PROBABILISTIC EXPOSURE ASSESSMENT OF PATULIN IN APPLE JUICE FOR PRESCHOOL CHILDREN IN FLANDERS

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INTRODUCTION

The mycotoxin patulin (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one) is produced by a large number of fungi including *Penicillium expansum*. This mould is commonly identified as the 'blue mould rot', a serious post-harvest disease of apples. As a consequence, patulin is frequently occurring in apples and apple products, like apple juice (Moake et al., 2005). The aim of the present study was to estimate the consumers' exposure to patulin through organic, handcrafted and conventional apple juice, based on a probabilistic method. The findings were then compared with the Tolerable Daily Intake (TDI) aiming to help risk managers in the regulatory decision making process. Since, a Scientific Cooperation task (SCOOP task) has shown that small children have a higher patulin intake compared to other population groups and because apple juice and apple nectar are the main sources of patulin intake in most countries, the focus of this probabilistic exposure assessment is on this particular population group and this specific matrix (Directorate – General Health and Consumer Protection, 2002). Secondly, the study aimed to evaluate the effectiveness of a reduction of the regulatory limit for patulin in apple juice.

MATERIALS AND METHODS

Data on the apple juice consumption were obtained from a large-scale epidemiological study investigating nutrition habits of preschool children in Flanders (2.5-6.5 years old), using 3-day estimated diet records and parents as a proxy (Huybrechts et al., 2006). The contamination data used in the present study were derived from a previous study (Baert et al., 2006) in which 177 apple juice samples were analyzed on their patulin content (65 organic, 90 conventional and 22 handcrafted apple juices).
The exposure to patulin was modeled by multiplying consumption data with contamination data of organic, conventional and handcrafted apple juice (patulin intake (µg/kg bw/day) = concentration of patulin in apple juice (µg/kg) x apple juice consumption (g/kg bw/day) x 1 (kg) / 1000 (g)).
In order to characterize the variability of interindividual consumption and patulin concentrations, a non-parametric approach was used. This means that a distribution is used whose mathematics is defined by the required

shape (Vose, 2000). In this study, a discrete uniform distribution (RiskDuniform) was used for both the observed consumption and contamination values. A discrete uniform distribution is a special case of the Discrete distribution where all possible values have the same probability of occurrence (Vose, 2000).

Uncertainty characterization was performed using non-parametric bootstrap. The bootstrap theory assumes that the distribution F (of e.g. patulin concentration in apple juice) can be reasonably approximated by the distribution F' of n observed values. This is of course a more reasonable assumption, when more data are collected. For a sufficiently large number of times, n random samples are taken with replacement from the distribution F' and each time a statistic of interest is calculated from the sample (Vose, 2000). Contamination data below the Limit Of Detection (LOD) were replaced by random samples from a uniform distribution with α as a minimum (α = uniform distribution between zero and the LOD) and β as a maximum (β = a uniform distribution between zero and the LOD) (RiskUniform (RiskUniform (0; LOD); RiskUniform (0; LOD))).

Propagation of the characterized variability and uncertainty was then performed using Monte Carlo simulation. It was executed with @RISK, a risk analysis software (@RISK 4.5 for excel professional edition, Palisade, UK). Latin Hypercube sampling was used to randomly sample the probability distribution functions of input parameters (consumption and contamination data) and the samples were used to calculate the intake by the described model. One thousand simulations were carried out to describe the consumption and contamination variability in the population and 1000 bootstrap iterations were carried out to estimate the uncertainty (expressed as a confidence interval).

In the study it was assumed that children consume a certain type of apple juice (e.g. organic) and do not consume different types. It was also assumed that the consumption data were the same for the different consumers (organic, handcrafted and conventional consumers).

RESULTS AND DISCUSSION

In a previous study, higher patulin concentrations were found in organic apple juice (8.8 μg/L) compared to conventional (4.1 μg/L) and handcrafted apple juice (4.4 μg/L) (Baert et al., 2006). It was one of the aims of the present study to evaluate the implications of these higher concentrations with regard to public health. The estimated exposures to patulin together with the 90% CI are summarized in Table 1 for the 3 types of apple juice tested. For the three types of apple juice, the 83rd percentile shows an intake of 0 μg/kg bw/day, indicating that 83% of the children have no intake of patulin. For organic apple juice the best estimate for the 99.5th percentile is higher than the TDI (of 0.43 μg/kg bw/day). The lower limit of the 90% CI of the 99.9th percentile is also higher than the TDI. Also for conventional apple juice, the best estimate shows that the TDI will be exceeded in less than 0.1% of the cases. The best estimate of the exposure through handcrafted apple juice is below the TDI. Although higher average exposures are observed for organic apple juice compared to the other two practices, their CI overlap, which indicates that the average exposure to patulin of children will be roughly the same for the 3 kinds of apple juice tested.

Table 1. Statistics on exposure (µg/kg bw/day) for different apple juices (AJ) (best estimation [90% confidence interval])

	Organic AJ	Conventional AJ	Handrafted AJ
P0	0 [0-0]	0 [0-0]	0 [0-0]
P50*	0 [0-0]	0 [0-0]	0 [0-0]
P83	0 [0-0]	0 [0-0]	0 [0-0]
P85	0.0001 [0-0.024]	0 [0-0.017]	0.0001 [0-0.022]
P90	0.039 [0.014-0.069]	0.030 [0.011-0.049]	0.037[0.013-0.066]
P95	0.072 [0.027-0.117]	0.059 [0.031-0.085]	0.065 [0.027-0.102]
P97.5	0.135 [0.053-0.229]	0.095 [0.057-0.133]	0.102 [0.047-0.151]
P99	0.350 [0.143-0.822]	0.156 [0.106-0.206]	0.150 [0.084-0.229]
P99.5	0.615 [0.249-1.472]	0.202 [0.141-0.287]	0.195 [0.109-0.290]
P99.9	1.471 [0.526-3.066]	0.328 [0.210-0.548]	0.298 [0.156-0.460]
Mean	0.019 [0.010-0.032]	0.009 [0.006-0.013]	0.010 [0.005-0.015]
Probability to exceed the TDI	0.009 [0.003-0.018]	0.001 [0-0.003]	0 [0-0.002]

* 50^{th} percentile

Comparison of the percentage of children exceeding the TDI for the 3 types of apple juice (Table 1) indicates that the probability of exceeding the TDI is higher for organic apple juice than for conventional and handcrafted apple juice. With 90% certainty, it can be stated that between 0.3 and 1.8% of the children consuming organic apple juice exceed the TDI while for conventional and handcrafted apple juice this is between 0 and 0.3% and between 0 and 0.2% respectively.

In Europe, the maximum limit for patulin is 50 µg/kg (European Commission, 2003). However during the study on the occurrence of patulin in apple juice in Flanders, two organic apple juices were found with patulin contents above 50 µg/kg (Baert et al., 2006). Therefore, the influence of a strict implementation of the current legislation on patulin exposure of young children, was tested by removing these two samples from the dataset. In the regulation 1425/2003 it was also foreseen that the commission would review the maximum levels for patulin by 30 June 2005 at the latest in order to reduce them (European Commission, 2003). However, until now no new legislation is in preparation or has been published. In order to test the effect of lowering the Maximum Limit (ML), the exposure was simulated with a limit reduced by 50%, resulting in a new limit of 25 µg/kg which is also the current ML for solid apple products. For this test, all samples above 25 µg/kg were removed from the dataset. Since concentrations above 25 µg/kg were only found for organic apple juice, both techniques were just tested for organic apple juice alone. Making a comparison of the percentage of the population exceeding the TDI (Table 2), shows that a strict implementation of the limit of 50 µg/kg reduces the percentage of children exceeding the TDI, (between 0.1% and 1.2%). A restriction to 25 µg/kg reduces the exposure higher than the TDI further, towards 0 to 0.3% of the population under study. Therefore it can be concluded that in order to reduce the exposure to patulin, a reduction of the legal limit to 25 µg/kg is necessary and causes a six fold reduction of the probability to exceed the TDI. However, it needs to be stressed that the implementation of a ML is only effective when this limit is followed and as a consequence control on the implementation is necessary.

Table 2. Comparison of the statistics on exposure for organic apple juice for different hypotheses to reduce the level of contamination of apple juice (best estimation [90% confidence interval])

	No reduction	Contamination <50µg/l	Contamination <25µg/l
Probability to exceed the TDI	0.009 [0.003-0.018]	0.005 [0.001-0.012]	0 [0-0.003]

CONCLUSIONS

Monte Carlo simulation showed that 0.9% [90% confidence interval (CI): 0.3 -1.8%] of the children consuming organic apple juice exceed the TDI. For conventional and handcrafted apple juice this was respectively 0.1% [90% CI: 0-0.3%] and 0% [90%CI: 0-0.2%]. Reduction of the maximum limit (ML) for patulin in apple juice from 50 to 25 µg/kg reduces the percentage of the children exceeding the TDI to 0% [90%CI: 0-0.2%], indicating that a reduction of the legal limit to 25 µg/kg is necessary to reduce the exposure to patulin.

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