Primary aromatic amines (PAAs) in black nylon and other food-contact materials, 2004–2009
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Primary aromatic amines (PAAs) were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in migrates from 234 samples of food-contact materials, including black nylon (polyamide) kitchen utensils \((n=136)\), coloured plastics (28), and clear/printed multilayer film/laminates (41), from retailers, importers, and food producers. A further 29 utensils in use were obtained from colleagues. Very high PAA migration was found from black nylon kitchen utensils to the food simulant 3\% acetic acid: the ‘non-detectable’ limit (20\(\mu\)g aniline equivalents kg\(-1\) food) was exceeded by up to 2100 times. All the other materials were compliant. The majority of the non-compliant utensils came from China. The predominant PAAs were aniline and 4,4\₀-methylenedianiline (4,4\₀-MDA). The frequency of violations decreased from the year 2004 (55\%) to the autumn of 2005 (13\%), possibly due to increased demands for in-house documentation, but they remained almost constant from 2005 to 2009. The validity of the results was shown by recovery studies, participation in proficiency testing, and comparative testing of utensils by two laboratories. Migration modelling was used to compare how various compliance migration test conditions influenced the final test results. Long-term release of PAAs was fitted by diffusion modelling experiments and long-term release was also seen as expected from used utensils. Toxicologists consider these migration levels of the suspected carcinogenic PAAs as a problem of major concern.

Keywords: survey; regulations; risk assessment; liquid chromatography/mass spectrometry (LC/MS); food-contact materials; migration; diffusion; colours; food simulants

Introduction

Primary aromatic amines (PAAs) and their uses

PAAs are defined as chemicals having a primary amine \(-\text{NH}_2\) attached to an aromatic ring and several of them have genotoxic, carcinogenic, and allergenic effects according to toxicological \textit{in vitro} and \textit{in vivo} studies. Epidemiological studies of the PAA 4,4\₀-methylenedianiline (4,4\₀-MDA) also indicate a risk of bladder cancer (Vitenskapskomiteen for mattrygghet (VKM) 2006). In the ‘Plastics Directive’ for food-contact materials (FCM), PAAs originating from isocyanates or azo-colours were regulated by a specification with a specific ‘non-detectable’ migration limit for the sum of PAAs of 20\(\mu\)g aniline equivalents (eq.) kg\(-1\) food (including uncertainty) of food or food simulant (European Commission 2002a). In May 2009, the limit was changed to a sum-limit of 10\(\mu\)g kg\(-1\) food or food simulant (plus uncertainty) and only PAAs appearing on the plastics positive lists are excluded from this restriction (European Commission 2007a). Examples of PAAs include some monomers and starting chemicals, additives and non-authorized compounds, whereas other PAAs can be formed as degradation products of the finished food-contact plastic. For food-contact materials, the most studied sources are PAA formation from aromatic isocyanates and azo-colours. Isocyanates are used to make polyurethane adhesives and lacquers, from where residuals or breakdown products can migrate, e.g. from flexible plastic films into food simulants (Lawson et al. 1996, 1999; Brede et al. 2003). Azo-colours can be used in printing inks for plastic, paper and board (Brede and Skjevrak 2004; Skjevrak et al. 2005), and are also used as food additives, where both free and bound forms of PAAs have been characterized (Voyksner et al. 1993; Lancaster and Lawrence 1999). Numerous other uses of PAAs are also described in textbooks and patents on polymers, elastomers and additives, but there is hardly any published migration data on these (Monsanto 1989; Bart 2005; Lattimer and Williams 2002).

Other studies of PAAs and kitchen utensils

It was the black colour, suspected to be an azo-colour, that in 2004 brought the attention of the Norwegian authorities to black kitchen utensils made of polyamide (interchangeably called nylon and PA). Of the 15 black and blue utensils tested, aniline migration was
measured from eight of them, as well as in the black raw material of polyamide 6.6. The utensils, primarily of a Swedish brand, also contained 30% glass reinforcement. The conclusion was that the aniline came from the black colour, and that migration would continue over time as the consumer used the utensil from the black colour, and that migration would violate by three out of 50 polyamide utensils (Skjevrak et al. 2005). Migration at 100°C into water in 4 h and at 175°C into olive oil in 30 min was tested. From laminates, small amounts of other PAAs were also found, probably from the printing ink.

Other European Union enforcement laboratories from, for example, Slovenia (Perharic et al. 2006), Germany, the Netherlands, the Czech Republic, Finland, France, Spain, the United Kingdom (Food Standards Agency (FSA) 2007), Ireland, and Italy have also since 2005 tested black nylon kitchen utensils. The findings are reflected in the number of Rapid Alerts, issued by the European Commission through the Rapid Alert System for Food and Feed (RASFF), on PAAs, which was approximately two in 2003, nine in 2004, 29 in 2005, 29 in 2006, 25 in 2007, 16 in 2008, and 23 in the first 9 months of 2009 (European Commission 2009). With this background, in November 2006 and April 2007 the European Commission sent a delegation to China to inspect the Chinese FCM enforcement system and quality of methods (European Commission 2007b).

**Analytical methods**

Since 2001, the European Standardization body (CEN TC194/SC1/Utensils in contact with food/TG9) has worked on improving the sensitive, but semi-quantitative spectrophotometric sum method for PAAs (Brauer and Funke 2001). Members of the CEN group have also developed suitable quantitative and specific verification methods, including an SPE-HPLC-UV method on ten PAAs (Brauer and Funke 2002), an LC-MS/MS method for 20 PAAs made by our laboratory (Mortensen et al. 2005), and a derivatization-SPE-GC-MS method on eight PAAs (Brede et al. 2003). Recently, the two LC-based verification methods have been through an intercalibration test, which is an important step towards having an official CEN method (Kappenstein et al. 2009). According to the CEN group, the spectrophotometric method is suitable for deeming samples compliant, when migration is less than 2 μg·kg⁻¹, whereas a verification method must be used to deem samples non-compliant, because some authorized PAAs are covered by separate limits, e.g. 4,4'-methylenedibis-(3-chloro,2,6-diethyl)-aniline CAS No. 106246-33-7 with a Quantum Maximum per unit area (QMA) restriction of 0.05 mg 6 dm⁻².

An agreed and validated CEN method was nevertheless desired by both the Chinese authorities and the European Commission (European Commission 2007b).

**The present study**

In the present study, 234 food-contact materials (black nylon kitchen utensils, various coloured utensils and clear/printable flexible films/laminates) were tested by LC-MS/MS for the specific migration of 20 PAAs which could potentially come from either aromatic isocyanates or azo-colours by direct LC-MS/MS. From 2004 to 2009, unused FCM items were sampled from Danish retailers, importers and food producers in a series of six enforcement campaigns, one of which was targeted at suspected violations, while the others were market surveys. Kitchen utensils previously used by consumers were also analysed for PAA migration. Furthermore, predictive migration modelling was used to compare how various compliance migration test conditions influenced the final test results. The diffusivity parameters used for wet nylon was derived from a long-term migration study.

**Materials and methods**

**Origin of samples and sampling procedures**

In 2004, eleven samples of black nylon kitchen utensils were purchased in Danish retail shops and analysed as part of a validation study of the analytical LC-MS/MS method (Mortensen et al. 2005). In the 2005a campaign (2005 targeted market control), new, unused food-contact materials were sampled upon suspicion by local food inspectors, in case of insufficient own-check documentation. Only food-contact materials with a known or an anticipated risk of containing PAAs were picked: 28 coloured plastic kitchen utensils (Table 2) were sampled from Danish importers, while multilayer materials as printed/coloured plastics (18 samples) and clear film (23 samples) were taken from Danish food-producing companies. Six of the multilayer materials were co-extruded and another four were plastic laminated with board. Twenty-two black nylon kitchen utensils were sampled from importers.

In the 2005b campaign (2005 market control), food inspectors collected 33 black nylon kitchen utensils available from Danish importers at the time. In the 2007, 2008 and 2009 campaigns (32, 23 and 15 samples, respectively), market control samples of black nylon were tested by the regional laboratory. In 2005, used kitchen utensils purchased in 1997–2003 were donated...
by colleagues from our institute, together with information on the age of the utensils, the types of food they had been used for, for how long, at what temperatures, and how often each week. A survey of the number of PA kitchen utensils sampled per year is shown in Figure 1. Details about all kitchen utensils are given in the attached datasheet.

In the enforcement campaigns, the food inspectors were instructed to wrap samples in clean aluminium foil and place them in plastic bags. For the multilayer plastic films, either the whole reels of film or the middle part of a roll were sampled, three to 15 layers beneath the outside layer, and sent to the authors. Upon reception in the laboratory, flexible films were frozen at \(-20^\circ C\). All other samples were kept at room temperature until analysis.

Chemicals and equipment

LC-MS/MS (Quattro Ultima, Waters/Micromass, Manchester, UK), thermostated heating cabinets for migration testing, glassware and equipment for automated Soxlet extraction (Büchi, Flawil, Switzerland), Fourier transform infrared spectrometry (FTIR) equipment for the determination of polymer type (Spectrum One, Perkin Elmer, Skovlunde, Denmark), glassware and chemicals were as previously described (Mortensen et al. 2005). In the 2007 analysis, standard substances (Dr. Ehrenstorfer, Augsburg, Germany) were exchanged with the regional laboratory, together with a mixed stock solution of all the PAAs except p-PDA, which was made up freshly every day. This mix was used for both the quantification and the verification of PAA.

Principle of LC-MS/MS analysis

The accredited LC-MS/MS method previously described was used to quantify 20 PAAs in the aqueous food simulants, distilled water and 3% acetic acid (3% HAc) at the National Food Institute (Mortensen et al. 2005). In brief, migrates were injected directly onto a C\(_{18}\)-phase column using pentafluoropropionic acid (PFPA) as the ion-pairing agent, in a gradient water/methanol mobile phase. PAAs were ionized in positive electrospray mode (ESI\(^+\)), followed by detection of one product ion from one precursor ion (MRM mode). To verify the identity of a PAA, an extra daughter ion was quantified and the target/qualifier ion ratio determined.

The above method was used with minor modifications and accredited at the regional laboratory as well. The column was an Acquity UPLC HSS T3 (2.1 x 100 mm, 1.8 \(\mu\)m particle size), from Waters (Milford, MA, USA) analogue to the C\(_{18}\) column. Analytes in 3.3 \(\mu\)l injection volumes of the food simulant were separated in only 5 min using 0.1% formic acid as a substitute for PFPA.

Sample preparation

Samples were prepared in accordance with European technical legislation (European Economic Community (EEC) 1982, 1985) and European Standard EN13130-1 (European Committee for Standardisation (CEN) 2004), unless otherwise stated. Migration experiments were performed without exposure to light. Each sample consisted of three sub-samples, taken from identical materials or articles.

Film

Pouches (1 dm\(^2\), 2 dm\(^2\) surface area) were made (cut with the same printed pattern on them) and filled with 100 ml food simulant.

Fillable articles

Fillable articles with original lids were filled, otherwise the sub-sample was fully immersed into a food simulant.

Kitchen utensils

Typically, kitchen utensils were too large to fit into the beakers of the Büchi instrument, which is why subsamples were cut in the largest possible pieces and fully immersed in the food simulant. In the comparative study (verifications of non-compliant samples), three sub-samples were cut from the same utensils at both laboratories. Single-use articles, such as film, were exposed once, and analysis made on each sub-sample. For repeated-use articles, for which it is the third migration test that should comply with legislation, sub-samples were pooled together in the first test and migrates analysed. If contents were > 5 \(\mu\)g aniline eq. kg\(^{-1}\) (in the 2004 and 2005a studies) or > 20 \(\mu\)g aniline eq. kg\(^{-1}\) (2005b and 2007–2009 studies), then a...
second test was performed with pooled sub-samples as in the first test. In the third migration test, which should comply with legislation, each sub-sample was exposed and analysed separately. Surface areas were measured by drawing the surface area and cut edges of the article onto paper, followed by cutting and weighing the paper. The ratio between exposed article surfaces to the volume of food simulant in the control tests was between 1.8 and 11.5 dm² kg⁻¹.

Migration conditions for enforcement tests
Samples that had clear instructions on maximum contact times, temperatures and/or food types were tested accordingly following legislation in the technical directives (EEC 1982, 1985). Other samples were exposed to worst-case migration conditions, i.e. 3% HAc, and exposure times of 0.5–1 h depending on the type of utensil. If usage temperatures were estimated to be above 175°C, the testing times were increased by a factor of 4 to compensate for the maximum temperature of aqueous simulants of 100°C (EEC 1982). Typical testing conditions for kitchen utensils were 2 or 4 h at 100°C in 3% HAc under reflux.

Conversion of units into aniline equivalents and into μg kg⁻¹
In order to compare a result with the PAA limit (before 1 May 2009), the concentration of a specific amine (μg PAA 1⁻¹) must be transformed into μg aniline eq. kg⁻¹ of food or food simulant. This was done in the following way:

\[ n_{\text{PAA}} = n_{\text{aniline}} = \sum n_{\text{PAA}} \times M_{\text{aniline}} \times M_{\text{PAA}}^{-1} \]

where \( n \) is the number of moles; \( M \) is molecular mass (g mol⁻¹); and \( m \) is mass (g). The migration test is performed exposing an area of material (\( A \)) to a portion of food simulant, which contains a total mass (\( m \)) of amine. \( C_A \), the migration per unit area, can then be combined with the actual surface area-to-mass ratio in use (dm² kg⁻¹) to obtain \( C_M \), the migration per unit mass of food simulant:

\[ C_A = m_{\text{aniline}} A^{-1} \text{ (μg aniline eq. dm}^{-2} \text{)} \]

\[ C_M = C_A (\mu g \text{ aniline eq. } \times \text{dm}^{-2}) \times A(\text{dm}^2 \text{ in real use } \times \text{kg}^{-1}) \]

Depending on the type of article, either \( C_A \) or \( C_M \) should be compared with the limit. The conventional surface area-to-mass ratio of 6 dm² kg⁻¹ was used for non-fillable articles (kitchen utensils) and where no recommended volume of food-to-surface area was given (EEC 1982). In this paper, all results are, for reasons of simplicity, expressed in μg kg⁻¹ and compared with the 20 μg kg⁻¹ limit after multiplying the test result in μg dm⁻² with the conventional factor of 6. Strictly speaking, according to legislation, the test result for utensils in μg aniline eq. *dm⁻² should be compared with a legislative limit of 20/6 = 3.3 μg aniline eq. *dm⁻². Since 1 May 2009, the limit has been 10 μg ΣPAA*kg⁻¹ or 10/6 = 1.67 μg ΣPAA*dm⁻².

Long-term tests and modelling MDA migration from nylon utensils
Two reinforced nylon utensils (samples 3 and 5) were tested repeatedly in new portions of 3% HAc at 100°C, each for three times for 2 h in an open system (extra food simulant was added regularly to compensate for evaporated simulant). The second and third tests were performed 7 days after the first test. Subsequently, samples were brought into contact with new portions of pre-warmed food simulants in closed migration cells for another 114, 2, 116 and 2 h at 95°C. Finally, two 10-min tests were performed.

Diffusion-controlled migration modelling was performed for samples 3 and 5 for 4,4’-MDA, using Migratext Lite 2001 software with 2004 updates (Forschungs-GmbH für Analytik und Bewertung von Stoffübergängen (FABES), Germany). Input data included the molecular weight of the specific PAA (4,4’-MDA = 198 g mol⁻¹), solubility of the PAA in the food simulant (fully soluble), sample geometry (spoon with a thickness = 2.1 mm; input = half thickness due to double-sided migration), times, temperatures, correction term to the activation energy (\( r = 0 \)), and contact conditions (full immersion). \( A_p \) values (a diffusivity-related constant) are linked to the polymer type, and officially recognized values are listed in the European Union Practical Guide (European Commission 2003). For polyamide, however, only an \( A_p \) value for dry PA6 is listed, whereas PA in contact with water will swell and exhibit different diffusivity and hence a different \( A_p \) value. Consequently, modelling experiments with various combinations of \( A_p \) values and initial PAA concentrations in the nylon (\( c_{p,0} \) values) were tested and compared with the measured data from samples 3 and 5 (Figure 2). This was done with the aid of Otto Piringer (FABES 2006–2009). The best fit for wet, reinforced PA was obtained with \( c_{p,0} = 1630–1660 \) mg kg⁻¹ plastic and \( A_p = 6.5 \) (\( r = 0 \)), which was also used at 175°C. The residual amount of 4.4 MDA in sample 5 after the test was determined to 310 mg kg⁻¹ plastic by total extraction, which is not too far from the expected residual concentration of 135 mg kg⁻¹ calculated by the model. The fitted \( A_p \) value was considered sufficiently precise to use for modelling the influence of various test scenarios on the final test result.
Results and discussion

Quality control

The accuracy of the results was assured by various levels of quality control of the analysis. Participation in the 2007 Food Analysis Performance Assessment Scheme (FAPAS) proficiency test of PAAs as well as the regional laboratory’s participation in an interlaboratory comparison (Kappenstein et al. 2009) showed good results for both laboratories. In the 2007 enforcement campaign, the National Food Institute verified results from three non-compliant samples and obtained the same results within the uncertainty as the regional laboratory. The criterion for identification was fulfilled if the ratio of the two daughter ions was within 20% of the ratio of the standards (cf. the guideline for substances covered by Directive EC/96/23; European Commission 2002b).

A summary of the method performance for the analysis of aniline and 4,4’-MDA in the measuring range 2–25 µg l⁻¹ in various 3% HAc migration solutions from plastic laminates is given in Table 1 (Mortensen et al. 2005). The within-laboratory reproducibility (sᵣ) for 20 PAA was calculated using their repeatability (intra-day) variances (sᵣ) and between-day variances (sᵅ) in the following manner:

\[ s_R = (s_r^2 + s_{\text{day}}^2)^{1/2} \]

Note that sᵣ was calculated from seven determinations measured by a single technician using a single HPLC column on the same day; sᵅ was determined by two technicians using two different HPLC columns to make a total of 132 determinations on 3 different days.

The uncertainty typically included contributions from the LC-MS/MS detection (Table 1), control charts, the migration step, and individual sample in-homogeneity, established from the standard deviation between the three sub-samples from one batch, the latter parameter usually being an

Table 1. Validation data for LC-MS/MS PAA method (Mortensen et al. 2005).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Matrix</th>
<th>LOD</th>
<th>LOQ</th>
<th>Recovery range (%)</th>
<th>RSDᵣ (%)</th>
<th>Measurement uncertainty (%)</th>
<th>Accreditation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>3% HAc food simulant</td>
<td>0.8</td>
<td>2.6</td>
<td>89–101</td>
<td>5–14</td>
<td>5–26</td>
<td>Yes</td>
</tr>
<tr>
<td>4,4-MDA</td>
<td>0.3</td>
<td>0.8</td>
<td>4.7</td>
<td>96–106</td>
<td>7–17</td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

Note: LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation.

Figure 2. Long-term test of nylon utensils: comparing measured migration of 4,4’-MDA as a function of time at 100°C from samples 3 and 5 with modelling experiments using \( A_p = 6.5 \).
important contributor. No confidence interval factor was multiplied onto the uncertainty. The result that was compared with the limit of 20\(\mu\)g aniline eq kg\(^{-1}\) food was calculated as:

\[
\text{Result} = \text{average (three sub-samples)} + \text{uncertainty.}
\]

**Enforcement campaigns**

A total of 201 plastic samples were collected over about 5 years from Danish importers, food-production companies and retail shops.

**Multilayer film**

No PAA migration was found from the 41 laminates tested in the targeted 2005 campaign. However, even if aromatic polyurethane had been used, the laminates would have cured during the rather long storage time at the food producers' facilities. At high temperature, e.g. sterilization at 121\(^{\circ}\)C, it is however possible that aromatic isocyanate-based adhesives could degrade and form PAAs (Lattimer and Williams 2002).

**Coloured plastic utensils**

Table 2 summarize sample characteristics and test results. PAA were detected in one sample (out of 28), which also gave rise to discoloration and bad smell.

PAA was shown in the first migration test only, but below the legal limit. It was observed that the food simulant became pink, the same colour as the pink café glass made from polypropylene.

**Black kitchen utensils**

A total of 136 samples were taken for official control over a 5-year period. Details of all black kitchen utensils are given in the supplementary data file (available online). Figure 1 shows the overall picture of a number of samples analysed each year and the frequency of violations found in the enforcement campaigns and for the older used kitchen utensils.

Repeated official enforcement campaigns seem to have lowered the amount of non-compliant samples on the Danish market, from around 54\% in the 2004 study and 45\% in the 2005a targeted campaign to 12\% in the 2005b market control. However, from the 2005b campaign to 2009, the frequency of violations remained almost constant in the market control campaigns: about 10\% of the samples analysed were non-compliant. The decrease in 2005a to 2005b from 45\% to 12\% violations in the targeted control versus market control could indicate that sufficient versus insufficient own-check documentation is related to the compliance of the product. The results also show that while own-check control has some effect, violations are still found for samples stated to be compliant in the documents kept by the importer.

The majority of samples with violations came from China (migration of both 4,4'-MDA and aniline), with Sweden (migration of aniline) being the second largest contributor.

Table 3 and 4 show the concentration range of measured PAAs in the third migration test of all 136 official control samples. The 'rare' PAAs (Table 4) were only present in samples with high PAA migration.

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**Table 2. Test for PAA migration from coloured plastics: 28 samples in the 2005a targeted market control campaign.**

<table>
<thead>
<tr>
<th>Types of plastic</th>
<th>PP, melamine, PS, PET, PPCO-B, LDPE, PC, unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colours</td>
<td>Black, white, grey, brown, dark blue, pink, red</td>
</tr>
<tr>
<td>Shapes of coloured plastic</td>
<td>Single-use containers, lids and fast-food boxes ((n=14)); repeated-use containers, bowls, plates, cups, utensils ((8)); 'inserts' for, for example, cake/chocolate boxes ((3)); others ((3))</td>
</tr>
<tr>
<td>First migration test with a PAA content &gt;2(\mu)g kg(^{-1})</td>
<td>One pink café PP glass (results in about 25(\mu)g aniline eq kg(^{-1}))</td>
</tr>
<tr>
<td>Number of organoleptic violations (smell/colour)</td>
<td>One pink café PP glass</td>
</tr>
</tbody>
</table>

Notes: PP, polypropylene; PS, polystyrene; PET, polyethylene terephthalate; PPCO-B, polypropylene (crystalline oriented); LDPE, low density polyethylene; PC, polycarbonate.

**Table 3. PAA migration from 136 official control samples of kitchen utensils (sum values and common PAAs).**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Number of samples with migration in the range ((\mu)g kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; LOD (2 or 5)</td>
</tr>
<tr>
<td>Aniline eq.</td>
<td>103</td>
</tr>
<tr>
<td>ΣPAA</td>
<td>103</td>
</tr>
<tr>
<td>Aniline (3. test)</td>
<td>107</td>
</tr>
<tr>
<td>4,4'-MDA (3. test)</td>
<td>112</td>
</tr>
</tbody>
</table>

Note: LOD, limit of detection.
In 2007–2009, the reporting limit was increased from 2 to 5μg aniline eq. kg⁻¹.

The test results for the majority of black nylon kitchen utensils showed one of two typical types of migration patterns in samples that were positive in the third test. One group of samples has an extremely high migration of 4,4'-MDA, accompanied by a broad selection of other PAAs exceeding the 20μg aniline eq. kg⁻¹ limit up to more than 1000 times. Other samples showed migration of up to 100μg kg⁻¹ of aniline only.

Modelling the influence of test conditions on compliance with the legislative limit

In Figure 2, the measured migration over time from the nylon utensils shows that 4,4'-MDA continues to migrate over long periods of use, which also was observed for aniline (data not shown).

The curve was based on the obtained values in migration experiments and an initial concentration in the polymer (c_p,0) of about 1650 mg kg⁻¹ plastic (0.17%), and A_p = 6.5. In comparison, Brede and Skjevrak (2004) fitted migration of aniline from Swedish utensils with A_p = 3.645 (c_p,0,measured = 82.5μg kg⁻¹ plastic, i.e. 0.0000083%). Our values lie close to the A_p values of 6–8, which were found for wet (swollen) polyamide (FABES 2006–2008), but far above the A_p = 2.0 for dry polyamide given in the European Commission’s Practical Guide section about mathematical modelling (European Commission 2003).

Choosing the right test conditions for enforcing the ‘non-detectable’ migration of substances is not straightforward. Generally, in the first test, migration increases with time and temperature. For repeated-use articles, the migrant may in principle be completely extracted during the first and second test leaving nothing to migrate in the third migration test. The result of the migration test is therefore very dependent on the selection of proper migration test times and temperatures. In European Union plastics regulation, 175°C is the highest test temperature for FCM, e.g. used when frying. When testing such high-temperature FCM with aqueous food simulants, testing times are increased by a factor of 4 to compensate for the maximum obtainable temperature of 100°C (EEC 1982). For compliance testing, enforcement laboratories typically use test times of 2 h at 100°C (simulating 0.5 h above 175°C) or 4 h at 100°C (simulating 1 h above 175°C) for aqueous simulants, but test periods as short as 10 min have also been used. To consider how various migration test conditions influence the PAA migration in the important third test, we use the fitted A_p and C_p,0 values to show the influence of testing at different times and temperatures: 10 min, 30 min and 1 h at 175°C and 2 and 4 h at 100°C. It is hypothesized that the fitted A_p value for reinforced nylon is valid also at 175°C.

Columns 1 and 2 in Figure 3 indicate a reasonable agreement between the modelled and the measured migration of 4,4'-MDA in 2 h at 100°C in the first, second and third migration tests of the nylon spoon (sample 3) in contact with 3% HAc. Columns 3–6 show the modelled migration at 100°C versus 175°C at various test times.

The test conditions of 2 h at 100°C (Figure 3, column 2) seem to underestimate real-life conditions of 0.5 h at more than 175°C (column 5). By coincidence, for this spoon (sample 3), the test conditions applied during the majority of tests in our investigations, 4 h/100°C, seem to simulate real use in 1 h at above 175°C quite well.

In the first migration test, the expected increase in migration with an increase in migration time is

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Table 4. PAA migration from 136 official control samples of kitchen utensils (rare PAAs).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>&lt; LOD</th>
<th>&gt; LOD to &lt;10</th>
<th>10 to &lt;20</th>
<th>20 to &lt;200</th>
<th>200–1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Phenylenediamine (p-PDA)</td>
<td>128</td>
<td>1</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m-Phenylenediamine (m-PDA)</td>
<td>135</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-Toluenediamine (2,4-TDA)</td>
<td>126</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2,6-Toluenediamine (2,6-TDA)</td>
<td>133</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Anisidine (o-ASD)</td>
<td>128</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzidine (Bnz)</td>
<td>126</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>o-Toluidine (o-T)</td>
<td>129</td>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-Dimethylamine (2,4-DMA)</td>
<td>134</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2,6-Dimethylamine (2,6-DMA)</td>
<td>134</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3,3-Dimethylbenzidine (3,3-DMB)</td>
<td>126</td>
<td>7</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Aminobiphenyl (4-ABP)</td>
<td>128</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Chloro-o-toluidine (4-CoT)</td>
<td>133</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Chloro-aniline (4-CA)</td>
<td>134</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2-Methoxy-5-methylaniline (2-M-5-MA)</td>
<td>135</td>
<td>1</td>
<td></td>
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<td></td>
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</tbody>
</table>
observed. The picture becomes less intuitively understandable in second and third migration tests, when the residual amount of 4,4'-MDA, e.g. in a plastic tested at 175°C, has been significantly reduced in previous test(s). This phenomenon is well illustrated by comparing the result of the 10 min and the 1 h test in the third test at 175°C, the first one being the highest. The seemingly more severe test conditions – combinations of highest temperature and longest test times – are not always the worst-case test conditions for repeated use items.

The network of national European Union reference laboratories has recommended for these types of utensils testing for 2 h at 100°C (Simoneau 2009).

**PAA migration from used kitchen utensils**

Long-term release of PAAAs was found when testing *used* kitchen utensils (Table 5). The frequency of violations was lower (7%) than for new utensils, but one reason could be that the utensils had already lost PAAAs to the food.

The usage time of the utensils was estimated from the collected questionnaires as: age (in years) multiplied by 52 (weeks per year) multiplied by number of uses (per week) multiplied by 15 min; assuming an average of 15 min food contact per usage period. One to two times per week was calculated as 1.5 times per week, and once a month was counted as 0.25 times per week. The year the utensil was bought counted as 0.5 year. Utensils were tested with 3% HAc at 100°C for 30 or 40 min. For example, the utensil showing a migration of 4,4'-MDA of 333 µg kg⁻¹ in Table 5 and purchased late in 2003 was used weekly for 78 times or for about 19.5 h. Using the above model to find the percentage of 4,4'-MDA expected to migrate in the time period 19.5–20 h after the start of use, it was calculated that 65% of the PAA migrated from the utensil before the test period. An average migration from 1 dm² of exposed spatula of 140 µg 4,4'-MDA per 15-min use period could be estimated to have taken place during the first 20 h of use. The uncertainty of this estimate is of course very high.

Many utensils deemed legal leach measurable amounts of PAAAs over a long time and it can even be questioned whether the present limit offers the intended protection of consumers when dealing with non-threshold carcinogens. For non-detectable substances, most authorities consider the result of the third test of a repeated-use article as conclusive, even if the result of the first test is far above the detection limit. On this point, it is however foreseen that European

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**Figure 3.** Influence of various modelling/testing scenarios on the concentration of 4,4'-MDA in the third migrate using migration modelling: sample 3, $A_p = 6.5$ and $C_{p,0} = 1.66$ g kg⁻¹ plastic.

**Table 5.** PAA migration from 29 kitchen utensils in private use.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Number of samples with migration in the range (µg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;2</td>
</tr>
<tr>
<td>Aniline</td>
<td>23</td>
</tr>
<tr>
<td>4,4'-MDA</td>
<td>28</td>
</tr>
</tbody>
</table>
Union regulation will soon be changed in favour of improved consumer protection.

Had the result from the first migration test been used to evaluate migration (because PAAs are not supposed to be detected at all), there would have been more violations (>20 µg kg⁻¹) and almost 50% of violations with a limit of detection (LOD) of 2 µg kg⁻¹, which is the prescribed European Union LOD for, for example, pesticides, veterinary drugs, and mycotoxins present in foods (EC/96/23; European Commission 2002b). With the current European Union limit of 10 µg PAA kg⁻¹ (European Commission 2007a), the major change is that the new limit is in µg PAA (versus µg aniline eq.), which means that PAAs with molecular weights higher than aniline will now have lower limits. For 4,4’-MDA this means almost a doubling of the analytical result as seen in the supplementary data file (available online).

Risk assessment
Exposure and risk characterization
Based on results from the migration tests performed in the 2004 study and in 2005 enforcement campaigns showing migration in mg kg⁻¹ food simulant amounts, together with an expectation of a continuing long-term migration from several of such samples, a worst-case average daily intake of 10–20 µg 4,4’-MDA kg⁻¹ body-weight (bw) day⁻¹ was anticipated when 300 g of contaminated food were consumed. For small children, the dose could be even higher because of a larger food intake per kg bw. However, this high intake will not continue and a lifetime dose of 1 µg kg⁻¹ bw day⁻¹ is considered to be more realistic, as a low ‘mean’ concentration over the whole usage time of such utensils, and because not all utensils are highly contaminated.

In 2004, the Norwegian Food Safety Authority conducted a survey on 4,4’-MDA migrating from cooking utensils collected on the Norwegian market (Brede and Skjevrak 2004). The highest migration of 4,4’-MDA was 1089 µg dm⁻² (6530 µg kg⁻¹), which migrated from a balloon whisk. In a worst-case situation, the exposure would represent a daily intake of 15.6 µg kg⁻¹ bw day⁻¹. It was emphasized that these exposure assessments are associated with great uncertainty.

4,4’-MDA is genotoxic and carcinogenic in experimental animals, and should be considered as a non-threshold carcinogen, which means that there might be a risk at even very low exposure levels.

Various approaches have been used to calculate the cancer risk, although these approaches are associated with great uncertainties. The European Food Safety Authority (EFSA) has proposed to use a margin of exposure (MOE) approach for quantitative risk assessment (EFSA 2005). MOE is the ratio between a defined point on the dose–response curve for the adverse effect, usually based on animal experiments in the absence of human data, and the estimated human intake. Using a benchmark dose lower limit (BMDL) of 1.7 mg kg⁻¹ bw day⁻¹ for 4,4’-MDA (VKM 2006), a MOE was calculated by dividing the BMDL10 with the level of human exposure. For the Danish survey, MOE was 1700 (=1.7 mg kg⁻¹ bw day⁻¹/1 µg kg⁻¹ bw day⁻¹), and for the Norwegian survey it was 109 (=1.7 mg kg⁻¹ bw day⁻¹/15.6 µg kg⁻¹ bw day⁻¹). Both values are much lower than 10 000, meaning that EFSA views the substance as a high priority for risk management. In addition, the theoretical excess human cancer risk can be calculated based on a T25 value (a dose representing 25% extra, non-spontaneous tumours) of 6.1 mg kg⁻¹ bw day⁻¹ calculated from animal studies. The excess cancer risk due to exposure from 4,4’-MDA in kitchen utensils was estimated in this way for the Danish survey to be 1.5 × 10⁻⁴ (=0.001 mg kg⁻¹ bw day⁻¹/6.1 mg kg⁻¹ bw day⁻¹) and for the Norwegian survey to be 2.3 × 10⁻³ (=0.0156 mg kg⁻¹ bw day⁻¹/6.8 mg kg⁻¹ bw day⁻¹).

Conclusions
We consider both the above risk estimates to be of major concern although based on ‘worst-case’ considerations. Moreover, PAAs have sensitizing and allergenic effects, which were not evaluated. For this reason, the Danish Authority withdrew the contaminated articles and advised Danish consumers to throw out any black nylon utensil they had in their possession. However, as this study shows, PAA migration from kitchen utensils on the market continues to be a problem, despite the high amount of time and money invested by European enforcement authorities in eliminating this problem. The 2005 campaigns show that while own-check documentation seems to have some effect in decreasing the rate of violations, it is not capable of fully preventing them, and in particular not for products originating from countries outside the European Union. The sources of contamination should be traced back to the raw material producers in order to prevent raw material producers from continuing to sell – knowingly or unknowingly – plastic raw materials contaminated by carcinogenic PAA.

Acknowledgements
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FABES, for fitting the experimental data to the migration model.

References


