

Migration of Nonylphenols from Polymer Packaging Materials into Food Simulants

LENKA VOTAVOVÁ, JAROSLAV DOBIÁŠ, MICHAL VOLDŘICH and HELENA ČÍŽKOVÁ

Department of Food Preservation and Meat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

VOTAVOVÁ L., DOBIÁŠ J., VOLDŘICH M., ČÍŽKOVÁ H. (2009): **Migration of nonylphenols from polymer packaging materials into food simulants.** Czech J. Food Sci., 27: 293–299.

p-Nonylphenol (NP) is widely used in many industrial applications (detergents, latex paints, pesticides, and plastics), and its presence in the environment has acquired an increasing concern since it was shown to be, besides its persistence and toxicity, an estrogenic compound. Seven samples of stretch PVC films and two PVC dishes for food packaging obtained from food producers were analysed for the presence of NP. Four of the PVC films contained NP at the concentrations of 0.44 mg/g, 1.03 mg/g, 1.28 mg/g, and 1.72 mg/g, respectively, while NP was not detected (the detection level being 5 µg/g) in the remaining films and two dishes. The NP positive films were used for the studies of NP migration into the food simulants. The levels of NP migration into the food simulants: distilled water, 3% acetic acid solution, and 95% ethanol were 0.017–0.091 mg/g (3.2–5.3%), 0.013–0.079 mg/g (2.9–4.6%), and 0.125–0.449 mg/g (21.5–35.0%), respectively. The potential safety risks estimated from the results obtained as well as the possible sources of the NP contamination in the analysed stretch PVC food films are discussed in the following article.

Keywords: nonylphenol; PVC; packaging; migration; food simulant

Nonylphenol (NP, Figure 1) and its derivatives are so-called “emerging contaminants” due to their widespread use, persistence in the environment, and toxicity (NÚÑEZ *et al.* 2007). Nonylphenol (a mixture of predominant *para* and *ortho* isomers with different degrees of branching of the nonyl side chain) is the starting material for the production of a number of derivatives with various uses, the most important being nonylphenol ethoxylates (NPEO_{*n*}, where *n* is a number of ethylene oxide units), which are used as surfactants in cleaning agents, wetting agents, and emulsifiers in the textile industry or polymer emulsifiers (BANDIERA

2006; CHEN *et al.* 2007). Another nonylphenol derivative tris (4-nonylphenol) phosphite is widely used as a stabiliser in rubber and plastics. Barium and calcium salts of nonylphenol are used as heat stabilisers in plastics. Phosphate esters of nonylphenol can be used as flame retardants (HOWE *et al.* 2001). The unmodified NP is used less frequently to assist in keeping other slightly soluble or insoluble materials in solution (CUNNY *et al.* 1997).

Nonylphenol derivatives have been in use for more than 50 years, e.g. more than 500 000 tons of alkylphenol polyethoxylates are produced annu-

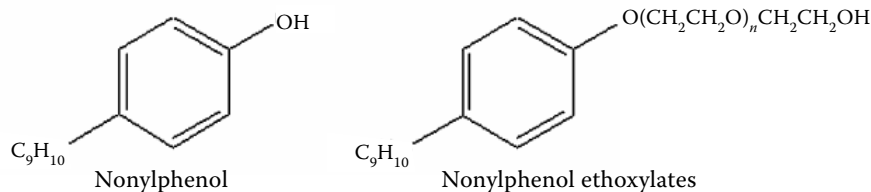


Figure 1. Chemical structures of NP and NPEO_n (NUNEZ *et al.* 2007)

ally worldwide, 60% of which end up in the environment (AZEVEDO *et al.* 2001).

Nonylphenol exhibits low acute toxicity in mammals. The oral LD₅₀ value (dosage lethal to 50% of treated animals) in rats is 1300 mg/kg, but it is more acutely toxic to aquatic organisms (BANDIERA 2006). NP, NPEO₁, and NPEO₂ have been reported to cause a number of estrogenic responses in aquatic organisms, and thus they have been classified as endocrine disruptors (EDCs) by several organisations.

The possible routes of human exposure to NP and NPEs are the dermal contact and inhalation by the workers involved in their manufacture and use, dermal and inhalation exposure of consumers due to household pesticide products, dermal contact with cleaning products and cosmetics, mucous membrane contact with spermicides; inhalatory exposure through the air, via the environment, and oral exposure through drinking water and food via the environment (NIELSEN *et al.* 2000). Food products were analysed also as a potential source of NP and NPEs in the human nutrition. According to a German study (GUENTHER *et al.* 2002) analysing 60 different foodstuffs purchased from supermarkets in Germany, NP is ubiquitous in foods, the levels found ranging from 0.1 to 19.4 µg/kg on the fresh weight basis. The concentration was not dependent on the fat content in the food, higher concentrations of NPs were found in all the analysed samples including both the fatty foods, e.g. butter (14.4 µg/kg), lard (10.2 µg/kg), or liver sausage (13.0 µg/kg), and also the nonfatty food, e.g. marmalade (7.3 µg/kg), apples (19.4 µg/kg), or tomatoes (18.5 µg/kg). NP was present in water from HDPE and PVC containers at 180 ng/l and 300 ng/l, respectively (LOYO-ROSALES *et al.* 2004).

The NPEO_n, which are used as nonionic surfactants in disinfectants and cleaning agents or as emulsifiers in pesticide formulations, are supposed to be the probable origin of foodstuffs and bottled water contamination. In particular, high concentrations of NP in apples and tomatoes can be attributed to the pesticide applications. The

lipophilic NP would be accumulated in the wax coats of fruits and vegetables, respectively. Plastic packaging materials may be another source. The major source of NP residues in food packaging comes from the oxidation of trisnonylphenyl phosphite which is used as an antioxidant in polymeric materials, e.g. polyvinyl chloride (PVC), polyolefins, and acrylics (CASAJUANA *et al.* 2004). In the Czech Republic, the migration of NP from PVC film food packaging was used during the last several years as the argument in the campaign against the general use of PVC for food packaging (ARNIKA 2005).

The objectives of this work were to evaluate the presence of NP in PVC films and to determine the migration of nonylphenol into food simulants.

MATERIAL AND METHODS

Samples. Seven types of stretch PVC films and two PVC dishes for food packaging were used (Table 1). The samples of the packing materials were stored in their original packaging at the room temperature in the dark prior to analysis. Procedural blanks were run before every PVC film sample extraction.

Chemicals. Technical nonylphenol was obtained from Sigma-Aldrich (technical grade, mixture of ring and chain isomers). Methanol, *n*-heptane, acetic acid, and ethanol were HPLC analytical grade from Sigma-Aldrich (Steinheim, Germany). Water was purified with a Milli-Q water purification system from Millipore (USA). All the glassware was washed with acetone followed by heating at 200°C for 4 h prior to use.

Standard solution. A stock solution of 2.5 mg/ml of technical nonylphenol was prepared in methanol and diluted to concentrations of 0.002–2.5 mg/ml. The calibration graphs for these standards were linear within the range tested. The quantification was based on the external standard.

Analytical method. A Hewlett-Packard 6890 Series GC system equipped with an auto-injec-

Table 1. Characterisation of the samples and the NP content in the analysed PVC films

Sample	Sample No.	Thickness (μm)	Unit weight (g/dm^2)	Residue NP (mg/g)
PVC films	1	8	0.1306	1.28 ± 0.11
	2	11	0.2426	< 0.005
	3	10	0.1906	1.72 ± 0.10
	4	10	0.1944	1.03 ± 0.12
	5	13	0.3239	< 0.005
	6	12	0.2693	0.44 ± 0.10
	7	8	0.1738	< 0.005
PVC dishes	8	–	3.0681	< 0.005
	9	–	4.1119	< 0.005

tor and HP 5973 mass-selective detector (Agilent Technologies Inc., Palo Alto, USA) was used for the gas chromatography-mass spectrometry (GC-MS) analysis. The data collection and integration were performed with HP ChemStation software. Chromatographic separations were performed using a DB-5MS capillary column (30 m \times 0.25 mm i.d., 0.25 μm film – J&W Scientific Inc., Folsom, USA). The samples (1.0 μl) were injected in the pulsed splitless mode, the pulse time being 0.3 min and the pressure 70 kPa. The injector temperature was 280°C. Helium carrier gas flow rate was 0.7 ml per minute. The column temperature was programmed from 50°C to 290°C at 10°C/min with the initial isotherm of 2 min and the final isotherm of 10 minutes. The total run time was 22.5 minutes. All the mass spectra were recorded at 70 eV. Quantification of the analytes was carried out in the selected ion monitoring (SIM) mode. The selected masses were m/z 107, 135, 149, 191, and 220 for 4-nonylphenol. The unequivocal confirmation of the identification of the compounds was carried out by the comparison with the authentic standards considering both the full mass spectra and the retention times and with the help of the NIST02 standard mass spectral library.

Quantification was performed using the external standard method. Five-point calibration curves were constructed for the analytes across the range of concentrations from 0.002 mg/ml to 2.5 mg/ml. Analyte calibrations were linear ($R^2 > 0.998$) from the detection limits past the upper ranges of the calibration concentrations.

Extraction of NP. Nonylphenol in the PVC film sample (1.0 g) was extracted with 150 ml of methanol by boiling under reflux for 2 hours. The solution

was concentrated to dryness and redissolved in 2 ml of methanol, then analysed by GC/MS.

Migration test conditions. The solvents selected for the migration of NP from the PVC films were: distilled water (food simulant A according to EU directives 82/711/EC, 93/8/EEC, and 97/48/EC), 3% acetic acid (food simulant B according to EU directives 82/711/EC, 93/8/EEC, and 97/48/EC) and 95% ethanol (evaporable food simulant of olive oil, i.e. food simulant D, according to EU directive 97/48/EC). A gram of the film was cut and immersed in 100 ml of the pre-heated solvent. The migration was allowed to proceed for 10 days at 40°C. The extraction of nonylphenol from the respective food simulant was carried out with *n*-heptane (50 ml). The *n*-heptane solution was then collected in a flask and evaporated to dryness in a rotary evaporator. The dry residues were redissolved in 2 ml of methanol and analysed using GC/MS.

RESULTS AND DISCUSSION

The standard of technical nonylphenol (0.242 mg per ml) was separated by GC system. Gas chromatographic analysis of technical NP resulted in the separation of nine peaks of the NP isomers. The peaks were identified and quantified using the retention times with their characteristic ions of SIM and response factors, respectively. A typical SIM chromatogram of the standard solution of technical nonylphenol isomers (0.242 mg/ml) is shown in Figure 2.

A similar mixture of NP isomers was also obtained by GC/MS analysis of the methanol extract

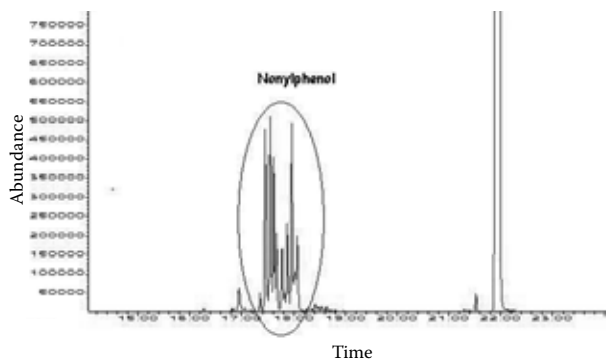


Figure 2. Typical SIM chromatogram of standard solution of technical nonylphenol isomers (0.242 mg/ml)

of PVC films. The NP isomers were detected in four PVC film samples, in the remaining ones their contents were under the detection limit (< 0.005 mg/g).

For the subsequent migration analyses, the positive samples were only used, and the results of the migration tests are shown in Table 2. Examples of the SIM chromatograms of nonylphenol isomers that migrated from sample 1 into the food simulants are shown in Figure 3.

The migration of NP from the PVC films was influenced by the test conditions (distilled water, 3% acetic acid, and 95% ethanol). The amounts of NP migrating from PVC films into a fat food simulant such as 95% ethanol (0.024 – 0.071 mg/dm²) were

higher than those migrating into distilled water and 3% acetic acid (0.004 – 0.017 mg/dm²).

The relationship between the migration of NP from the PVC films into the food simulants (distilled water, 3% acetic acid and 95% ethanol) as related to the polymer mass and NP content in packaging materials is shown in Figure 4. The worse correlations ($R^2 = 0.7023$ for the migration into 95% ethanol, $R^2 = 0.7308$ for the migration into distilled water, and $R^2 = 0.8087$ for the migration into 3% acetic acid) may reflect differences in the polymer film parameters, i.e. the polymer composition, film thickness, etc. These differences can also explain the higher NP migration into 95% ethanol as determined for sample 1 (0.449 ± 0.051 mg/g of polymer) compared with that for sample 3 (0.371 ± 0.042 mg/g of polymer) despite the lower content of NP in film 1 than in film 2 (1.280 ± 0.11 mg/g and 1.723 ± 0.10 mg/g, respectively). Nevertheless, the results obtained document rather a low migration of NP into the food simulants. The same could be expected for real foodstuffs. While methanol at the reflux temperature can extract more than 99% of NP from a PVC film within 2 h (findings of the authors obtained during the evaluation of the used extraction method recovery) or *n*-heptane can elute 62–100% of NP from a PVC film at 25°C within one hour depending on NP residual concentration in the

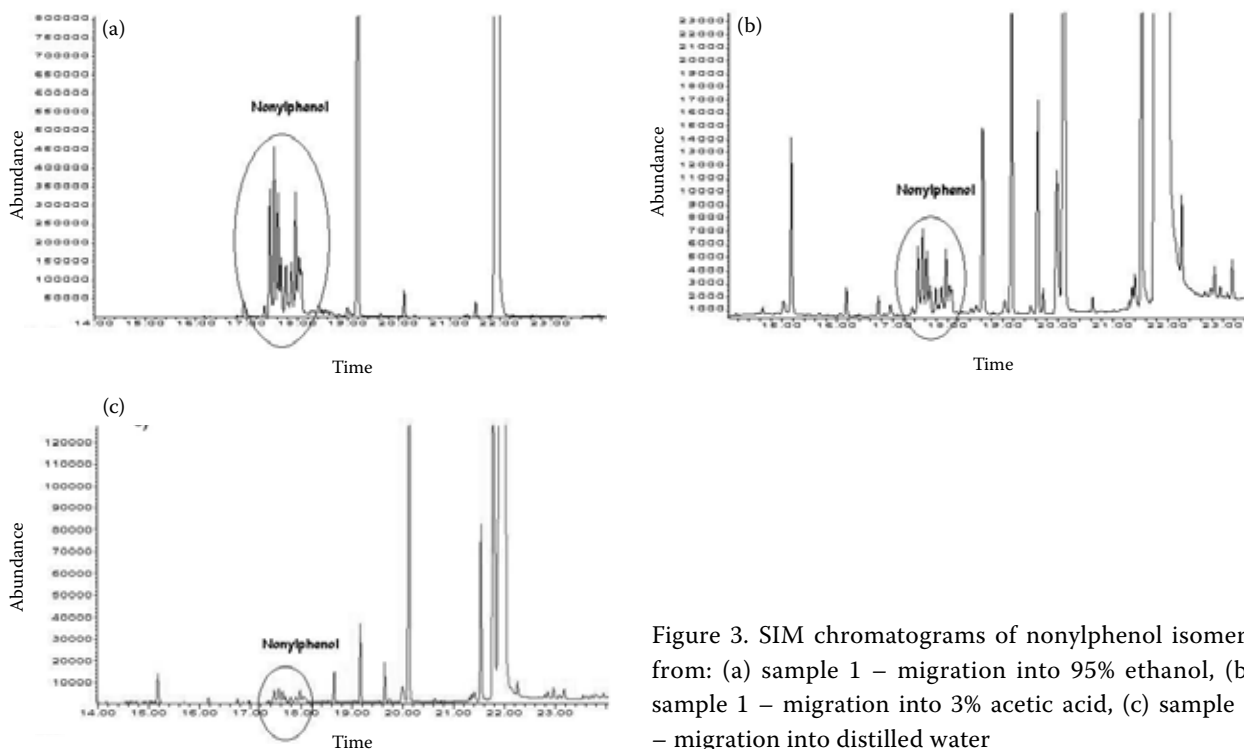


Figure 3. SIM chromatograms of nonylphenol isomers from: (a) sample 1 – migration into 95% ethanol, (b) sample 1 – migration into 3% acetic acid, (c) sample 1 – migration into distilled water

Table 2. The specific migration of NP of the four NP positive PVC films

Sample No.	Food simulant	Released amount of NP (mg/g of polymer)	Detection (%)	NP migration	
				(mg/dm ²)	(mg/kg)*
1	95% ethanol	0.449 ± 0.051	35.1	0.059 ± 0.007	0.352 ± 0.040
	distilled water	0.041 ± 0.014	3.2	0.005 ± 0.001	0.032 ± 0.012
	3% acetic acid	0.040 ± 0.014	3.2	0.005 ± 0.001	0.032 ± 0.014
3	95% ethanol	0.371 ± 0.042	21.5	0.071 ± 0.008	0.423 ± 0.052
	distilled water	0.091 ± 0.018	5.3	0.017 ± 0.004	0.104 ± 0.017
	3% acetic acid	0.079 ± 0.011	4.6	0.015 ± 0.002	0.090 ± 0.012
4	95% ethanol	0.262 ± 0.038	25.5	0.051 ± 0.009	0.305 ± 0.053
	distilled water	0.044 ± 0.019	4.3	0.009 ± 0.004	0.051 ± 0.018
	3% acetic acid	0.038 ± 0.017	3.7	0.007 ± 0.004	0.044 ± 0.021
6	95 % ethanol	0.125 ± 0.033	28.6	0.024 ± 0.006	0.144 ± 0.036
	distilled water	0.017 ± 0.010	4.1	0.005 ± 0.004	0.032 ± 0.019
	3% acetic acid	0.013 ± 0.011	2.9	0.004 ± 0.002	0.027 ± 0.014

*According to Directive 2002/72 (EC 2002) migration values for packaging films expressed in mg/dm² can be converted to mg/kg by multiplying using factor 6

polymer material (INOUE 2001), the found levels of migration into distilled water, 3% acetic acid, and 95% ethanol were much lower, i.e. up to 5%, 4%, and 32%, respectively. These migration levels are about ten times higher compared with those found by INOUE *et al.* (2001) for distilled water and 4% acetic acid but at significantly shorter time of exposure (30 min at 60°C).

The legislation in force Commission Directive 2002/72/EC does not mention NP in the positive

list of permitted additives into polymer materials intended for food contact, and therefore there is no limit for their migration into foods. In agreement with this legislation, no food packaging materials containing NP in levels above the limit of detection by commonly used analytical methods (approximately 5 µg/g) should be used for food packaging (2002/72/EC). On the other hand, NP is a very common pollutant and there is rather a great chance to meet it in food packaging films

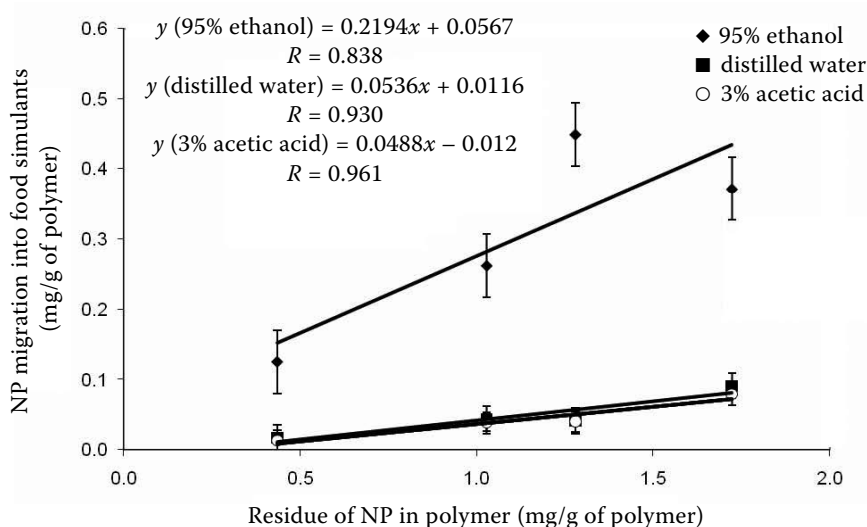


Figure 4. The correlation between the release of NP into the food simulants (95% ethanol, distilled water, 3% acetic acid, 40°C, 10 days) and the content of residual NP in PVC films

utilised in practice. It is obvious that NP is not used as a direct additive into polymers, but more likely it may originate as a component of a more complex additive preparation (e.g. stabiliser). In this context, NP in polymer packaging materials is not an additive but just one of many contaminants. Moreover, the levels of NP migration are more important in the evaluation of the packaging safety than the NP content in the respective polymer.

As concerns the above mentioned facts, one question must be considered. How to evaluate the danger of NP occurrence in the food packaging materials? May in this aspect the food packaging legislation be too strict compared with other aspects of the consumer safety?

The Directive 2003/53/EC of the European Parliament and of the Council of 18 June, 2003 proposed a risk limitation strategy for nonylphenol and nonylphenol ethoxylate. The permitted amounts of NP are equal to or lower than 0.1% by mass (i.e. 1.0 mg/g) for the industrial and institutional cleaning, domestic cleaning, textiles and leather processing, emulsifiers in agricultural teat dips, metal working, manufacturing of pulp and paper, cosmetic products, co-formulants in pesticides and biocides (2003/53/EC).

Concerning the results of this study, the amounts of NP migrating from PVC films into fat food simulants were 0.35–0.42 mg/kg and those migrating into non-fat food simulants 0.030–0.10 mg/kg (Table 2). The human exposition to NP coming from the packaging materials can be evaluated from the following estimation: If the packaging film sample No.1 is used for packaging fatty food, for the wrapping of a 100 g portion of cheese 2 dm² of PVC film are used, and the portion can contain 70 µg ± 8 µg of NP. When eaten in one day by a person of 75 kg, weight, it will correspond to the value of (0.9 ± 0.1) µg/kg bw/day. This value is only seven times higher than the general regular daily NP intake for the adults as estimated by GUENTHER *et al.* (2002) according to the German consumption data. Assuming the NOAEL of 10 mg/kg bw/day (NAGAO *et al.* 2001), it indicates the safety margin of about 11200. This consideration confirms that the intake of NP coming from packaging does not represent any real risk for the consumers. The presence of NP is rather a qualitative defect. There is also some information indicating that the plastic films containing NPs are not of a very reliable origin, and during their production low end additives are used.

NP coming to foods from the packaging is not a topic for panicmongering. A realistic and reasonable approach to nonylphenol is described e.g. DEGEN and BOLT (2003). There is a consensus that endocrine active compounds may cause adverse effects at high doses but the real risks following from xenoestrogens under the realistic exposure conditions are still under discussion. Compared to other endocrine disruptors, NP is rather weak, estrogenic and natural phytoestrogens being usually present at much higher dietary levels than NP. On the other hand, it is evident that, due to a high content of additives, plasticised PVC represents generally higher health risks when compared to other polymer packaging materials.

References

- ARNIKA (2005): News Release of Association Arnika 15. 12. 2005: Analýzy opět prokázaly kontaminaci PVC obalů na potraviny zakázanými škodlivými látkami. Available at: <http://pvc.arnika.org/tz.shtml?x=1042425>
- AZEVEDO D., LACORTE S., VIANA P., BARCELÓ D. (2001): Occurrence of nonylphenol and bisphenol-A in surface waters from Portugal. *Journal of the Brazilian Chemical Society*, **12**: 532–537.
- BANDIERA S.M. (2006): Reproductive and endocrine effects of *p*-nonylphenol and methoxychlor: A review. *Immunology, Endocrine & Metabolic Agents – Medicinal Chemistry*, **6**: 1–26.
- CASAJUANA N., LACORTE S. (2004): New methodology for the determination of phthalate esters, bisphenol A, bisphenol A diglycidyl ether, and nonylphenol in commercial whole milk samples. *Journal of Agricultural and Food Chemistry*, **52**: 3702–3707.
- CHEN L., ZHOU H., DENG Q. (2007): Photolysis of nonylphenol ethoxylates: The determination of the degradation kinetics and the intermediate products. *Chemosphere*, **68**: 354–359.
- CUNNY H.C., MAYES B.A., ROSICA K.A., TRUTTER J.A., VAN MILLER J.P. (1997): Subchronic toxicity (90-day) study with para-nonylphenol in rats. *Regulatory Toxicology and Pharmacology*, **26**: 172–178.
- DEGEN G.H., BOLT H.M. (2003): Comments on “Endocrine disrupting nonylphenols are ubiquitous in food”. *Environmental Science & Technology*, **37**: 2622–2623.
- GUENTHER K., HEINKE V., THIELE B., KLEIST E., PRAST H., RAECKER T. (2002): Endocrine disrupting nonylphenols are ubiquitous in food. *Environmental Science & Technology*, **36**: 1676–1680.

- HOWE S.R., SURANA P., JAKUPCA M.R., BORODINSKY L. (2001): Potential dietary exposure to *p*-nonylphenol from food-contact use of tris(nonylphenyl)phosphite (TNPP). *Food Additives and Contaminants*, **18**: 1021–1039.
- INOUE K., KONDO S., YOSHIE Y., KATO K., YOSHIMURA Y., HORIE M., NAKAZAWA H. (2001): Migration of 4-nonylphenol from polyvinyl chloride food packaging films into food simulants and foods. *Food Additives and Contaminants*, **18**: 157–164.
- LOYO-ROSALES J.E., ROSALES-RIVERA G.C., LYNCH A.M., CLIFFORD P., RICE C.P., TORRENTS A. (2004): Migration of nonylphenol from plastic containers to water and a milk surrogate. *Journal of Agricultural and Food Chemistry*, **52**: 2016–2020.
- NAGAO T., WADA K., MARUMO H., YOSHIMURA S., ONO H. (2001): Reproductive effects of nonylphenol in rats after by gavage administration: a two-generation study. *Reproductive Toxicology*, **15**: 400–404.
- NIELSEN E., ØSTERGAARD G., THORUP I., LADEFOGED O., JELNES O., JELNES J.E. (2000): Toxicological evaluation and limit values for nonylphenol ethoxylates, ticsesyl, phosphates and benzoic acid. Environmental Project (Miljøprojekt), No. 512. The Danish Environmental Protection Agency, Denmark.
- NUNEZ L., TURIEL E., TADEO J.L. (2007): Determination of nonylphenol and nonylphenol ethoxylates in environmental solid samples by ultrasonic-assisted extraction and high performance liquid chromatography-fluorescence detection. *Journal of Chromatography*, **1146**: 157–163.
- 82/711/EC Council Directive of 18 October 1982 laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs. 1982.
- 93/EEC Commission Directive of 15 March 1993 amending Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of the constituents of plastics materials and articles intended to come into contact with foodstuffs. 1993.
- 97/48/EC Commission Directive 97/48/EC of 29 July 1997 amending for the second time Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs. 1997.
- 2002/72/EC Commission Directive of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. 2002.
- 2003/53/EC Directive 2003/53/ec of the European Parliament and of the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement). *Official Journal of the European Union*, L 178: 24–26.

Received for publication September 29, 2008

Accepted after corrections April 6, 2009

Corresponding author:

Ing. LENKA VOTAVOVÁ, Ph.D., Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav konzervace potravin a technologie masa, Technická 5, 166 28 Praha 6, Česká republika
tel.: + 420 220 443 014, fax: + 420 220 444 404, e-mail: lenka.votavova@vscht.cz
