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Identification of Diisobutyl Phthalate (DIBP) Suspected as Possible Contaminant in Recycled Cellulose for Take-away Pizza Boxes

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Italian law specifically requires the use of regenerated, rather than recycled, cellulose in the production of pizza boxes. We investigated the frequent failure to comply with this law: the identification and determination of phthalates, which have widespread use, contribute to the quality control of various food packaging materials.

Diisobutyl phthalate (DIBP) has similar structural and application properties to di-n-butyl-phthlate (DBP), and it is used as a substitute for DBP.

We standardized an analytical method that allows the calculation of an 'exposure index' (EI) for DIBP in take-away pizza boxes. The technique, which relies on Solid-Phase Micro Extraction/Gas Chromatography/Mass Spectrometry (SPME/GC/MS), allows the definition of a large range of DIBP content in the headspace within various take-away pizza boxes.

Data concerning pizza boxes purchased in 16 restaurants in 2006 were reported, and the 'EIs' were estimated to range between 6 and 72 μg. Copyright © 2007 John Wiley & Sons, Ltd.

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KEY WORDS: DIBP; phthalates; take-away pizza boxes; SPME; GC/MS

INTRODUCTION

To protect the environment, new ways of recycling packaging materials are appearing on the market. Paper and board, partly or fully produced from recycled fibres are already used in contact with certain foodstuff in many countries in Europe. However, as described by Binderup *et al*.[1] recovered paper is also used in direct contact with dry foodstuff and also with fatty foodstuff like pizzas and other fast food items.

Recovered paper and board may vary in origin and could include paper containing printing inks, adhesives and other substances, which are not intended to come into contact with foodstuffs.

In Italy, the use of recovered paper is forbidden for the manufacture of 'box for take-away pizza', and only regenerated cellulose must be used.[2] Regenerated cellulose is a sheet material obtained from refined cellulose derived from unrecycled wood or cotton, as well-officially explained.[3] Italian regulation does not include diisobutyl phthalate (DIBP) in the list of substances authorized in the manufacture of materials and articles made of regenerated cellulose intended to come into contact with foodstuff. More recently, di-n-butyl phthalate

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(DBP) and butyl benzyl phthalate were also cancelled from the list.[4]

Due to its similarities to DBP, DIBP can be used as a substitute for DBP as plasticizer in adhesives, printing inks and coloured laminated films; it is frequently used as a gelling aid in combination with other plasticizers and for polyacetate dispersions. These phtalates, such as bisphenol A or diisopropyl naphthalenes, may migrate to food packaged in paper products that contain these contaminants, and the recycled fibre-based paper produced by recovered paper is, following our institutional analytical work, the most probable and frequent source of release. Binderup *et al*.[1] cited a list of chemicals deduced from available chemical analyses of recovered paper. The list contains phtalates, solvents, azo-colourants, diisopropyl naphthalenes, primary aromatic amines, polyciclyc aromatic hydrocarbons, benzophenone and others.

Figure 1 shows the chemical structure of DIBP.

DIBP has been reported to have anti-androgenic effects,[5] and has similar structural and application properties as DBP.

Endocrine-disrupting activity has been described for phthlates,[6–9] and DBP and di-2-ethylhexyl phthalate, which are common chemical residues in food packaging materials, were investigated in paper and board containers used for the take-away food. The oestrogenicity of paper and board extracts was studied.[10]

Because of these characteristics, the migration of DIBP (or DBP) from the 'box for take-away pizza' could be considered a potential problem, especially given that pizzas are placed in these boxes in a very hot condition. For take-away service, the only container used in Italy is a corrugated board box, usually supplied as a six-corner carton blank, erected at the vending point. Italy is a leader in the production of these containers.[11]

In any case, the safety and quality of pizza must be guaranteed and specifically provided by Italian law, and the identification and quantification of released DIBP (or DBP) give a measure of the producer attention to health prevention, as suggested by Regulation (EC) No 1935/2004.[12]

A possible method useful to identify various contaminants in recycled paper and paperboard for food packaging was published by Song *et al.*[13] using ultrasonic extraction system.

Using a more actual and fast technique, as SPME/GC/MS, we analysed the volatile compounds released from take-away pizza boxes purchased on the national market, and we identified the presence of DIBP, often with DBP, as a marker of recycled cellulose. Here, we describe our method, which allows comparison of various board boxes for the levels of DIBP that they release.

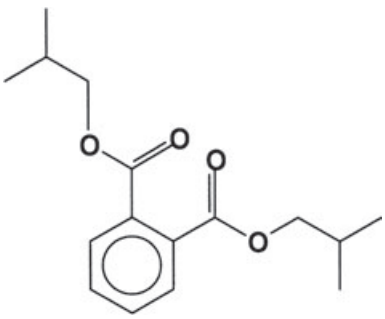

Figure 1. Chemical structure of phthalic acid, diisobuthyl esther (or DIBP). CAS RN. 84-69-5.

EXPERIMENTAL

Chemicals

DIBP standard, purity ≥98% (GC), was purchased from Fluka (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and 96% ethanol was obtained from Merck (Darmstadt, Germany).

Samples

Sixteen samples of take-away pizza boxes were collected and classified with code numbers, as reported in Table 1, to ensure privacy, according to the procedure PR 06 included in the Quality Management System adopted by the Analytical Research Laboratories, Food and Environment, University of Milan, Italy (Quality Management System Det Norske Veritas, Cert. 14110-2004-AQ-MIL-SINCERT).

The samples were purchased from 16 Italian pizza restaurants in northern Italy.

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Table 1. Data from ER and its derived EI related to various take-away pizza box samples purchased on the Italian market

Code number	S* (dm^2)	ER† ($\mu g/dm^2$)	EI‡ (μg)
H1	25.9	0.36	9.3
G2	25.9	0.71	18.4
A1	25.9	1.61	41.7
C3	25.9	0.76	19.7
B1	25.9	2.79	72.3
D1	25.9	1.90	49.2
D2	25.9	0.86	22.3
E1	25.9	0.46	11.9
E2	25.9	0.76	19.7
E4	25.9	0.78	20.2
C1	25.9	0.39	10.1
C2	25.9	0.33	8.5
F1	25.9	0.25	6.5
G1	25.9	0.29	7.5
I 1	17.28	0.43	7.4
I 2	17.85	0.43	7.7

*S, internal area of the take-away pizza box.
†ER, DIBP experimental release, representing the quantity of DIBP released from the unity area (1 dm^2) of board under the adopted analytical standard conditions.
‡EI, DIBP exposure index, representing the calculation of DIBP quantity (μg) released in the real volume of the box in which the pizza is exposed.

Preparation of standards for calibration curve

A stock standard solution of DIBP was prepared at a concentration of 1033 mg/l in ethanol. Working standard solutions were prepared by serial dilutions of the stock standard solution with ethanol to give DIBP concentrations of 0.41, 2.06, 20.66 and 41.32 mg/l. An aliquot of 0.5 ml of each standard was then transferred to a 1 l glass to give DIBP concentrations in the jar atmosphere in the range of 0.21–20.66 μg/l.

Sample preparation

A board pizza box disk sample (8 cm ∅) was inserted into a 1 l headspace glass jar fitted with a cap and equipped with a Teflon septum. The jar was then set in the oven at 60°C for 30 min to achieve headspace equilibrium; afterwards, a fibre was inserted and exposed to the headspace at 60°C for 60 min. The fibre was then immediately inserted into the injection port of a Shimadzu 2010 gas chromatograph coupled to a Shimadzu QP-2010 MSD quadrupole mass spectrometer at 250°C for 10 min. The concentration of DIBP in microgram per litre reached in the jar atmosphere represented, operating under the adopted standard conditions, the level produced by release from two surfaces of the board, i.e. from the total area of 1 dm^2.

Equipment

GC/MS analysis was performed using a Shimadzu 2010 gas chromatograph coupled to a Shimadzu QP-2010 MSD quadrupole mass spectrometer. Suitable separation of analytes was achieved using an Equity™-5 [poly (5%diphenil/95%dimetylsiloxane)] capillary column, 30 m length × 0.25 mm i.d., 0.25 μm phase (Supelco, Milan, Italy). The operating conditions for the GC/MS were as follows: helium flow = 1.0 ml/min, and oven temperature = 60°C for 1 min, increased to 240°C at a rate of 3°C/min. The temperature of the ion source was set at 200°C, the electron energy was set at 70 eV, and the interface temperature was set at 250°C.

An SPME fibre coated with 2 cm of 50/30 μm divinylbenzene/carboxen/PDMS (Supelco) was used.

RESULTS AND DISCUSSION

The calibration curve reported in Figure 2 was generated in the range of 0.20–20.66 μg/l of DIBP, and the detection response was linear over the tested concentration range: the coefficient of correlation was $R^2 = 0.989$.

Repeatability was assessed by measuring the peak area produced with standard solutions and proceeding as described for the preparation of the calibration curve: the coefficient of variation (CV%) ranged from 6.3 to 3.5% for the concentration data limits of 0.206 and 20.66 μg/l, respectively, used for the calibration curve. Each measure obtained for the board samples as described in the experimental

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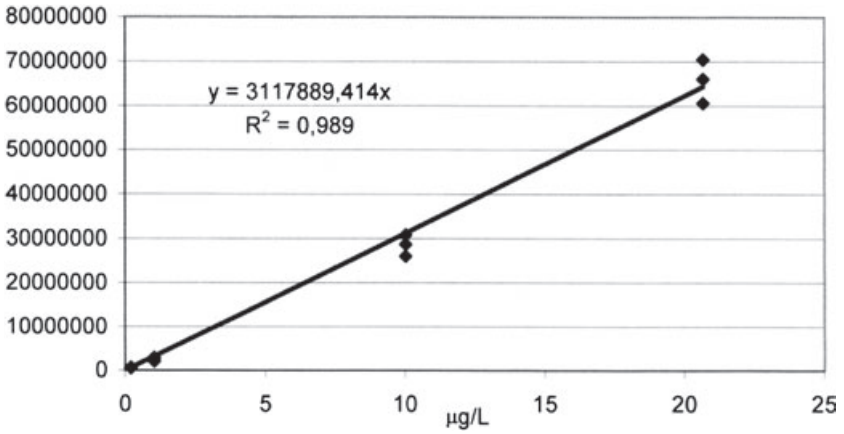

Figure 2. Calibration curve generated in the range 0.20–20.66 µg/l of DIBP.

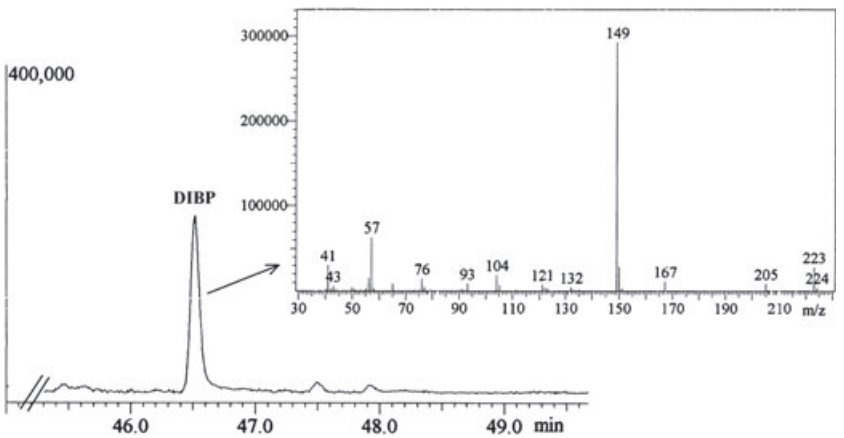

Figure 3. Example of the SPME/GC/MS chromatogram corresponding to DIBP concentration of 1.03 µg/l used for calibration curve and MS spectrum of DIBP.

section was preceded by a blank measure to avoid possible memory interferences.

Figure 3 shows an example of the SPME/GC/MS chromatogram corresponding to a DIBP concentration of 1.03 μg/l used for the calibration curve and MS spectrum of DIBP.

Figure 4 shows the SPME/GC/MS trace of sample G2, which exhibited the DIBP and DBP peaks.

The results of comparative tests of the 16 take-away pizza boxes purchased on the Italian market are summarized in Table 1 as ER, i.e. 'experimental release' (μg/dm^2), and represent the quantity of DIBP released from the unity area (1 dm^2) of board under the adopted analytical standard conditions (resulting in 1 l headspace). All data reported were corrected for the blank contribution. A temperature of 60°C was conventionally used for the assessment because it is considered the minimum temperature level reached in a box containing a just-cooked pizza.

To interpret the data in the contact of a complete pizza box, a more useful comparison was achieved when the whole internal surface of the box (S) was considered. Indeed, the 'exposure index' (EI) reported in Table 1 represents the calculation of

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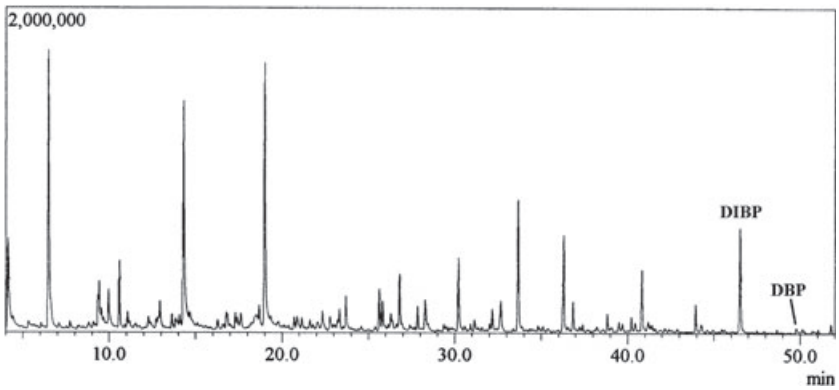

Figure 4. SPME/GC/MS trace of sample G2, where are evidenced peaks of DIBP and DBP.

DIBP quantity (μg) released in the effective headspace, i.e. in the real volume of the box in which the pizza is exposed. This 'EI' was calculated by EI (μg) = ER ($\mu g/dm^2$) × S (dm^2).

CONCLUSIONS

Here, we show the results of application of a simple analytical criterion adopted to compare the release of DIBP from samples of take-away pizza boxes purchased in Italian pizza restaurants. The suggested and standardized analytical conditions permit the identification of this contaminant often identified in boxes largely used for take-away, and also enable the calculation of an 'EI'. This index, useful in making a valuable comparison between different samples, also makes it possible to evaluate DIBP risk contamination of hot pizza introduced into the box for take-away. The 'EI' represents a useful standardized risk measure.

In addition, this method avoids the problem that arises when measurements are made by simply introducing the fibre in the free internal volume of the box; here, we quantified the concentration of the pollutant in the headspace, which can be more relevant to the real-life scenario.

Moreover, the identification of DIBP in take-away pizza boxes in Italy has recently revealed the frequent non-observance of the specific law regulating the compulsory use of regenerated cellulose, rather than recycled cellulose, for boxes intended for carry-out pizza. Based on our analysis, related to the sample collected in 2006, it is evident that a very large range of DIBP is released, falling between the 'EIs' of 6–72.

ACKNOWLEDGEMENTS

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