

Contamination of dry foods with trimethyldiphenylmethanes by migration from recycled paper and board packaging

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Abstract

Contamination of foods with trimethyldiphenylmethanes is reported and the origin is shown to be migration from food packaging materials of which the use of recycled carbonless copy paper was found to be the major source. This chemical is one of the solvents used in the carbonless copy paper and its presence in food and the environment has not been previously identified. In this paper we have pursued previous studies on diisopropylnaphthalenes and hydrogenated terphenyls contamination from packaging and now report the identification of this new food contaminant and present evidence of its source. Solid foods such as egg pasta, barley coffee and rice were analysed by GC/MS and a mean concentration of 18 µg/kg of trimethyldiphenylmethanes was found. Extracts from carbonless copy paper were analysed by proton NMR to characterize the trimethyldiphenylmethanes. Since trimethyldiphenylmethanes are found in solid food together with diisopropylnaphthalenes, and considering their similar chemical character, they may follow the same migration pathway as one another.

Keywords: Food contamination, organic contaminant, gas chromatography-mass spectrometry, NMR, trimethyldiphenylmethanes, carbonless copy paper

Introduction

Food safety is an increasingly important public health issue. Governments all over the world are intensifying their efforts to improve food safety. These efforts are made in response to an increasing number of food safety problems and rising consumer concerns. The Persistent Organic Pollutants (POPs) are compounds that accumulate in the environment and the human body. Exposure to POPs may result in a wide variety of adverse effects in humans. A large number of organic environmental chemical contaminants (approximately 50 000) may be adventitiously present in food as a result of human activity, or may stem from natural sources. They include polycyclic aromatic hydrocarbons (PAHs), phthalates and brominated flame retardants. They may be introduced into the food chain at any point from growth of the plant to harvesting

through to packaging, storage and processing. PAHs, phthalates and monoaromatic hydrocarbons (MAHs) are widely considered to have the potential for causing adverse health effects in humans. Although some data concerning dietary exposure to these chemicals are available, the relationship between human dietary intake, metabolism and excretion, in particular the extent to which dietary intake is absorbed, is less well characterized. Since contamination generally has a negative impact on the quality of food, and may imply a risk to human health, the European Union has taken measures to minimize the presence of contaminants in food-stuffs. The UK Food Standards Agency is undertaking research programmes on *Food Safety Aspects of Organic Environmental Chemical Contaminants, and Chemical Contaminants from Food Contact Materials and Articles*. The objective is to assess

the extent of human exposure to chemicals that can be present as contaminants in food, including those introduced through animal feed, food processing and packaging materials. These programmes investigate possible harmful effects of these chemicals on food, as well as ways of protecting consumers from them. A specific project concerns the *Migration from recycled paper and board to dry foods*. This work aims to investigate the migration of chemicals from recycled paper and board packaging into dry foods and to produce guidelines for industry.

The economic and environmental benefits of paper recycling are significant. However, the use of recycled fibres for food packaging may be problematic as long as the fundamental science affecting migration from cellulosic fibres, such as recycled paper and board, is poorly understood, as are the characteristics that cause chemicals to resist washing processes during recycling, but subsequently allow their migration to packaged dry foods.

During the past decade many organic compounds were identified as food contaminants and their source attributed to paper or board packages. Foodstuff contamination by diisopropyl-naphthalenes (DIPN) was first considered by Sturaro et al. (1994). Six isomers were identified and their origin and migration pathway was established. Also the hydrogenated terphenyls (HTP) were examined (Sturaro et al. 1995), and recycled carbonless copy paper (CLCP) was indicated as the major source of both contaminants. Other contaminants were identified and new analytical methods proposed (Gilbert et al. 1994; Johns et al. 1995; Castle et al. 1997; Boccacci Mariani et al. 1999; Song et al. 2000; Summerfield and Cooper 2001; Triantafyllou et al. 2002; Nerin and Asensio 2004). In this paper we pursue the aim that was outlined in the previous work, and describe the identification of a new contaminant, its properties and likely sources.

Materials and methods

Food, packaging materials and carbonless copy paper samples

Twenty-six food samples, packed directly in cardboard without any functional barrier, were purchased from retail outlets. These samples included egg pasta, some brands of rice and barley coffee, etc. Packaging and foods were analysed just after purchasing. The pasta samples chosen were of small size. The carbonless copy paper samples were obtained from printers manufacturing stationery products.

Materials

2,6-Diisopropyl-naphthalene (2,6 DIPN) standard was purchased from Aldrich, Chimica, Milan, Italy. *Diisopropyl-naphthalene industrial mixture* was obtained from the producer. *Isopropyl-diphenylmethane (IDM)* isomers were synthesized by mixing diphenylmethane with propan-1-ol or propan-2-ol in presence of 98% sulphuric acid at room temperature overnight. Extraction and enrichment of the reaction products in hexane allowed the GC/MS acquisition of retention times and mass spectra of mono-substituted compounds. *Isopropyl-methylbiphenyl (IMB)* isomers were synthesized by mixing 4-methylbiphenyl with propan-1-ol in presence of 98% sulphuric acid at room temperature overnight. Extraction and enrichment of the reaction products in hexane allowed the GC/MS acquisition of retention times and mass spectra of disubstituted biphenyl.

Extraction procedures

Solvent extraction of food. A total of 3.5 g of each food sample were placed into vials together with 2 ml of acetone. After stirring for 15 min, 2 μ l of each organic solution was introduced into the GC/MS.

Solvent extraction of cardboard. A total of 500 mg of each cardboard sample was placed into vials together with 2 ml of acetone. After stirring for 15 min, 2 μ l of each organic solution were introduced into the injector of the GC/MS system for target compound determination.

GC/MS analysis of food, packaging and carbonless copy paper samples

All determinations were performed using a GC/MS system comprising a HP 6890 gas chromatograph coupled with an HP 5973 N quadrupole mass spectrometer. Chromatographic separation of DIPN was obtained using a HP 5MS capillary column with the following dimensions: length 30 m; internal diameter 0.25 mm and film thickness 0.25 μ m. The column, with a constant helium flow of 0.7 ml/min, was operated with a temperature programme from 80°C for 1 min, then at 10°C/min to 250°C and then isothermal for 12 min. The injector temperature was 250°C. All injections were carried out under splitless conditions for 0.3 min. The mass spectrometer was in SCAN acquisition mode in order to detect the ions produced by electron ionization (70 eV) at the ion source temperature of 230°C, in the mass range from 50–250 Daltons, with a scan rate of 6.76 scan/sec. All quantifications were performed by extracting, from total ion current, the signal

at m/z 197 for DIPN and at m/z 195 for trimethyldiphenylmethanes (TMDPM).

HPLC apparatus and conditions

HPLC analysis of carbonless copy paper extracts were performed by using a Waters (Milford, MA, USA) HPLC system consisting of a model 600 MS pump and a model 996 photodiode array (PDA). Data handling was carried out with Millennium 32 software. The compounds were separated using a Delta-Pak RP-18 100 Å column (150 mm × 3.9 mm i.d. and spherical particles of 5 µm) and methanol – water (80–20) as mobile phase at a flow of 0.50 ml/min.

NMR analysis

A total of 20 mg of coated back carbonless copy paper were extracted with 0.5 ml of deuterated chloroform. After 15 min sonication, the extract was filtered, analysed by means of GC/MS, and also submitted to NMR analysis. Proton NMR spectra were recorded at 25°C on a Bruker AMX-300 instrument, using SiMe₄ as internal reference. Two-dimensional ¹H–¹H correlated COSY (Aue et al. 1976) and NOESY (Bodenhausen et al. 1984) spectra were acquired with the spectral window utilized for the 1D spectra. A mixing time of 450 ms was utilized for NOESY spectra.

Results and discussion

Food and packaging

Most of the analysed foods showed the presence of DIPN in a range of 20–300 µg/kg, (Table I). Foods such as sugar and salt did not show any evident contamination, confirming previous findings (Sturaro et al. 1994, 1995).

Seven food samples, namely three types of egg pasta, three brands of rice and a brand of barley coffee, showed two small sharp peaks having similar MS spectra (see Figure 1), together with aliphatic hydrocarbons and DIPN.

The same data were observed on the corresponding packaging, which indicated that the cardboard could be the origin of migration. The peaks ratio was about 2.5 (Figure 2A), but the mass spectra were slightly different (Figures 2B and 2C).

The fragment ion that was present only in the minor component was at m/z 118. Moreover, a slight difference was observed in the ratio m/z 210 vs. m/z 195. Assuming a molecular weight of 210 Da and excluding compounds such as isopropyl-diphenylmethane and isopropyl-methylbiphenyl which were synthesized and analysed without success, the attention was focused

Table I. Ranges of DIPN and TMDPM present in analysed foods.

	TMDPM* µg/kg	DIPN mg/kg
Cardboard packages		
Barley coffee	523	25.3
Egg pasta A	122	13.6
Egg pasta B	334	5.2
Egg pasta C	378	3.0
Rice A	937	10.7
Rice B	998	72.9
Rice C	85	2.3
Foods		
Barley coffee	21	1.6
Egg pasta A	18	0.1
Egg pasta B	10	0.3
Egg pasta C	34	0.8
Rice A	25	0.9
Rice B	11	0.8
Rice C	9	0.5

*TMDPM content is expressed as DIPN concentration due to the unavailability of certified standard.

on the trimethyldiphenylmethanes (TMDPM). Certified standards of these compounds and trade products were not available. By matching the MS database information on similar compounds and applying the mass spectra fragmentation mechanisms proposed by Peterman and Delfino (1990) for some components of Santosol 100 (Monsanto), the fragmentation mechanism in Figure 3 could explain the MS data and molecular structure. Other evidence was obtained by correlating the GC/MS data with the reported results of Bhaskaran and Bhat (2002) concerning the synthesis of 3,4-dimethyldiphenylmethane. In that synthesis the presence of a small amount of 2,3-dimethyldiphenylmethane was observed.

Carbonless copy paper

After analysing a large number of carbonless copy papers from printers, the predominant dye solvent observed was composed of DIPN, together with linear and branched hydrocarbons. The new compounds that had been found were observed only in two cases. The dye solvents were extracted from this carbonless copy paper by using the same technique used for cardboard. The extract was analysed by GC/MS, and revealed a high percentage of two components having the same ratio and MS spectra as the newly detected contaminant in foods. The same extract was analysed by means of the HPLC-PDA, and showed a key difference between the new contaminant and the DIPN spectra. In fact, the maximum UV peaks were at a lower wavelength compared with DIPN, with clear

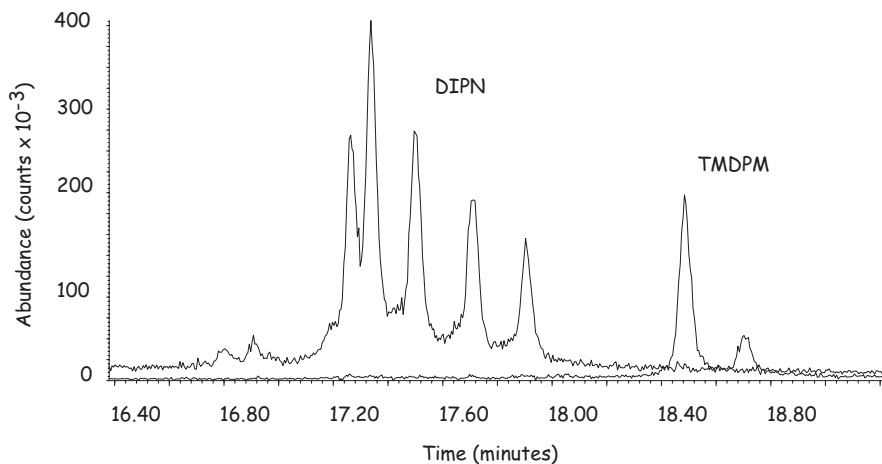


Figure 1. Chromatographic behaviour of the cardboard extract from packaging from barley coffee. The partial chromatogram shows the ion current at m/z 197 for DIPN and m/z 195 for TMDPM.

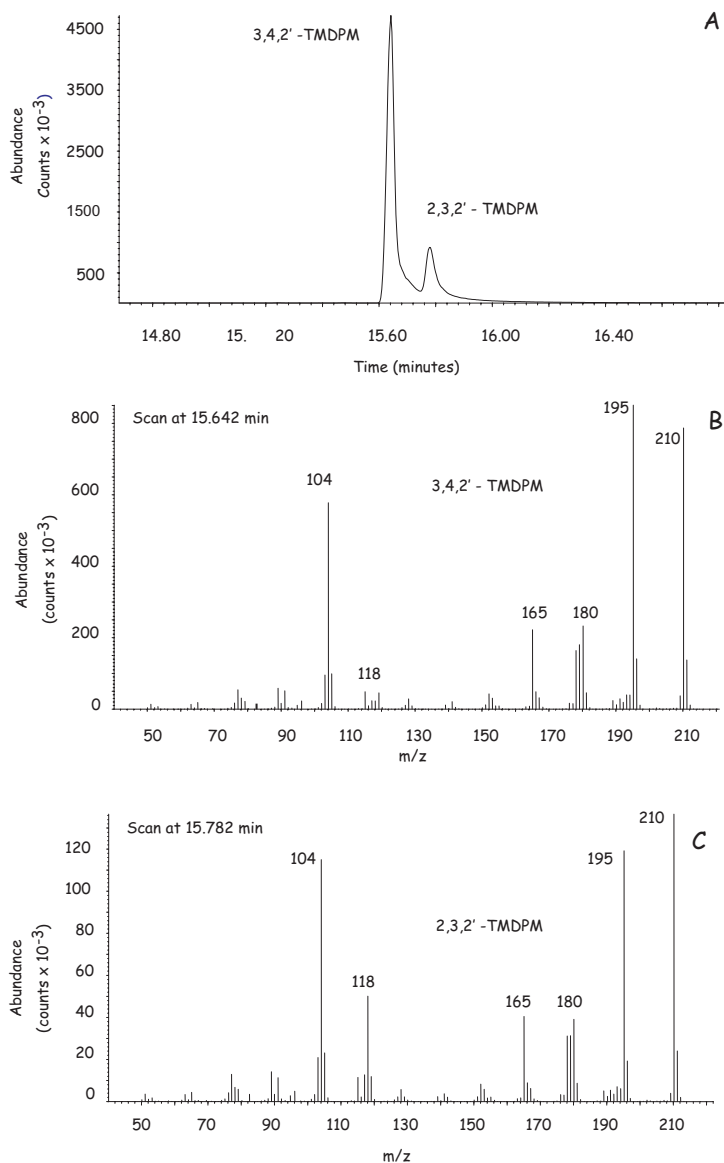


Figure 2. (A) Chromatographic profile of TMDPM isomers extracted from carbonless copy paper. (B) Mass spectrum of 3,4,2'-TMDPM isomer. (C) Mass spectrum of 2,3,2'-TMDPM isomer.

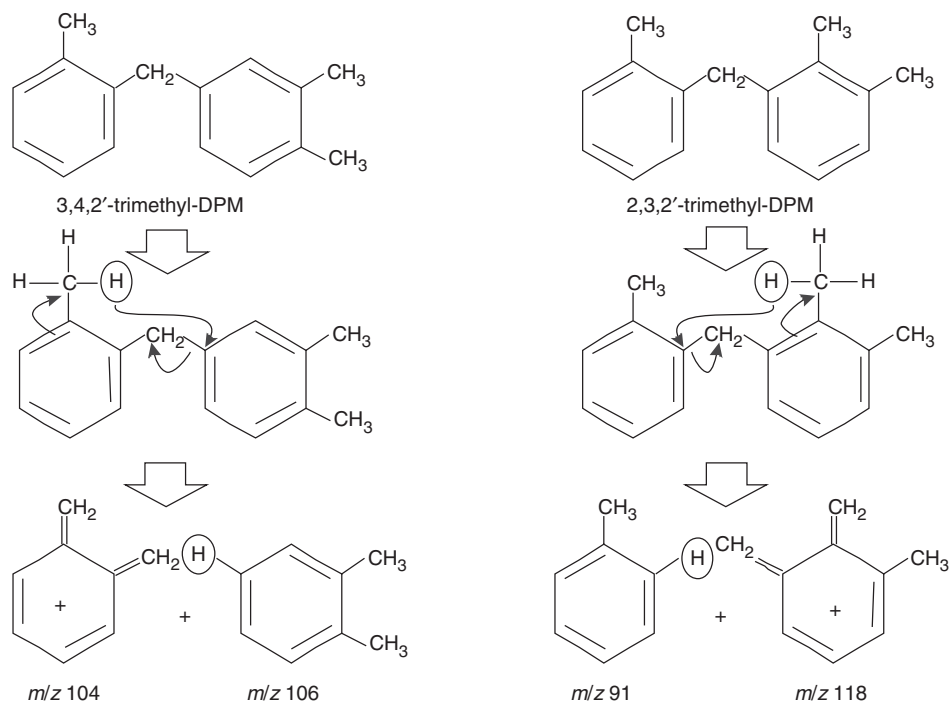


Figure 3. Proposed mass spectral fragmentation mechanisms of 3,4,2'- and 2,3,2'-TMDPM.

evidence of uncondensed aromatic rings (<210 nm and 267 nm vs. 228.5 nm and 273 nm for 2,6 DIPN).

NMR evidence for trimethyldiphenylmethanes (TMDPM)

The proton NMR spectrum of the extract in deuterated chloroform provided evidence for the presence of two major components, namely hydrocarbons (55%), TMDPM (40%) and some TMDPM-related impurities (less than 5%). TMDPM displays aromatic and aliphatic signals, whose integration is in agreement with the formula depicted in Figure 4.

In particular, the singlet at 3.91 ppm is typical of a methylene group (7) bridging two substituted phenyl rings, and the system centred at 2.20 ppm is associated with ortho-positioned methyl groups (8, 9 and 10) incorporated in aromatic rings. The three upfield shifted aromatic signals belong to a tri-substituted phenyl ring having two ortho-positioned protons (5 and 6), whose scalar coupling is pointed out by the multiplicity in the mono-dimensional spectrum (two doublets) and the cross coupling in the COSY map. The NOESY experiments, which correlate protons magnetic interactions through the space, prove that the bridging methylene group (7) is correlated with the nearby proton in position (6') and to the unique methyl group (10). On the other phenyl, the bridging methylene group (7)

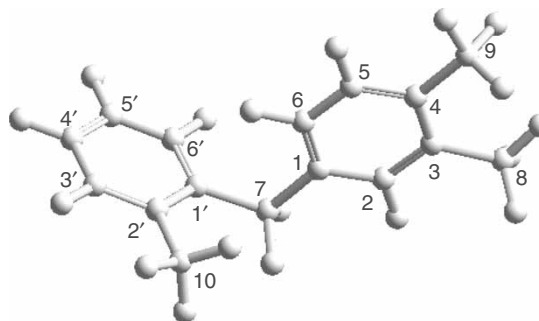


Figure 4. 3D representation of 3,4,2'-TMDPM.

is correlated with protons in positions (2) and (6). The latter proton is coupled with the proton in (5), which in turn correlates with the methyl group (9). Lastly, the methyl group (8) is correlated with the proton in (2).

^1H NMR (δ , ppm; CDCl_3): 2.08–2.31 (9H, 3 CH_3), 3.91 (m, 2H; CH_2), 6.83 (d, 1H; $^3J = 7.9$ Hz), 6.90 (s, 1H), 7.02 (d, 1H; $^3J = 7.9$ Hz), 7.09–7.17 (4H).

Conclusions

Carbonless copy paper dye solvents which can be present in wastepaper can be transferred, by recycling paper mills, into the recycled cardboard final product or released directly into the aquatic

environment. They are hydrophobic and can migrate outside the cardboard if it is in contact with lipophilic substances as is the case with some foods and finally can accumulate in organisms or sediments. Most of these dye solvents are moderately toxic, and experts are therefore urged to assess acute and chronic toxicity, metabolism and persistence. When diisopropyl naphthalenes were discovered in some solid foods, and their contamination pathway was clarified, DIPN and carbonless copy paper producers exhibited scepticism, but the irrefutable data convinced them and some governmental institutions to tackle the problem. The TMDPM is only one of the solvents used in the carbonless copy paper, and its presence in food or environment has never previously been identified. Since it is found in solid food together with DIPN, considering the similar chemical character as chemical stability and lipophilicity, it may follow the same fate as the majority of dye solvents. The mean concentration of 18 µg/kg in food is very low, but nothing is reported in the current literature concerning its fate and toxicity. However, putting together carbonless copy paper dye solvents, TMDPM, DIPN (Sturaro et al. 1994), HTP (Sturaro et al. 1995) and other chemicals, many of which have not been identified or studied yet, the total contamination of some packed food may be relevant and worth being considered as a public health concern.

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