Occurrence of patulin in organic and conventional apple-based food marketed in Catalonia and exposure assessment

Ester Piqué a,b,*, Liliana Vargas-Murga a,c, Jesús Gómez-Catalán a,b, Joaquin de Lapuente b,d, Joan Maria Llobet a,b

a GRET-CERETOX and Toxicology Unit, Public Health Department, School of Pharmacy, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain
b Nutrition and Food Safety Research Institute (INSA UB), Food and Nutrition Torribera Campus, Avda. Prat de la Riba 171, 08921 Santa Coloma de Cramenet, Spain
c Biothani Europe, S.L: Can Lleganya, Sant Feliu de Buixalleu, Spain
d GRET-CERETOX and Experimental Toxicology and Ecotoxicology Unit, Barcelona Science Park, Badirí i Reixac 10-12, E-08028 Barcelona, Spain

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A B S T R A C T

In the last years, consumption of organic foods has become increasingly popular. Nevertheless, safety of organic foods is still unclear, and needs to be thoroughly evaluated. Patulin is a mycotoxin mainly present in rotten apples and apple-based products. The aim of this study is to analyse the content of patulin in apple juices and purees derived from organic and conventional production systems, in order to assess the risk to consumers, particularly in children. A total of 93 apple-based products marketed in Catalonia were analysed, 49 of which were derived from conventional and 44 from organic farming. The results showed higher incidence of positive samples and higher concentration of patulin in organic apple purees when comparing with conventional ones. In the case of juices, significant differences were found between conventional and organic samples, but applying a multivariate analysis the type of agriculture did not seem to have a relevant contribution to patulin occurrence, being cloudiness the main factor involved. The estimated daily intake of patulin for infants and young children (0–3 years old), children (4–18 years old) and adults (19–66 years old), were below the provisional maximum tolerable daily intake (PMTDI) of 0.4 μg/kg bw in all scenarios considered.

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1. Introduction

Patulin is a mycotoxin produced by some filamentous fungi of the genus Aspergillus, Penicillium and Byssochlamys. These fungi grow in fruits and vegetables, but rotten apples and apple-based products are considered the main source of this fungal toxin, and Penicillium expansum the principal responsible. Patulin was firstly proposed for therapeutic purpose because of its antibiotic properties. However, in 1960s it was reclassified as mycotoxin due to its toxicity (Puel et al., 2010).

Patulin causes gastrointestinal effects as distension, ulceration and haemorrhage in acute and short-term in vivo studies. Recent studies have also demonstrated that patulin alters the intestinal barrier function. In chronic studies in rats, patulin causes neurotoxicity, immunotoxicity and genotoxicity. Reproductive and teratogenicity in vivo studies showed that patulin is embriotoxic. Regarding its potential as a human carcinogen, it has been classified as group 3 (Not classifiable as to its carcinogenicity to humans) by International Agency for Research on Cancer (IARC) (Moake et al., 2005). Patulin has electrophilic properties and high reactivity to cellular nucleophiles. At cellular level it can cause enzyme inhibition and chromosomal damage. Patulin causes cytotoxic and chromosome-damaging effects mainly by forming covalent adducts with essential cellular thiols (Fliege and Metzler, 2000; Glaser and Stopper, 2012; Pfeiffer et al., 1998). Based on reproductive and carcinogenicity studies, a provisional maximum tolerable daily intake (PMTDI) of 0.4 μg/kg body weight/day was established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) (WHO, 1995).

The European legislation has established a maximum level of 50 μg/kg of patulin in apple juices, 25 μg/kg for apple puree and 10 μg/kg for foods that are intended for infants and young children (European Commission, 2006a). Over the past years, several studies have been carried out in different countries in order to monitor the human exposure to patulin through apple-based products and
to determine the associated risks (Marin et al., 2011). Some of these studies have found samples with content of patulin above maximum allowed level particularly in organic food (Baert et al., 2006; Bonerba et al., 2010; Piemontese et al., 2005; Ritieni, 2003; Spadaro et al., 2007).

In the last years, consumption of organic foods has become increasingly popular for a number of reasons, the most significant of which is the perception of health and wellness benefits associated with naturally grown foods. Specifically, organic foods are promoted as being safer, better-tasting, environmentally and farmer friendly. In addition, consumers are choosing organic foods because several food crises have caused a health concern. Consequently, organic farming has risen in Spain, especially in Catalonia, as well as in whole Europe, since the nineties. However, the health benefits of consuming organic compared to conventional foods are still unclear. Current scientific literature cannot state that organic food is healthier than conventional alternatives and future research is needed (Hoeckens et al., 2010,2009).

The most important factors that influence the presence of mycotoxins in crops or raw materials are the insect attack, damage to vegetables during harvest and the temperature and humidity during storage (Reddy et al., 2010). Typical organic farming practices are characterised by very strict limits on chemical synthetic pesticides (fungicides, herbicides, insecticides) and synthetic fertilizer use. This fact could lead to more susceptible food to fungal attack and mycotoxin contamination (Magkos et al., 2006). The aim of the present study is to evaluate the occurrence of patulin in organic and conventional apple-based products in Catalonia and the risk of their intake for population. None of the studies previously conducted in Catalonia have compared these production systems regarding patulin contamination.

2. Materials and methods

2.1. Chemicals and reagents

All the selected chemicals including patulin and 5-hydroxymethyl furfural (5-HMF) standards, anhydrous sodium sulphate, sodium carbonate, pectinase and acetic acid were purchased from Sigma–Aldrich (Madrid, Spain). Acetonitrile (HPLC grade) was obtained from Panreac (Barcelona, Spain). Acidified water was prepared adjusting the pH to 4 with acetic acid. Deionised water was obtained using a Milli-Q water purification system (Millipore Iberica S.A.U., Madrid, Spain).

Patulin stock solution (200 μg/mL) was prepared in ethyl acetate and stored at –20°C. In order to prepare the working solution (10 μg/mL), 250 μL of stock solution were evaporated and dissolved in 5 mL of ethanol. The exact concentration of the working solution was calculated by UV at 276 nm, against a solvent blank, using the molar extinction value and also was stored at –20°C. Finally, a patulin calibrant solution (1000 ng/mL) was prepared evaporating an appropriate volume from the working solution and then dissolved in acidified water and was stored at 4°C.

2.2. Samples

A total of 93 apple-based products from 24 different brands (18 local and 6 imported), were purchased in Catalan supermarket and bio shops from November 2011 to May 2012: 47 apple juice (25 conventional and 22 organic) and 46 apple puree (24 conventional and 22 organic). These samples included 27 baby foods (4 conventional juices, 4 organic purées and 19 conventional purées) intended for infants and young children (0–3 years old). Characteristics of samples have been included in a supplementary file.

Juices and purées of the same product and brand but from different batch were considered as different samples. All samples were stored in their original packages at room temperature until they were analysed. The samples were opened and thoroughly homogenised. One aliquot was processed and four aliquots were stored at –20°C for duplicating analysis.

2.3. Analysis of patulin in apple-based products

Analytical method in apple juice was based on AOAC method 995.10 with little modifications (Brause et al., 1996). Briefly, 2.5 mL of juice was extracted twice with 10 mL of ethyl acetate. The organic extracts were cleaned up with a solution of sodium carbonate and then dehydrated with anhydrous sodium sulphate. After evaporation with nitrogen, the dry residue was dissolved in 250 μL of acidified water and was injected into HPLC for chromatographic analysis. A previous step with pectinase was done in puree analysis (Marin et al., 2011). The pectinase treatment consisted on adding 100 μL of pectinase enzyme solution (>3800 units/mL) to 2.5 g of homogenised sample. The enzyme was left to act during 1 h at 40°C. After that, the samples were centrifuged 5 min for 6000 rpm, and 2.5 mL of the supernatant was processed as described above for juices.

2.4. Chromatographic analysis

The HPLC system consisted on a Gilson Inc (Middleton, WI, USA) model G-234 auto-injector, a G-832 temperature regulator, two G-306 pumps, a G-805 monometric module and a G-811C mixer. Separations were performed on a Nucleosil 100 C18 column (5 μm, 25 × 0.46 cm; Teknokroma, Spain) connected to a Teknokroma BDS guard column. The detector was a Gilson Inc (Middleton, WI, USA) model G-118 UV/Vis detector. The composition of mobile phase was water: acetonitrile (90:10, v/v). The volume of injection was 50 μL, the flow rate of the mobile phase was 1.0 mL/min and wavelength of detection was 276 nm.

The chromatograms were evaluated using Gilson UniPoint system software version 2.0. The peak area was used as the quantitative signal. Quantification was carried out by external linear calibration.

2.5. Validation studies

A column test was performed in order to confirm the peak separation of patulin and its principal interference 5-HMF. A solution containing 2 mg/L of 5-HMF and 50 μg/L of patulin was prepared and injected into HPLC.

Linearity was checked by injection into HPLC of patulin standards in the range from 10 to 1000 μg/L which is equivalent to range 1–100 μg/kg of patulin in apple juices and 2–200 μg/kg in apple puree, the correlation coefficient obtained was 1.00.

Recovery experiments were done by spiking negative samples at concentrations of 10, 50 and 100 μg/kg. After 1 h the spiked samples were processed. The precision was calculated from the relative standard deviation (RSD) (Table 1).

In order to establish the limit of quantification (LOQ), 6 negative samples for juices and 6 more for purées were spiked at different concentrations. The LOQ was determined as the lowest concentration allowing a quantification with the acceptable accuracy (recovery 50–120%) and precision (RSD <30%) set by European legislation (European Commission, 2006b). The LOQ for juices was 3 μg/kg and for purées was 6 μg/kg.

2.6. Statistical analysis

All results were reported corrected for recovery. Samples with a concentration of patulin higher than the LOQ were considered positive, thus samples with concentrations lower than LOQ were considered negative. For samples with a concentration below the LOQ, a concentration of LOQ/6 was used for calculating the average, following the recommendations of European Union (Majerus and Kapp, 2002).

To compare patulin mean concentrations Mann–Whitney and Kruskal–Wallis tests were used. The Chi-square test and Fisher Exact test were used to compare frequencies. A logistic regression analysis for patulin occurrence has been done considering type of agricultural practices, cloudiness and presence of vitamin C as categorical independent variables. Statistical analyses were performed using the programme Statgraphics plus 5.1.

2.7. Exposure assessment

In order to characterise the exposure to patulin, three population groups have been considered: infants and young children (0–3 years old), children (4–18 years old) and adults (19–66 years old). Intake of patulin was obtained multiplying consumption data (mean or 95th percentile) and the mean patulin concentration of

<table>
<thead>
<tr>
<th>Spiking level (μg/kg)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>84.7</td>
<td>7.6</td>
</tr>
<tr>
<td>50</td>
<td>82.5</td>
<td>2.4</td>
</tr>
<tr>
<td>100</td>
<td>90.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Apple puree</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>63.2</td>
<td>10.8</td>
</tr>
<tr>
<td>50</td>
<td>64.4</td>
<td>1.8</td>
</tr>
<tr>
<td>100</td>
<td>86.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

RSD, relative standard deviation.
organic or conventional apple-based products. It was assumed that a consumer uses only one of the two categories of products, conventional or organic apple-based products. The consumption data for apple-based products reported by Cano-Sancho et al. (2009) were considered.

3. Results and discussion

3.1. Occurrence of patulin in apple products

The mean concentration in the 47 apple juice samples was 5.5 µg/kg (Table 2), and none of them exceeded the maximum levels set by (European Commission, 2006a). These results are in accordance with some studies recently performed in Spain that found mean concentration between 4.2 and 8.1 µg/kg (Cano-Sancho et al., 2009; Marin et al., 2011; Murillo-Arbizu et al., 2010). A comparison of percentage of positive samples obtained in the present study (45%) with these previous studies (7–70%) is difficult as a consequence of different criteria considering positive samples.

Factors that could influence the patulin occurrence in apple juice are the type of agriculture (Piemontese et al., 2005), cloudiness (Baert et al., 2007a), and the addition of vitamin C (Sant’Ana et al., 2008). These factors were first analysed separately by means of univariate tests. A higher incidence of patulin contamination \( p = 0.014, \) Chi-square Test) and a higher concentration \( p = 0.0028, \) Mann Whitney Test) were found in organic juices when comparing with conventional ones. A higher incidence of patulin contamination \( p = 0.002, \) Fisher Exact Test) and a higher concentration \( p = 0.001, \) Mann Whitney Test) were found in cloudy juices than in clear ones, and finally, results indicated that patulin contamination was not related to vitamin C addition \( p = 0.12, \) Chi-square Test; \( p = 0.26, \) Mann Whitney Test). The multifactorial analysis of the effects of these factors was difficult by the strong correlation between them, specifically between the agriculture type and cloudiness. In our samples there were no conventional cloudy juices. This asymmetry reflects the fact that actually there is a very low penetration of conventional cloudy apple juices in the Catalan market. The comparison between clear juices from both agriculture types showed no significant differences \( p = 0.61, \) Fisher Exact Test; \( p = 0.63, \) Mann Whitney Test) whereas the comparison for cloudy juices was not possible. As a multivariate analysis option a logistic regression model was applied in order to determine which factors have a significant predictive capacity to classify negative and positive samples. The results of logistic regression showed that the main factor contributing to patulin positive result in apple juice was cloudiness (Odds Ratio = 30; CI 95 = 1.6–560) whereas the type of agriculture practices and vitamin C addition did not make a significant contribution.

Some authors observed that levels of patulin in apple juice are influenced by the addition of additives into the formulations, and described the patulin instability and their fast degradation when ascorbic acid was present (Sant’Ana et al., 2008). However, the real responsible of patulin degradation seems to be the free radicals generated by ascorbic acid oxidation, this process needs oxygen but food package has low oxygen content, consequently the addition of ascorbic acid to products prior to filling, cannot reduce the content of patulin in an effective manner (Drusch et al., 2007). Ours results are in accordance to that.

Results obtained regarding cloudiness, both separately and analysed by logistic regression agree with those found in Belgium and Portugal (Baert et al., 2006; Barreira et al., 2010; Tangni et al., 2003). The solid parts of cloudy juices are richer in proteins compared to the liquid phase, and probably, patulin could interact with these proteins (Baert et al., 2007a).

Most patulin generation occurs postharvest but it can also take place during harvest. Organic farming practices could favour insect damage and fungi infections. The physical damage provides an entrance for patulin-producing fungi into the fruit, and thus the majority of infections by P. expansum in apples are due to injuries caused by insects within apples (Moake et al., 2005). This hypothesis about agricultural practices could not be confirmed in the present study. Univariate analysis suggested that there were significant differences between conventional and organic samples, but applying a multivariate analysis the type of agriculture seems not to have a relevant contribution to patulin occurrence. However, this analysis could be biased because the strong correlation between cloudiness and organic juices.

Two of the 46 apple puree samples analysed (Table 3) exceeded the maximum level of 25 µg/kg fixed by EU, but none of the baby food samples exceeded the maximum level of 10 µg/kg (European Commission, 2006a). Mean concentration obtained considering only positive samples was 22.5 µg/kg and percentage of positive samples was 13%. Previous studies conducted in Spain showed lower mean concentration (considering positive samples) of patulin, ranged from 0 to 13.5 µg/kg, and lower percentage of positives, ranged from 0% to 5.2% (Cano-Sancho et al., 2009; Marin et al., 2011). There was a higher incidence of patulin contamination \( p = 0.008, \) Fisher Exact Test) and a higher concentration \( p = 0.007, \) Mann Whitney Test) in organic samples. As well as in juices, the patulin contamination was not related to vitamin C presence.

There was a high variability in patulin contamination between brands analysed and between batches from the same brand (supplementary file). One of the most important factors determining

### Table 2

<table>
<thead>
<tr>
<th>Production method</th>
<th>% Positives</th>
<th>Number of samples</th>
<th>Max (µg/kg)</th>
<th>Mean ± SD all samples (µg/kg)</th>
<th>Mean ± SD positive samples (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positivea/Total</td>
<td></td>
<td>10–25 µg/kg</td>
<td>25–50 µg/kg</td>
<td>&gt;50 µg/kg</td>
</tr>
<tr>
<td>Conventional</td>
<td>7/25</td>
<td>28</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>14/22</td>
<td>64</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Additives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without vitamin C</td>
<td>9/26</td>
<td>35</td>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>With vitamin C</td>
<td>12/21</td>
<td>57</td>
<td>7</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Clarity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear</td>
<td>10/35</td>
<td>29</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Cloudy</td>
<td>11/12</td>
<td>92</td>
<td>4</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>21/47</td>
<td>45</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

a Samples with patulin concentration > LOQ were considered as positive samples.
b LOQ, limit of quantification.
c Mean level was calculated using LOQ/6 for negative samples.
d Mean level considering only positives samples.
patulin contamination is the quality of apples, and this quality is heterogeneous among batches and brands (Moake et al., 2005).

### Table 3
Occurrence of patulin in apple puree.

<table>
<thead>
<tr>
<th>Positive / Total</th>
<th>% Positives</th>
<th>Number of samples</th>
<th>Max (μg/kg)</th>
<th>Mean ± SD all samples (μg/kg)</th>
<th>Mean ± SD positive samples (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LOQ - 10 μg/kg</td>
<td>10 - 25 μg/kg</td>
<td>25 - 50 μg/kg</td>
<td>&gt; 50 μg/kg</td>
</tr>
<tr>
<td>Production method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>0/24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Organic</td>
<td>6/22</td>
<td>27</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Additives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without vitamin C</td>
<td>5/17</td>
<td>29</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>With vitamin C</td>
<td>1/29</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>6/46</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

* Samples with patulin concentration > LOQ were considered as positive samples.
* LOQ, limit of quantification.
* Mean level was calculated using LOQ/6 for negative samples.
* Mean level considering only positive sample.

### Table 4
Exposure to patulin through apple juice consumption of Catalonian population.

<table>
<thead>
<tr>
<th>Population group (years)</th>
<th>Weight (kg)</th>
<th>Apple juice intake a (mL/day)</th>
<th>Patulin intake (ng/kg bw /day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conventional</td>
<td>Organic</td>
</tr>
<tr>
<td>Infants and young children (0–3) Mean consumers</td>
<td>12</td>
<td>20.40 b</td>
<td>4.0</td>
</tr>
<tr>
<td>Infants and young children (0–3) High consumers</td>
<td>12</td>
<td>135.50 c</td>
<td>26.3</td>
</tr>
<tr>
<td>Children (4–18)</td>
<td>40</td>
<td>64.53 b</td>
<td>3.8</td>
</tr>
<tr>
<td>Adults (19–66)</td>
<td>70</td>
<td>63.95 b</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* Apple juice intake of Catalonian population reported by Cano-Sancho et al. (2009).
* Mean of consumers.
* 95th Percentile.

### Table 5
Exposure to patulin through apple purees consumption of Catalonian population.

<table>
<thead>
<tr>
<th>Population group (years)</th>
<th>Weight (kg)</th>
<th>Apple puree intake a (g/day)</th>
<th>Patulin intake (ng/kg bw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conventional</td>
<td>Organic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Organic baby food b</td>
</tr>
<tr>
<td>Infants and young children (0–3) Mean consumers</td>
<td>12</td>
<td>22.2 b</td>
<td>1.9</td>
</tr>
<tr>
<td>Infants and young children (0–3) High consumers</td>
<td>12</td>
<td>130 c</td>
<td>10.8</td>
</tr>
</tbody>
</table>

* Apple puree intake of Catalonian population reported by Cano-Sancho et al. (2009).
* Mean of consumers.
* 95th Percentile.
* Organic purees intended for infants.
* Organic purees not intended specifically for infants.

3.2. Exposure assessment

Different scenarios were considered related to apple juice consumption and to loyalty to a particular production system. The worst case scenario regarded was a consumer above the 95th percentile and faithful to the most contaminated brand. As discussed in Section 3.1, in both juices and purees, the most contaminated brand belongs to the organic food category. The patulin intake derived from consumption of organic apple-based products was higher than from conventional ones (Tables 4 and 5). However, in all cases including the worst case scenario, the estimated daily intakes of patulin for all population groups studied were below the PMTDI (400 ng/kg bw). Two additional scenarios were added when purees were analysed, infants and young children who eat organic purees intended for infants (baby food) and, infants and young children who eat organic purees not intended specifically for infants. Conventional purees were not considered in these new scenarios since none of the 24 samples of conventional puree analysed were positive for patulin (Table 5). Considering to whom was destined the apple-based products, exposure to patulin arising from consumption of organic apple puree not intended specifically for infants was higher than that resulting from consumption of organic baby food apple puree. Infants and young children are considered to be more susceptible to all the toxins than adults because of their lower body weight,
higher metabolic rate, lower ability to detoxify, and incomplete
development of some organs and tissues (Drusch and Aumann, 2005).
Most of the studies undertaken in Europe showed that in-
fant and children were the main group exposed to patulin, in
comparison with adults (Baert et al., 2007b; Cano-Sancho et al.,
2009; González-Osnaya et al., 2007; Moukas et al., 2008; Murillo-
Arbiizu et al., 2009; Piemontese et al., 2005; Tangni et al., 2003;
Thuvalander et al., 2001). Our results were also accordance to that.
Consequently, they represent the highest risk group since they
are the most susceptible group, but also because of their large
dietary intake of apple-based foods. Considering as the worst scenario
an infant with a juice intake of 135.5 mL/day (95th percentile) of
the most contaminated brand (14 ng of patulin/mL) the patulin in-
take would be 153 ng/kg bw/day, which represents 38.5% of the
PMTDI. In the case of purees, for an infant who has a consumption
above the 95th percentile, 130 g/day, of the most contaminated
brand (21 ng patulin/g) the patulin intake would be 223 ng/kg
bw/day, more than a 50% of the PMTDI. Considering that an infant
could eat puree and apple juice, the patulin intake derived from
this pattern of consumption would be 377 ng/kg bw/day, which
is near to PMTDI. In a recent study performed by Brandon et al.
(2012) in Netherlands, the results obtained were in accordance to
ours, the highest exposure to patulin, 342 ng/kg bw/day, corre-
sponds to children aged 12–20 months with high consume of or-
ganic apple-based products (95th percentile).

4. Conclusions

In recent years, several surveys have been conducted in Europe
in order to analyse patulin levels in organic and conventional apple-
based products and to determine the associated risk for human
health. The present study is the first that has been carried out in
Catalonia. Incidence and levels of patulin were higher in organic
than in conventional food. Nevertheless, in apple juice the con-
tribution of agriculture practices and cloudyness cannot be separated
due to the lack of cloudy conventional juice in the analysed sam-
ples. Patulin intake in population groups studied does not repre-
sent a health concern; all the estimated intakes of patulin calculated
from the data of this study are below the PMTDI. Finally, it
is worth noting that vulnerable groups, such as infant and chil-
dren, with higher risk associated to the consumption of these apple
products, could occasionally overcome PMTDI.

5. Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in
the online version, at http://dx.doi.org/10.1016/j.fct.2013.07.052.

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