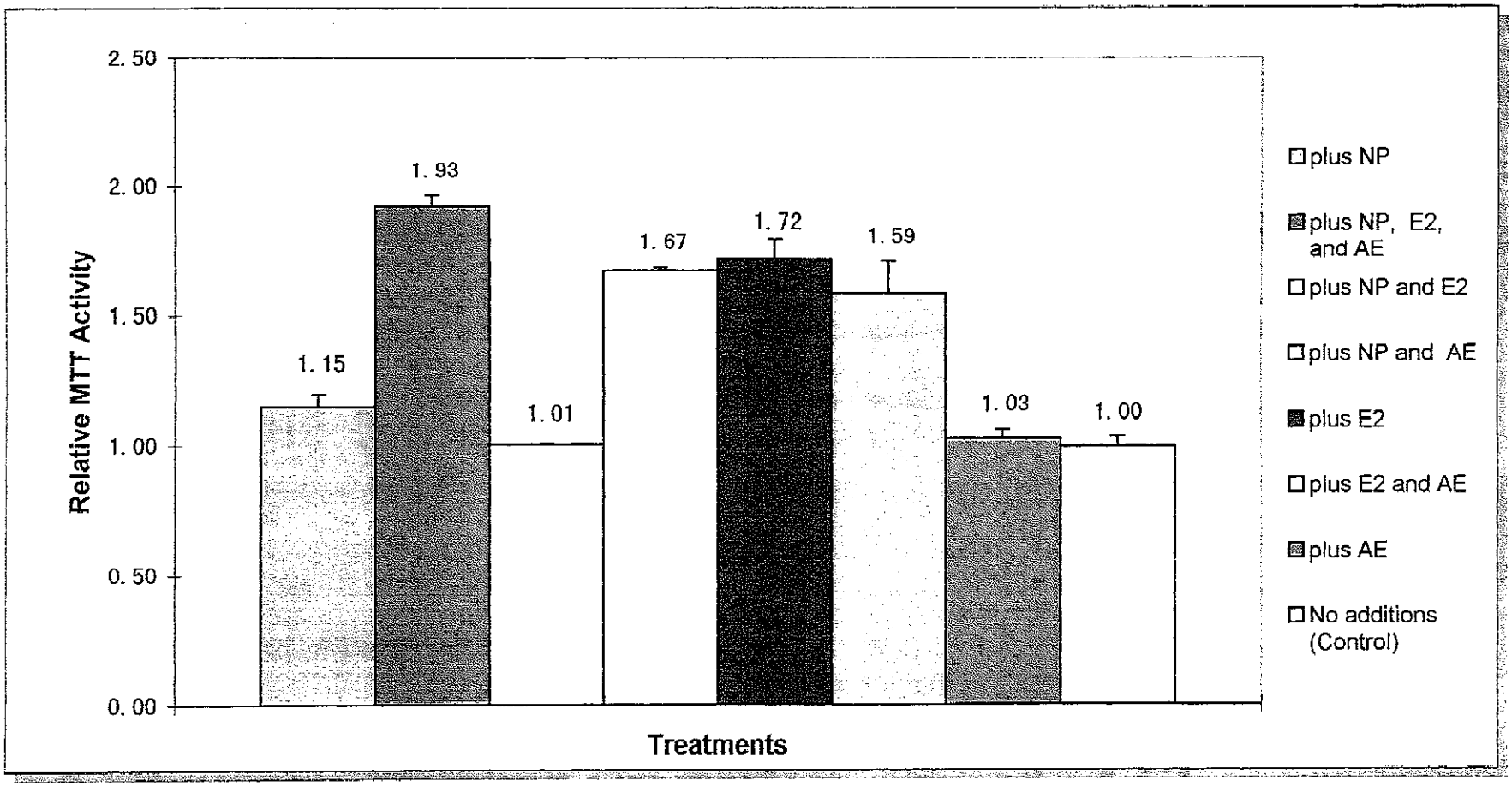

Figure 8. MTT activity of MCF-7 cells in the presence or absence of 1 µM *p*-nonylphenol (NP), 1 nM 17 β-estradiol (E2) and 5% (v/v) *Agaricus* extract (AE). Results based from at least four independent replicates are expressed as relative MTT activity, which is obtained by dividing the mean MTT activity of the cells incubated with the test compounds and/or AE by the mean activity of the control (no additions), ± SD. Relative MTT activity of control is therefore 1. The statistical analyses of this figure are shown in Table 3 (page 42).

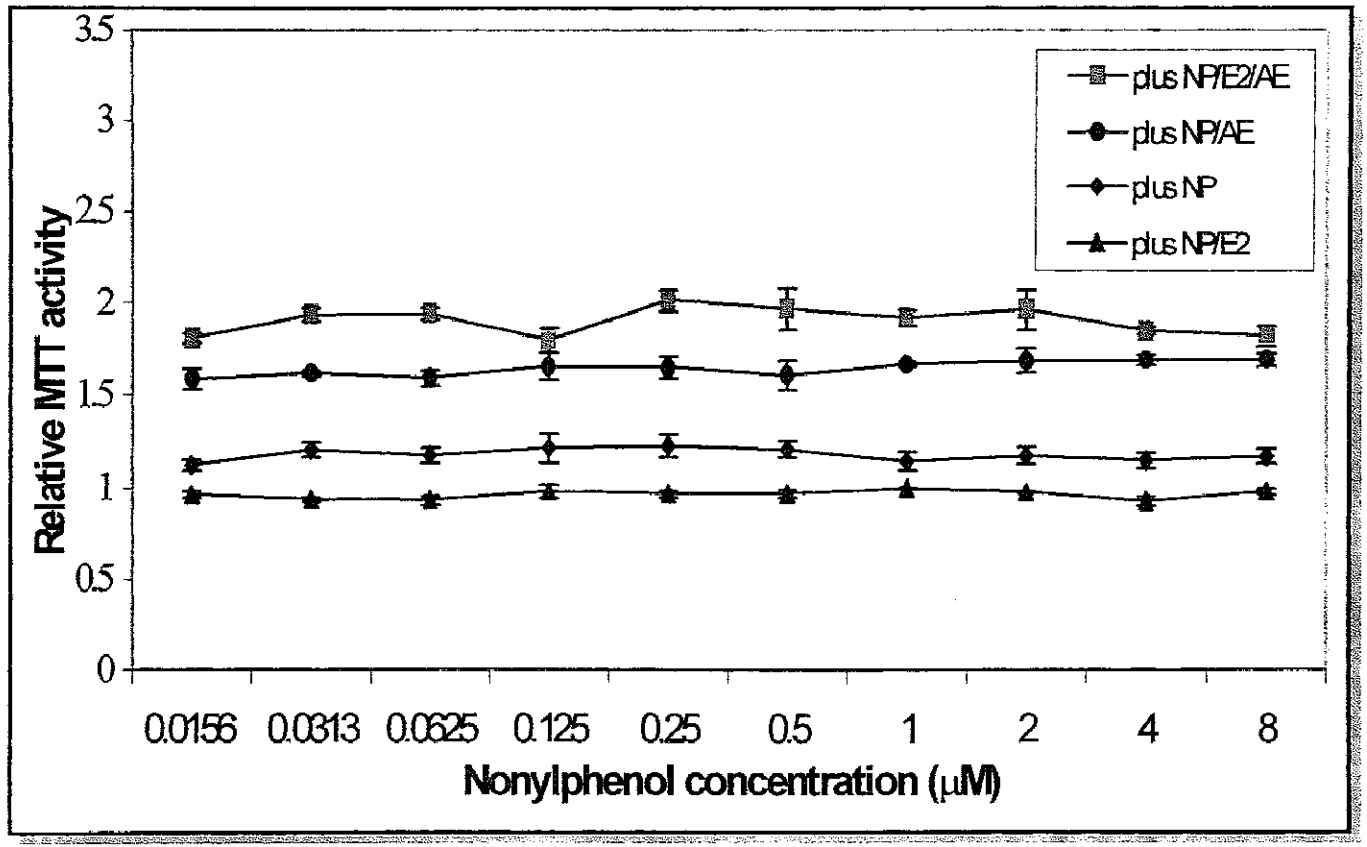

Figure 9. MTT Activity of MCF-7 cells in the presence of varying concentrations of *p*-nonylphenol (NP), plus or minus 1 nM 17 β-estradiol (E2) and 5% (v/v) aqueous *Agaricus* extract (AE). Results based from four independent replicates are expressed as relative MTT activity, which is obtained by dividing the mean MTT activity of cells incubated with the test compounds and/or AE by the mean MTT activity of the control (no additions), ± SD. Relative MTT activity of control is therefore 1. Two-way ANOVA showed significant differences between the <plus NP/E2/AE> and <plus NP/AE> treatments compared to control ($p < 0.0001$) while the differences in the MTT activity of cells incubated in different NP concentrations within the same treatments are not statistically significant ($P > 0.05$).

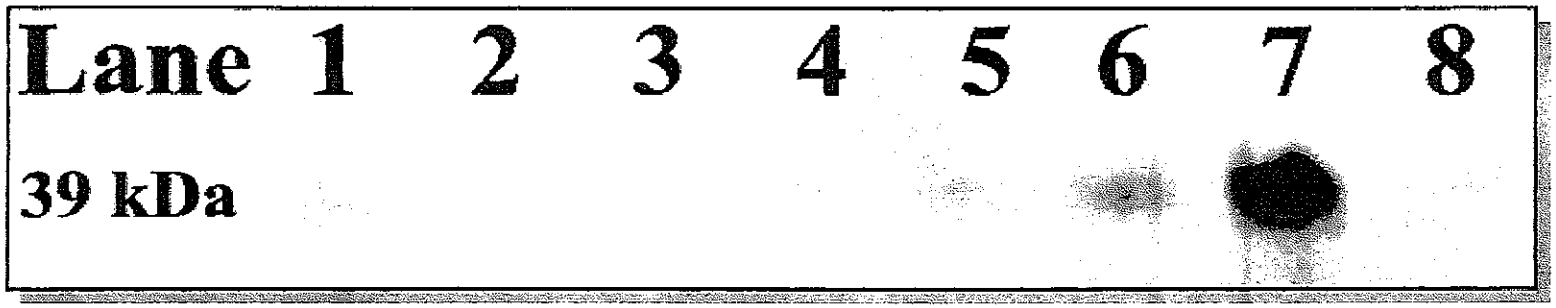

Figure 10. Western blotting results showing the expression of the c-Jun/API protein in MCF7 cells after 6 days incubation in the presence or absence of 1 μM *p*-nonylphenol (NP), 1 nM 17 β-estradiol (E2), and 5% (v/v) aqueous *Agaricus* extract (AE). Lane 1, plus NP only; 2, plus NP, E2 & AE; 3, plus NP & E2; 4, plus NP & AE; 5, plus E2 only; 6, plus E2 & AE; 7, plus AE only; 8, no addition (control). Results show enhanced expression of the c-Jun protein in the presence of 5% (v/v) aqueous *Agaricus* extract (Lane 7).

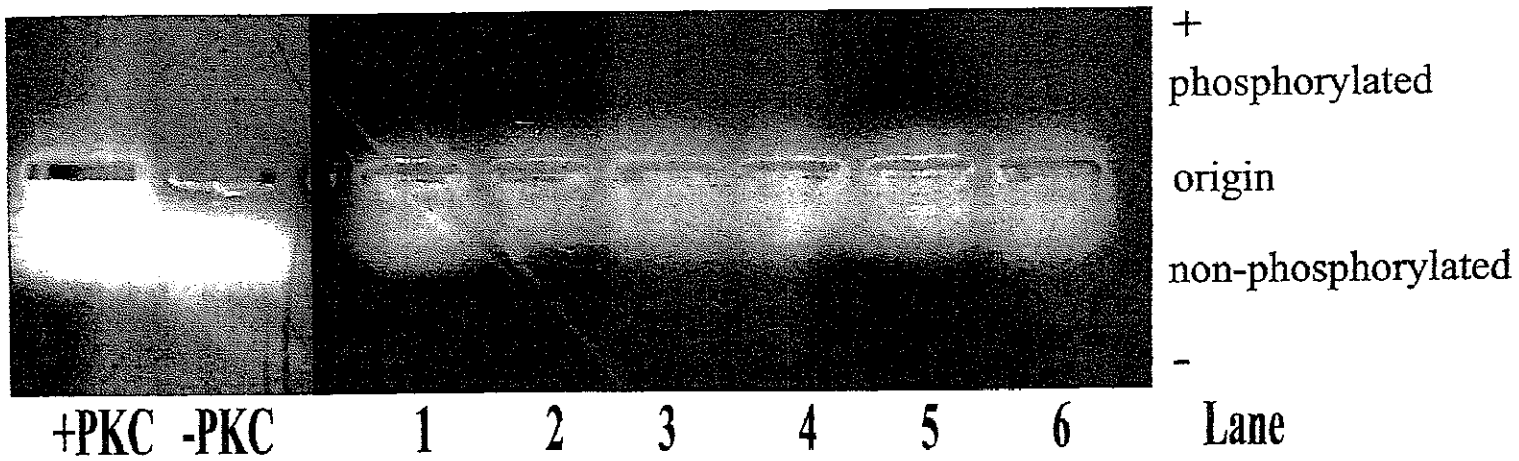

Figure 11. Protein kinase C (PKC) assay results showing PKC activity in MCF7 cells after 24 h incubation in the presence or absence of 1 μM *p*-nonylphenol (NP), 1 nM 17 β-estradiol (E2), and 5% (v/v) aqueous *Agaricus* extract (AE). Lane 1, no addition (control); 2, plus E2 only; 3, plus NP only; 4, plus AE only; 5, plus E2 & AE; 6, plus NP & AE; +PKC, positive control; -PKC, negative control. This means that the PKC enzyme is not a factor in the observed results.