Scientific paper

Presence of Nonylphenols in Plastic Films and Their Migration Into Food Simulants

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Abstract

Nonylphenols (NP) possess estrogenic activities and may cause increased risk of cancerous and reproductive abnormalities in several living organisms. Despite their harmful nature, NPs are used as additives in food packaging material, especially plastic, which is the main food contact material in today's packaging landscape. According to the fact that food packaging is one of the major potential food contaminant sources regarding NPs, there is a need for more information on identifying and quantifying of these chemicals when migrating into food.

Seventeen plastic films for food packaging were analyzed for the presence of NPs; nine of them were plastic films for deep freeze food packaging. We determined and quantified NPs by Gas Chromatography-Mass Spectrometry (GC-MS) using the internal standard method. All plastic films for deep freeze food packaging contained NPs within a concentration range from 11.9 to 43.7 mg/kg. We also studied the migration of NPs from three of the plastic films for deep freeze food packaging into food simulants. The migration of NPs from these plastic films into 95% ethanol and 10% ethanol was higher than the amount migrating into distilled water or 3% acetic acid.

Keywords: Nonylphenols; migration; plastic films; food packaging; GC-MS; food simulants

1. Introduction

NPs are widely used for the synthesis of nonylphenol polyethoxylates (NPnEO, where n is the number of ethoxylates units), which are one of the most important types of nonionic surfactants. In 1995, the annual worldwide production of NPnEO was estimated to be 500,000 tons.[1,2] NPnEOs are also used as dispersive agents during paper and leather manufacturing, emulsifiers for pesticide formulation, and antifogging agents in the polymer industry .[1–4] NPs are being used as stabilizers in the manufacture of plastics. The major source of NP contamination in the environment is the microbial degradation of NPnEO, although contamination by NP may also result from migration of the plastic products.[1–3]

NPs, which were listed as one of the 33 priority pollutants proposed in the EU Water Framework Directive (Decision 2455/2001/CE),[5] are a mixture of different branched and linear chain isomers (ortho-, meta-, para-), the most common being para isomer (4-NP).[2] Moreover, the presence of 4-NP in surface waters is regulated by the EU (Directive 2000/60/CE).[6]

NPs are toxic xenobiotic compounds classified as endocrine disrupters capable of interfering with the hormonal systems of numerous living species.[1,4] As an estrogenic endocrine disrupting chemical, NPs might alter, compete with, or displace natural hormones in organisms and change their functions. NPs were found to mimic natural hormone 17β-estradiol by competing for the binding site of the hormone receptor (Figure 1A), due their structural similarity (Figure 1 B).[7] NPs are capable of inducing the production of vitellogenin in a male fish, a protein normally only found in sexually mature females under the influence of natural estrogens.[1] *In vivo* data suggested that NP could induce uterine growth in immature female rats. The impact of NP on the environment include the feminization of aquatic organisms, decrease in male fertility and the survival of juveniles at concentrations as low as 8.2 μg/L.[4]

The potential undesirable health effects of NPs in humans are unknown and difficult to assess but concerns were raised about human exposure to NPs. It is believed to occur after dermal contact with cleaning products and cosmetics, oral exposure through contaminated water and food or during occupational exposure.[1,4] According to a study by Guenther,[8] NP was found to be ubiquitous in foodstuffs with concentrations ranging from 0.1 to 19.4 μg/kg, with an estimated daily intake of 7.5 μg/day for an adult. NPs were found in all 60 samples analyzed including apples, marmalade, meat, tomato, etc.[8] However, a Taiwanese study[9] suggested that the average daily NP intake in Taiwan is four times higher (28 μg/day) than those of the Guenther study. The pathways for NP contamination of food were probably via the use of cleaning agents or as emulsifiers in pesticides and surfactants in disinfectants.

One of the pathways for the NP contamination of food is foodstuff packaging material that might release NP as a break-down product of trisnonylphenyl phosphite (TNPP) and other nonylphenolic compounds (NPnEO).[3,10] TNPP is used as an antioxidant to stabilize the polymer coating used as a food contact material against the degradation by ultraviolet light. NPs are present in TNPP as a residual impurity and can be formed as a result of the acid hydrolysis of TNPP. Food packaging can interact with the packed food by diffusion-controlled processes, which mainly depend on the chemical properties of food contact material (FCM) and foodstuffs exposure to UV light, temperature and the storage time of the product. This interaction can lead to the migration of FCM compounds from the packaging into food.[10]

The aims of our work were to evaluate the presence of NP in randomly selected plastic films for food packaging and to estimate the migration of NP into food simulants. For NP determination a modified standard method (SIST ISO 18857-2: "Determination of selected alkylphenols") was used.[11]

2. Experimental

2. 1. Samples

Seventeen plastic films for food packaging were tested, nine of them being plastic films for deep-freeze food packaging (Table 1). The plastic film samples were randomly selected from various supermarkets in Slovenia and stored at room temperature in the dark until analysis. The types of plastic films were confirmed using an FT-IR Spectrometer (Spectrum 100, Perkin Elmer, USA). 1 dm^2 pieces of each film were cut and weighed (g/dm^2).

2. 2. Chemicals

All reagents and solvents used were at least of analytical grade. Ethanol, dichloromethane (DCM) and acetic acid (100%), were purchased from Sigma Aldrich (Germany), acetone from Riedel-de-Haän (Germany), anhydrous sodium sulphate from J.T.Baker (Deventer, The Netherlands). Ultrapure water was prepared with a Nanopure water purification system (Barnstead, Int., Dobuque, IA, USA). For identification and quantification, reference NP mix standard solution and ^{13}C-labeled (363 NP-$^{13}C_6$), an internal standard solution in acetone, according to standard ISO 18857-2, were obtained from Sigma Aldrich (Germany).

A N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) derivatization grade, supplied by Aldrich (Ger-

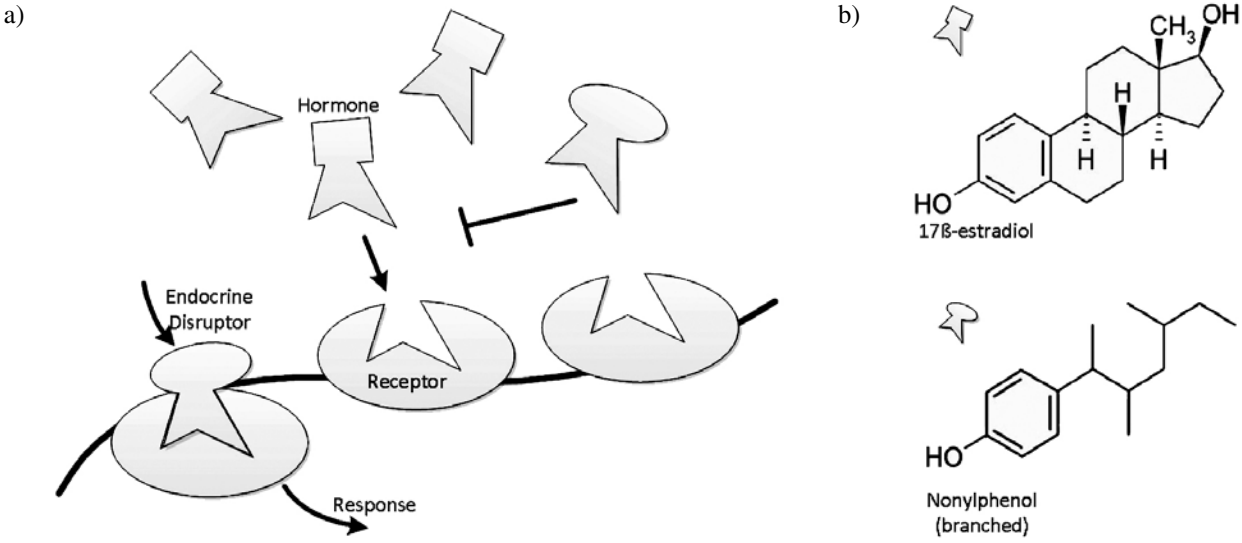

Figure 1: Competition for the estrogen binding receptor between 4-NP and 17β-estradiol in the cell, one of the mechanisms of action of NP (A), and a comparison between the chemical structures of hormone 17β-estradiol and branched 4-nonylphenol as suggested by Warhurst[7] (B).

many) was used as the silylation reagent. Nitrogen (99.996%, Messer, Austria) was used for solvent evaporation.

2. 3. Standard Solutions and Quantification

A reference standard working solution of NP isomer mixtures at 0.5 ng/μL and an internal standard working solution of 0.1 ng/μL were prepared in acetone and stored at –20 °C.

A set of five calibration standard solutions covering a concentration range between 0.1 and 5 ng/μL was used for quantitation. The internal standard, 363 NP-$^{13}C_{6,}$ was spiked into the standard solutions with a final concentration of 0.25 ng/μL. Concentrations of NP were calculated as a sum of the peak areas of all isomer peaks according to the internal standard method. Blank tests were performed to control any laboratory contamination of NP.

2. 4. Analytical Methods and Performance

A Polaris Q ion trap mass spectrometer (Thermo electron corporation, USA) equipped with a Trace GC ultra (Thermo electron corporation, USA) was used for analysis. Separation of the analytes was performed on a ZB-5ms capillary column (60 m × 0.25 mm i.d. and a film thickness 0.25 μm, Zebron, Phenomenex). 2 μL of the sample extracts were injected in a splitless mode by an AS 3000 auto sampler (Thermo electron corporation, USA) with splitless time of 1 min. The oven temperature programme was as follows: 1.1 min at 65 °C, 10 °C/min to 230 °C, 6 °C/min to 270 °C, and then 40 °C/min to 300 °C (1 min). The total run time was 22.5 min. The injector temperature was 255 °C. Helium 6.0 (Messer, Austria) was used as a carrier gas at a constant flow rate of 1 mL/min.

The GC-EI(+)MS/MS technique was used for the quantitative determination of NP. m/z 207 and m/z 221 ions were chosen as parent ions in the MS/MS determination for the trimethylsilyl derivate of NP mixture of isomers (NP-TMS). For TMS derivate of "single isomer" NP-$^{13}C_6$ internal standard, m/z 227 was chosen as the parent ion. A sufficiently wide mass window (mass window = 23) was used in order to cover the two base peaks of different isomers (m/z 207 and m/z 221) and the base peak of the internal standard (m/z 227). The mass fragments in the range m/z 207 to m/z 227 were exposed to secondary fragmentation by a collision induced dissociation with the helium gas in the ion trap, using a collision excitation voltage of 3 V. The following daughter ions were selected: m/z 179 and m/z 163 for NP and m/z 185 and m/z 169 for NP-$^{13}C_6$ internal standard, for quantification and identification.

Method performance was evaluated by analyzing the spiked samples (In-house QC samples) and method blank samples for each set of migration experiments. The sample blank was measured under the same analytical conditions for each set of migration experiments but without adding any plastic film. One aliquot was analyzed as a sample blank. The second aliquot was spiked with NP standard solution at the middle point of the calibration curve (0.75 ng/μL) and included in the analysis scheme (QC). Compounds were identified by matching the retention times and by the presence and relative responses of the selected daughter ions. Identification of the compounds was also confirmed using a full scan mass spectra.

Quantification was performed using the internal standard method. Quantification of NP (sum of isomers) was performed using the sum of peak areas of the m/z 179. The presence and relative response of fragment m/z 163 was observed for confirmation. For the internal standard NP-$^{13}C_6$ the area of fragment m/z 185 was used for quantification, and m/z 169 for confirmation.

Good linearity was obtained within the concentration range of 0.1 to 5 ng/μL ($R^2 > 0.98$).

2. 5. Sample Preparation and Migration Test Condition

In our initial study samples of 500 mg food packing film were cut into pieces and extracted with 50 mL of DCM by soaking for 10 days at room temperature. An aliquot (200 μL) of the sample extract was transferred to the test tube, and 250 μL of the internal standard solution (0.1 ng/μL) was added. The spiked sample extracts were then evaporated to dryness under a moderate stream of nitrogen.

According to the EU directives 82/711/EC, 93/8/EEC and 97/48/EC[12], the selected solvents for the migration of NP from the plastic film were distilled water (food simulant A for aqueous foods), 3% acetic acid (food simulant B for acidic foods), 10% ethanol (food simulant C for alcoholic foods) and 95% ethanol (food simulant D for fatty foods). 500 mg of the film was cut and immersed in 50 mL of the selected solvent. In accordance with chapter II in the EU directive[12], migration was carried out for 10 days at 40 °C for both types of samples.

Extraction of the analytes from simulants A, B and C was performed by liquid-liquid extraction with DCM. 10 mL of each of the sample simulants was spiked with 250 μL of internal standard solution (0.1 ng/μL) and extracted twice with 3 mL of DCM. Extracts were then dried with anhydrous sodium sulphate and evaporated to dryness under a moderate stream of nitrogen.

For the fat-food simulant, 500 μL of 95% ethanol solution extracts were spiked with the internal standard as described above, and concentrated to dryness.

2. 6. Derivatization

The dry residues of sample extracts were re-dissolved in 70 μL of acetone. For derivatization of the hydroxyl groups, 30 μL of MSTFA was added to the acetone so-

lution of the sample extract and mixed gently. After a reaction time of at least 5 min at room temperature, the derivatized sample extracts were transferred to a vial, and analysed with GC-MS.

3. Results and Discussion

Technical NP consist of a mixture of mainly branched para-isomers with up to 6% branched ortho-isomers.[2] Standard GC-MS analytical techniques do not lead to a satisfactory separation of mostly co-eluting isomers. [2,13,14] The typical GC-MS full scan chromatogram of NP-TMS in the plastic film sample 1, and the GC-MS/MS chromatogram with selected ions chromatograms, are shown in Figures 2 and 3.

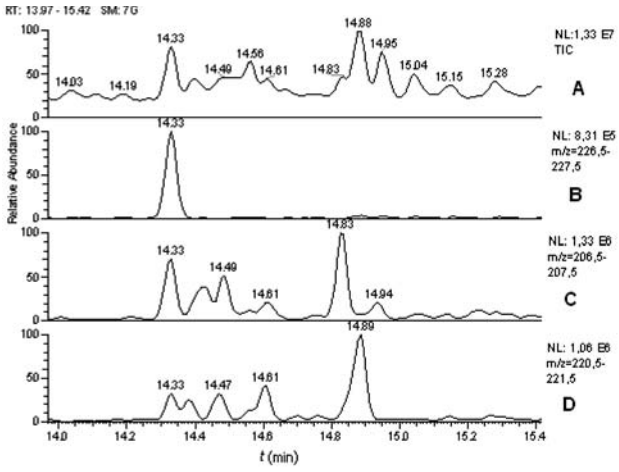

Figure 2: GC-MS full scan chromatogram of technical NP-TMS in plastic film sample extract (A) and related ion trace chromatograms of m/z 227 (B), m/z 207 (C) and m/z 221 (D).

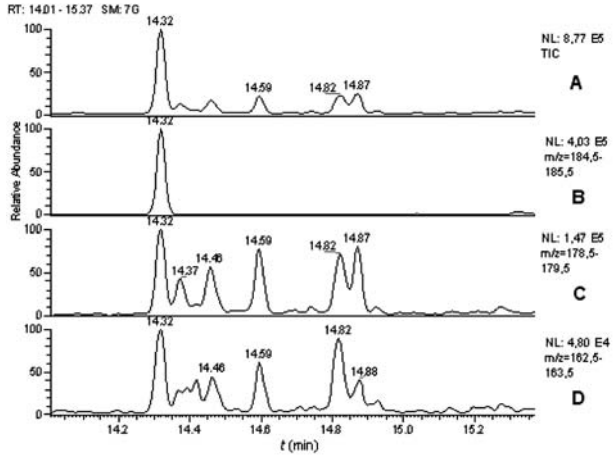

Figure 3: GC-MS/MS chromatogram of plastic film sample extract (A) and ion trace chromatograms of m/z 185 (B), m/z 179 (C), m/z 163 (D).

Besides dealing with the mix of isomers, the analytical challenge also represents the mass fragmentation of NP isomers in EI(+)MS mode. The mass spectrometric fragmentation pattern of NP depends on the grade and position of branching of the alkyl chain. The mass spectra of different NP isomers mainly vary in the base peaks.[2,13] In accordance with SIST-ISO 18857-2[11] for NP-TMS, m/z 207 is used for quantification, and m/z 221 and 179 may be used for identification. However, m/z 207 on its own is insufficient for quantitative determination, due to its low abundancy or the absence of some isomers (Figure 2). m/z 207 and m/z 221 should be used for quantification, even though there is a potential problem with the relative response. In order to improve the analytical approach of NP with GC-MS, we used the MS/MS technique with both m/z 207 and m/z 221 as the parent ions. Ion m/z 179 is produced with secondary fragmentation, which is the daughter fragment of both m/z 207 and m/z 221 parent ions (Figures 2 and 4). Quantification of NP isomers was than performed using the sum of the peak areas of the ion m/z 179. Identification was confirmed by the presence and relative response of fragment ion m/z 163.

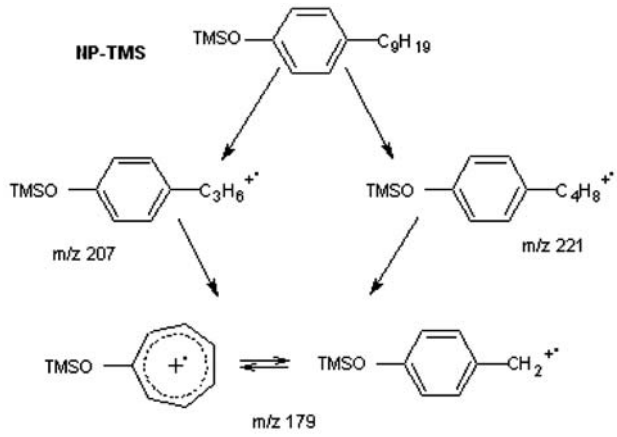

Figure 4: EI-MS fragmentation pathways of the 4-NP-TMS.

In our study, NP isomers were detected in all DCM extracts of deep freeze packaging film, whilst in the plastic films for non-deep freeze food packaging, their concentrations were under the detection limit. The levels of NP in the deep freeze food packaging are shown in Table 1, and were within the range of 11.9 mg/kg to 43.7 mg/kg. Slices of plastic material with and without external printing were extracted for three samples in order to determine whether the migrants originated from the plastic material itself or from the externally applied printing ink. The amounts of migrant released from the plastic films, with or without printing, are comparable (Table 1), the differences are within the range of method uncertainty.

Table 1: Concentration of NP found in DCM extracts of deep freeze plastic films in two different plastic types (PE = polyethylene; PP = polypropylene).

Sample No.	Plastic Type	NP (mg/kg) in film	NP (mg/kg) in unprinted film
1	PE	37.4	–
2	PE	32.7	–
3	PP	23.7	24.2
4	PE	17.3	12.7
5	PE	11.9	15.4
6	PP	31.8	–
7	PE	43.7	–
8	PE	12.0	–
9	PE	13.9	–

Three of the plastic films (sample nos. 1, 2 and 3) were further used for the studies of NP migration into food simulants (Table 2). Quality control samples (QC) and blanks were included during the analyses of every batch experiment (Table 3).

In the case of Sample no. 2, the amount of NP released from the plastic film into 95% ethanol was up to 64.9 mg/kg of polymer. This level was much higher than that migrating into distilled water, 3% acetic acid, and 10% ethanol. It is even higher than the amounts of NP migrating from plastic films into DCM. The highest amount of NP was in the thinnest film (Sample 1). According to FT-IR, the spectral acquisitions performed on both sides of the films, Samples nos. 1, 2 and 3, were homogeneous PE or PP films (Table 1).

Table 3: Results of the quality assurance measurements in final extracts.

Food simulant	Blank (ng/µL)	QC (ng/µL)	QC Recovery (%)
95% ethanol	< 0.10	0.75	100%
10% ethanol	< 0.10	0.71	95%
distilled water	< 0.10	0.86	115%
3% acetic acid	< 0.10	0.79	105%
DCM	< 0.10	0.72	96%

which were much thinner than the polymer films tested in our study.

Substances authorized for the production of food contact materials are specified in the EU positive list of starting materials and in the incomplete positive list for additives ("Plastic FCM Directive" 2002/72/EC and amendments: 2004/1/EC, 2004/19/EC, 2005/79/EC, 2008/39/EC, 975/2009).[16] The incomplete list of permitted additives into plastic materials intended for food packaging in this specific directive (Annex III) does not include the additive TNPP, so it may be used provided it complies with Article 3 of the Regulation (EC) No. 1935/2004.[17] On the other hand, NP in polymer packaging materials are not additives or starting substances but break down products or impurities. The presence of such compounds in the final products must also comply with the requirements of Article 3 of the Regulation (EC) No. 1935/2004, [17] which states that substances should not be transferred to foodstuffs in a quantity which could endanger human health or bring about an unacceptable change

Table 2: Migration of NP from the selected plastic films.

Sample No.	Food simulant	Weight (g/dm^2)	DCM extraction[a] (%)	4-NP migration (mg/kg of polymer)	(mg/dm^2)
1	95% ethanol	0.480	173	64.9	0.03
	10% ethanol		13.7	5.1	0.002
	distilled water		8.3	3.1	0.002
	3% acetic acid		5.9	2.2	0.001
2	95% ethanol	0.555	127	41.5	0.02
	10% ethanol		14.8	4.9	0.003
	distilled water		4.7	1.5	0.0009
	3% acetic acid		5.1	1.7	0.0009
3	95% ethanol	0.660	117	27.7	0.02
	10% ethanol		3.5	0.84	0.0006
	distilled water		2.2	0.52	0.0003
	3% acetic acid		2.6	0.61	0.0004

[a] In comparison with the results obtained using DCM extraction.

The results of migration levels into H_2O and 3% acetic acid are about ten times lower compared to those found by Votavová,[15] but migration levels into 95% ethanol are almost the same as found by Votavová. These differences might result from polymer composition and polymer thickness, since Votavová used stretch PVC films in the composition of the foodstuffs. A risk assessment should be carried out, in order to ensure compliance with Article 3 of the Regulation (EC) No. 1935/2004, for substances NP and TNPP present in food packaging. Furthermore, Regulation 1907/2006/EC[18] provides that NP and NPEO should not be placed on the market or used as a

substance or constituent of preparations in concentrations equal or higher than 0.1% (w/w) for the purposes of industrial, domestic, and institutional cleaning, textiles and leather processing, metal working, manufacturing of pulp and paper, cosmetic products or co-formulants in pesticides, and biocides. Although this Directive covers a wide application, there are no restrictions for the use of NP in plastic food packaging.

4. Conclusion

A GC-MS/MS method was successfully applied to the analysis of NP in plastic (PE and PP) films for deep-freeze food packaging. NPs were detected in all of the plastic films for deep-freeze food with concentrations of 11.9 to 43.7 mg/kg. In the other tested plastic films, concentrations of NP were under the limit of detection. The amounts of NP migrating from three of the deep freeze plastic films into fat food simulants were 27.7–64.9 mg/kg of polymer, and the amounts of NP migrating into non-fat food simulants were 0.5–5.1 mg/kg of polymer.

Although the NP relative potency as an endocrine disrupter is only 0.0023 of the natural estrogen 17β-estradiol,[4] chronic exposure to NP migrating from food contact materials might present a human health risk, and thus requires a risk assessment. With the increasing use of freezable food, consumers also are being increasingly exposed to NP migrating from the plastic films. The widespread presence of NP in plastic food packaging materials should be updated according to recent scientific knowledge.

5. Acknowledgment

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Povzetek

Nonilfenole (NP) uvrščamo med motilce delovanja endokrinega hormonskega sistema. Zaradi posnemanja delovanja naravnih hormonov lahko povzročijo povečano tveganje nastanka raka in reproduktivne nepravilnosti v živih organizmih. Kljub neugodnim lastnostim, se kot dodatki uporabljajo v embalažah za živila, predvsem v plastičnih folijah. Embalaža za živila je lahko eden izmed glavnih izvorov NP v živilih. Zato so podatki o vsebnosti NP v embalaži in predvsem podatki o prehajanju NP v hrano zelo pomembni.

Prisotnost NP smo preverili v sedemnajstih plastičnih folijah, ki se uporabljajo za embalažo živil. Devet izmed vzorcev je bilo plastičnih folij, ki se uporabljajo za hranjenje globoko zamrznjene hrane. Vsebnost NP smo določili s plinsko kromatografijo v povezavi z masno spektrometrijo (GC-MS) z metodo internega standarda. V vseh preiskovanih folijah, ki se uporabljajo za embalažo zamrznjene hrane smo zaznali NP v koncentracijskem območju od11,9 mg/kg do 43,7 mg/kg folije. Z migracijskimi poskusi smo preverili prehod NP iz treh plastičnih folij v modelne raztopine. V modelni vodni raztopini z 95 % in 10 % etanola je migrirala večja količina NP kakor v samo destilirano vodo ali v modelno vodno raztopino z 3 % ocetne kisline.