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## Abstract

Patulin is a mycotoxin produced by several filamentous fungi, which has been the object of a number of surveys in recent decades, due to its frequent occurrence in apple products made from decayed fruit. The legislated limit in apple products is 50 μg/kg and a provisional maximum tolerable daily intake (PMTDI) of 0.4 μg/kg bodyweight/day has been set.

It is of concern that the risk reducing practices adopted in large scale commercial apple juice production do not apply to the Swedish home producers of apple must. Potentially risky practices include incorrect storage conditions and not discarding bad apples. Home producers are presumed to consume large volumes and are thereby exposed to potential patulin. This study presents a chemical risk assessment of patulin in home-made apple must, including a minor study on the routines of eight Swedish apple must processors. Previous studies have focused on commercial and clear juice products, but there is no risk assessment available regarding small scale produced apple must.

It became apparent that not all processors are considered as food sale operations, but offer instead a service, which may put them outside current food inspection regimes and thus entail less responsibility for the final product.

Based on concentrations of patulin found in a previous study of raw cloudy apple juice, made from apples with 0% or 30% rot, intake for average and high consumers were estimated to be 0.009-2.04 μg/kg/bw/day and 0.003-0.65 μg/kg/bw/day among 4-year olds and adults, respectively. However, the simulated exposure calculations were based on patulin concentrations that may not fully represent the situation in Sweden. Sources of uncertainty in the exposure assessment are discussed. The main gaps in knowledge to perform a more accurate risk assessment for patulin in Swedish home-made apple juice include: concentrations of patulin in similar products; consumption data on home-made apple must; and patulin occurrence in Swedish apple cultivars. From the overview of apple must production gained during this study, suggested measures to reduce patulin risk in this product are to avoid storage of bruised and ground harvested fruit and remove rotten fruit and parts thereof before processing.

*Keywords:* Patulin, mycotoxin, apple, *Penicillium expansum,* risk assessment, exposure assessment, Sweden

## Sammanfattning

Patulin är ett mykotoxin som produceras av ett flertal filamentösa svampar och som har studerats de senaste åren på grund av dess förekomst i äppelprodukter gjorda av dålig frukt. Det högsta tillåtna gränsvärdet i äppelprodukter är 50 µg/kg medan tolerabelt intag, PMTDI, är 0,4 µg/kg kroppsvikt/dag.

Småskaliga musterier som pressar äppelmust av privatodlares skörd förmodas att inte ha samma kontroll eller kunskap kring risken för patulinkontaminering så som storskaliga industriella producenter. Exempel på faktorer som ökar risken för patulinkontaminering är felaktiga lagringsförhållanden samt att använda skadad fallfrukt i produktionen. De personer som levererar sin skörd för pressning förväntas dricka stora volymer och kan på så sätt eventuellt exponeras för patulin. En kemisk riskbedömning av patulin i äppelmust pressad av privatpersoners egen frukt presenteras här tillsammans med resultatet från en intervjustudie med åtta småskaliga musterier. Tidigare forskning har fokuserat på patulin i storskaligt producerad äppeljuice av annan karaktär, men det finns ingen riskbedömning gjord på äppelmust producerad småskaligt.

Intervjustudien visade att inte alla musterier klassificeras som livsmedelsföretag, utan anses erbjuda en tjänst. I och med det undantas musterierna kommunala livsmedelskontroller samt fullständigt ansvar för den färdiga produkten, likt en industriell producent.

Tidigare funna koncentrationer av patulin i råpressad äppeljuice gjord på 0 % respektive 30 % rutten frukt applicerades i riskbedömningen för att simulera olikascenarier. Den estimerade exponeringshalten hos medel- och högkonsumenter uppskattades till 0,009-2,04 µg/kg/kv/dag bland 4-åringar samt 0,003-0,65 µg/kg/kv/dag bland vuxna. Omständigheterna i originalstudien är inte likvärdiga de som råder hos privatodlare i Sverige, osäkerhet kring detta, samt de antaganden som har gjorts, diskuteras i rapporten. För att kunna göra en förbättrad exponeringsuppskattning i Sverige krävs en kartläggning av förekomsten av patulin i svenska äppelsorter, konsumtionsdata på hemgjord äppelmust samt uppdaterade uppgifter av patulinkoncentrationer i äppelmust eller liknande produkter. Privata odlare och äppelmusterier rekommenderas att vidta riskreducerande åtgärder, så som att undvika att plocka fallfrukt och skadad frukt för lagring samt att göra en noggrann kvalitetsbedömning, för att sortera bort ruttna äpplen, innan mustning.

*Nyckelord:* Patulin, mykotoxin, äpple, Penicillium expansum, riskbedömning, exponeringsuppskattning, Sverige

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# 1 Introduction

## 1.1 Background

Patulin is a mycotoxin produced by certain species of *Aspergillus*, *Penicillium* and *Byssochlamys* filamentous fungi (moulds) that grow on different types of foods including fruits. One of the toxin producing moulds, *Penicillium expansum*, is a common cause of rot in apples. (Harrison 1988) Major dietary source of patulin are apples and apple products made from damaged fruit, and toxic effects include gastrointestinal inflammation and genotoxicity. (Majerus and Kapp 2002, Puel et al. 2010)

Occurrence of patulin in apples was first reported in the 1950's (Brian et al. 1956). During the past decades, the mycotoxin has been found in commercial apple products and juice in several investigations. (Murillo-Arbizu 2009) This can in part be explained by the practice of using culled apples, unfit for sale as fresh fruit, for processed apple products. (Moake et al. 2005) In 1992 a smaller survey reported the alarming result that patulin exceeded the maximum limit of 50 µg/kg in 1 in 4 apple products in the U.K. This lead to increased awareness among manufacturers of the contamination risk in apple products. (British Soft Drinks Association, Sydenham et al. 1995, Bandoh 2012)

However, it is of concern that the monitoring conducted by commercial manufacturers does not apply to the home production of apple products. Pressing home picked apples into fresh apple must (sv. äppelmust) has become more popular, according to the manufacturers offering the service[1]. To avoid food waste, the fruit is not solely picked from the tree, but can additionally be ground harvested, which increases the risk for mould infection and subsequent patulin contamination. (FDA 2004, Godani 2014) It has

1. Interviews with personnel from must manufacturies, 2015-04-15 – 2015-05-13. Hereafter, this source is cited as (pers. comm.).

been observed that apples arriving at the must making stations (sv. musteri) may be of mixed condition. (pers. comm.)

People making apple must are presumed to consume large volumes and are thereby exposed to potential patulin in the product. Thus, it is uncertain if consumers of less regulated home-made products are exposed to higher levels of patulin than those who drink primarily processed products.

## 1.2 Aims and Scope

The purpose of this study was to perform a chemical risk assessment of patulin in home-made[2] apple must, together with a small study of routines and practices among apple must producers. The latter was obtained through interviews with personnel from eight apple must manufacturers. The secondary aims were to evaluate the outcome of the risk assessment, by identifying gaps in available data, and to suggest risk-reducing measures for the production of home-made apple must.

By way of introduction, the concept of chemical risk assessment is described, the source and occurrence of patulin in apple products is discussed, and current regulatory limits for patulin are summarised.

## 1.3 Description of Chemical Risk Assessment

Risk assessment is one of three components in a Risk analysis, together with Risk management and Risk communication. The procedure is science based and intended to identify and evaluate potential hazards with adverse effects resulting from human exposure, through compilation of existing data. (WHO 2009)

When assessing a risk, the decisive factors to observe are the toxicity of the agent, the amount of a chemical present in a medium and the amount of contact (exposure) a person has with the medium containing the contaminant. The hazard may be physical, biological or chemical. (WHO 1995) Once the problem is formulated, the work of risk assessment constitutes four steps, seen in **Table 1**.

2. Home-made meaning home picked apples pressed into must at a processing station.

**Table 1** *The work of Risk assessment, including problem formulation*

| Steps of Risk Assessment | Description |
|---|---|
| Problem | Establishment of scope and objective of assessment |
| Hazard Identification | Identification of biological, chemical or physical agent capable of causing adverse health effects in a particular food |
| Hazard Characterization | Description of inherent properties of the agent having potential to cause adverse health effects |
| Exposure Assessment | Estimation of amount of particular agent that may reach a target population. |
| Risk Characterization | Integration of the information from preceding steps, to characterize the risks to population in local circumstances. |

Source: WHO 1995

The global food trade leads to the need for harmonized safety requirements and fair trading practices. In Europe, the European Food Safety Authority (EFSA) was established to assess potential food associated hazards. By collecting and analysing scientific data, assessments can made based on the most scientific information available. (EFSA, 2015) In addition, every country world wide, typically has national organs to perform the work of risk analysis.

Outside of EU, food safety concerns led the World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) to found an international cooperation: Codex Alimentarius Commission (Codex). The work of Codex relies on risk analysis, performed by expert committees, e.g. Joint FAO/WHO Expert Committee on Food Additives (JECFA) that establishes food standards and maximum limits for substances. (Codex 2015)

Risk assessments can be hampered due to lack of sufficient existing data. Therefore, assessing exposure may require assumptions to be made, e.g. regarding occurrence. An assessment should be seen as yielding a prediction. Consequently, risk assessment results have associated uncertainties, which should be made clear. (WHO 1995)

## 1.4 Patulin in apple products

### 1.4.1 Defining and justifying scope of risk assessment

The product in question is apple must made from home grown apples in Sweden. Currently no specific risk assessments for this product are available. Home-made apple must is a small-scale[3] produced product, consumed by a limited, but increasing, number of people. (pers. comm.) The consumers, also being the home producers, of apple must are presumed to drink large volumes and may thereby be exposed to potential patulin in the product.

A risk assessment was performed to evaluate the potential risks of the consumption of apple must made from home picked apples. The reason for focusing on apple must and not commercial juice products is that certain pre- and postharvest handling practices by home producers may potentially increase patulin risks. Moreover, due to the character of the product being raw, measures to potentially reduce patulin during production such as fermentation, use of preservatives, clarification and dilution of product are rarely included in the process of home-made apple must. The assessment did not include other home-made apple products, given that the preparation practices for products such as raw apples, apple purée, dried apple slices, etc., represent a smaller safety concern, because rotten fruit or parts thereof are expected to be removed by the consumer prior to eating.

The U.S. Food and Drug Administration have stated that one rotten apple among 200 sound in the production of apple juice could lead to exceeding the legislated limit on patulin in a finished product. (FDA 2004) With this in mind, it was considered necessary to gain insight about production routines among small scale must manufacturers.

#### 1.4.2 Home-made apple must in Sweden

Apples are the most popular home grown crop in major parts of Sweden. In 2012, the harvested apples from home gardens, together with pears, were estimated to represent 9 % of the total consumption of these fruits. (Jordbruksverket 2014)

In recent years, alarms about food waste have lead to multiple efforts to make the most of all grown foods. (Alvarsson 2007, Godani 2012, Mattson 2014) The desire for a way to preserve all apples grown in private gardens is clear, in view of the interest for home-made apple must increasing every year. (pers. comm.)

Home-made apple must is generally prepared by private growers bringing their harvest of apples to local small scale manufacturers, operating during the general growing season from September until November. These processors are mostly classified as small scale, but do vary in size and services.

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3. Volumes typically made by individuals or informal groups (families, neighbours, up to small non-commercial cooperatives)

The manufacturers make must in return from the growers' own harvest, or alternatively resell equivalent amount of must in return for the volume of apples delivered. Some actors demand a minimum supply of 20 kg to press. In general, 1 kg apples yields 0.6 l must. (pers. comm.) The amount of apples becoming must per day varies between the actors, ranging from 100 kg to 3 tons. Some manufacturers offer optional pasteurization. A general processing scheme (that may vary between processors) is seen in **Table A**, Appendix.

A significant difference between the smallest must makers and commercial apple juice producers is that according to certain local authorities, the manufacturers only providing a must press are not classified as a food company, but as providing a service. The general regulation is: as long as the manufacturers do not resell the product to outsiders, or provide bottles, the practice is considered equivalent to home-made food and is thus not controlled by the local authorities. (pers. comm.) This could presumably mean, from a food safety perspective: 1) less monitored production; 2) manufacturers not required to be educated about potential risks; 3) manufacturers not being obliged to follow Good Manufacturing Practices (GMP) or having the same responsibility for the final product. In general, the slightly larger manufacturers (most common type) offer bottles, pasteurization and resell must to others. Thus, they are required to be registered as a food company. Under other local authorities, the manufacturers are assessed on a case to case basis, independent of produced volumes and sale of bottles. No matter of locality, if pasteurization and sale of bottles is performed, local food inspectors assess routines on both pasteurization and cleaning of bottles for sale. (pers. comm.) One local authority has requirements for the manufacturer to perform a hazard analysis. Education and awareness are not equal between manufacturers, some were educated about HACCP, and some rely on common sense while others maintain knowledge by reading on their own. Among processors, the majority know about soil derived bacterial contamination, and focus on this for their food safety management. Inspectors assess general hygiene and delivery routines, with similar focus; soil bacteria, without concern about toxin producing moulds. (pers. comm.)

### 1.4.3 Patulin in apple products processing chain

#### *1.4.3.1 Pre-harvest and harvest*

Patulin is produced by a variety of moulds, encompassing over 30 genera, including *Aspergillus, Byssochlamys* and *Penicillium.* (Moake et al. 2005) *P. expansum* is a psychotrophic plant pathogen whose spores have been reported in the U. K. to be wide spread in orchards, soils and various parts of apple trees- including on leaves and surface of the fruit. It can invade through stem ends and lenticles and grow internally. (Baert et al. 2012) In addition, insect damaged, mechanically harvested, or windfall fruit with bruised skin are considerably more susceptible to mould infection than sound fruit. (Codex 2002, Baert et al. 2011) Thus, patulin has been reported to be present in ground harvested apple cider, in concentrations between 40.2 and 374 μg/l, whereas not detected in cider derived from tree-picked apples (Elhariry et al. 2011). Also, high concentrations (mean value 485 μg/kg) have been found in home-made applesauce made from apples contaminated by *P. expansum*. (Brandon 2012)

The apple cultivar determines its mould resistance with its pH, firmness, resistance mechanisms against pathogens, and time of ripening, among others. (Morales et al. 2005, Ahmadi-Afzadi 2013, Zong et al. 2015) For example, the cultivar Cox Orange Pipplin has been reported to be more susceptible to mould, while Golden Delicious has been shown to possess higher resistance. (Moake et al. 2005) One must manufacturer described their problem with some cultivars tending to harbour internal rot. This susceptibility increases the risk for transfer of mould during storage and potential patulin in the finished product. (pers. comm)

#### *1.4.3.2 Post harvest and storage stability*

A feature in small scale must production that may entail increased risk of contamination, is the inconsistent harvest and storing conditions in private homes before delivery to the manufacturer. Incorrect storage, such as high humidity, dirty storage bins and increased temperatures can exacerbate any flaws of inhibiting mould growth in prior parts of the chain. (Sydenham et al. 1995, Moake et al. 2005) Therefore, it is essential to discard damaged fruit prior to storage; any skin wound makes the fruit more susceptible to mould infection. (Baert et al. 2011) One lesion of rot may reach high concentrations of patulin (2335 ng/g) and the toxin can subsequently also be detected in surrounding healthy tissue. (Sydenham et al. 1995, Bandoh et al. 2008) Affected fruit stored together with sound fruit has been reported to result in toxin migration from decayed portions to apparently healthy fruit tissues when in contact. (Marin et al. 2006, Celli et al. 2009, VKM 2014) Although patulin is associated with rotten tissue, bruised apples can develop high patulin levels without any visible mould. (British Soft Drink Associaiton) If apples are stored with debris, branches and leaves, potential stem invasion could occur. (Codex 2002) Furthermore, long-stored fruit is more susceptible to *P. expansum* infection. (Elhariry et al. 2010) The optimum temperature for *P. expansum* growth is near 25 °C, whilst patulin production has been reported to occur over a temperature range between 0-25°C. (Garcia et al. 2011, Lawley 2013) In one study, storage of apples at 25°C for 3 days lead to more than seven-fold greater average diameters of the fungal lesion, and patulin production, than cold storage of similar apples. (Welke et al. 2010)

Post delivery, the manufacturers do not need nor are able to keep controlled atmosphere storage since they generally handle the fruit within 7 days. However, the storing period could potentially be up to one month during off-season. (pers. comm.) Many manufacturers store either at room temperature, slightly chilled, in unheated spaces following the seasonal temperature or in few cases, the fruit is kept covered outdoors. (pers. comm.)

Seven out of eight manufacturers who were interviewed, routinely sort and discard rotten apples. One processor fully trusts their customers and performs a less rigourous selection procedure; another processor selects apples at random and cuts them open to check for internal rot; whereas a third sorts each apple. However, most manufacturers (and some food inspectors) did not see any harm in using fallen fruit. Thus, they typically allow ground harvested fruit, as long as intact or “lightly bruised”. (pers. comm.) This can be explained by the general opinion, including among food inspectors, that the point of apple must is to make use of the redundant fruit fallen to the ground.

A washing procedure before processing has proven to be efficient to reduce patulin. (Moake et al. 2005, Sydenham et al. 1995, Cunha et al. 2014) However, the interviews indicated that not all manufacturers can provide a water bath. When this is the case, private growers are expected to wash the fruit before delivery.

#### *1.4.3.3 Production*

Liquid apple products are processed in various ways. Clarification and filtration steps generally reduce patulin levels. (Moake et al. 2005, Cunha et al. 2014) It has been suggested that patulin forms adducts with protein particles in cloudy juice and that if filtered, as occurs in clear juice, patulin levels decrease. (Odhav 2001)

Addition of preservatives has been shown to reduce patulin levels. Studies report it being unstable in the presence of sulfur dioxide, although various degrees of effectiveness are noted. (Moake et al. 2005, see also Ough et al. 1980 and Steiner 1999) Also, ascorbic acid is reported to reduce patulin levels. (Drusch et al. 2005)

Fermented apple must (cider) is rarely associated with patulin problems, because fermentation by most common yeasts degrades patulin. (Moake et al. 2005)

Certain patulin-producing moulds such as *Byssoclamys nivea* are thermally stable, (Sant'Ana et al. 2010) and patulin itself is heat stable in acidic conditions. (Harisson 1987) Consequently, the most common pasteurization used for quality purposes (commonly 70-85°C, 10-30 min (pers. comm)) is not effective for ensuring that the product is free from patulin. (Elhariiy et al. 2011, Codex 2002)

The fate of patulin in stored products is not clear, with differing reports of patulin degradation or lack thereof during storage. When apple juice was stored for 3 weeks at 22°C, little reduction was seen, while canned apple juice stored for 5 weeks, showed decreased patulin levels. (Harrison 1987)

#### 1.4.4 Limits and regulations

Today patulin is one of the most regulated mycotoxins in the world, and most limits deal with apple derived products. (FAO 2004) Interest regarding patulin levels in apple products rose in 1992 as a result of an investigation made by the Ministry of Agriculture, Fisheries and Food (MAFF) in the UK, which reported that 26 % of the samples contained over 50 μg/kg. Recurrent monitoring has resulted in improved control routines and avoidance of damaged or poor-quality fruit for production and lead to reduced patulin contamination. (British Soft Drinks Association, Sydenham et al. 1995)

In 2003, 44 out of 48 countries were harmonized with established limits at 50 μg/kg. (FAO 2004) European countries were among the first to address the concern (Moake et al. 2005) and in 2000, The Scientific Committee on Food (SCF) endorsed the provisional maximum tolerable daily intake (PMTDI) of 0.4 μg/kg bw/day for patulin. As a result of *Assessment of the dietary intake of patulin by the population of EU Member States* in 2001, maximum levels were established in certain foods. (European Committee 2000) Established guidance values are found in Commission Regulation (EC) No. 1881/2006 and can be seen summarized in **Table B**, Appendix.
As a complement to the EU commision regulations, Sweden has a national legislated regulation on guidance values especially for fruit and berry products. (Livsmedelsverket 2012)

#### 1.4.5 Examples of patulin contamination

In contrast to apple must, the occurrence of patulin in apple juice has been investigated in multiple studies, with concentrations ranging from just above Level of Detection (LOD) to over 1000 μg/l. (Majerus and Kapp 2002, Baert et al. 2006, Murillo 2008) Subsequently the reported mean concentrations vary.

The issue of contamination has mainly been studied in commercial products. The scope and number of samples in available studies have varied greatly, but the contamination levels are generally within the established limits. (Majerus and Kapp 2002, Baert et al. 2006, Murillo 2008, Piqué et al. 2013)

One of the biggest surveys was a scientific cooperation study (SCOOP) conducted in 2001 by the Directorate – General Health and Consumer Protection of the EU. Of 4633 apple juice samples (freshly pressed, cloudy, clear and from concentrate), 57 % contained patulin and approximately 1% with levels higher than 50 μg/kg. Levels ranged between Level of Detection (LOD) 0.03 μg/kg to 1150 μg/kg. In that study, Sweden provided results from 39 juice samples, of which five were positive and contained low levels. (Thuvander et al. 2001, Majerus and Kapp 2002)

A recent survey on 100 commercial juices sold in Spain showed 66% contained patulin, 11% with levels exceeding 50 μg/l. The concentrations ranged between 0.7 and 118.7 μg/l, with a mean concentration of 19.4 μg/l. (Murillo-Arbizu et al. 2009) Other studies in Spain showed mean concentrations ranging from 0 to 13.5 μg/kg (Piqué et al. 2013).

Two surveys in Italy analysed 57 and 53 samples, yielding 49% and 47% positive samples, respectively. The first study showed concentrations ranging from 0.5-69.3 μg/l and the other was observed to not exceed the permitted levels. (Murillo-Arbizu et al. 2009) When 1049 juice samples were tested in Germany, 305 were shown to contain patulin, with concentrations between 0.28 and 145 μg/l. (Brandon et al. 2012)

A study by Tangni and others (2003) on apple juice in Belgium reported 35 out of 43 samples positive for patulin, with concentrations between 0.67–38.8 μg/l. (Murillo-Arbizu et al. 2009) When commercial apple juices on the Belgian market were analyzed again by Baert et al. (2006), 12 % were shown to contain patulin. The study observed differences in patulin contamination rates in handcrafted, conventional and organic apple juices. Merely 2/22 handcrafted juices contained patulin above the LOD (mean 10.5 μg/liter) while the number above LOD among conventional juices was 12/90 (mean 10.2 μg/liter). Organic juices were reported to contain higher patulin (mean 43.1 μg/liter) than in both handcrafted (mean 10.5 μg/liter) and conventional juice (mean 10.2 μg/liter).

A study on juices on the Italian market also found higher mean concentration in organic products than conventional. (Murillo-Arbizu et al. 2009) Comparisons of patulin contamination between organic and conventional products are inconclusive, but the concentrations and incidence in organic products are reported to be higher. (Cunha et al. 2014, see Piqué et al. 2013)

Cloudy juices are in some cases shown to contain higher mean patulin levels than clear juices. A study from Belgium calculating mean concentrations in cloudy and clear juices made from fresh apples reported 0.1 μg/kg in cloudy and <0.1 μg/kg in clear. (Baert et al. 2012) Another study showed a mean concentration at 30 μg/l in cloudy juices and 14 μg/l in clear juice samples. (Baert et al. 2006)

# 2 The Risk Assessment

## 2.1 Materials and method

The risk assessment was performed according to the method described by WHO. (WHO 2009) The data were generated through compilation of literature on toxicity, occurrence in similar products and dietary intake on patulin, through search of scientific literature in databases of PubMed, ScienceDirect, ProQuest and Web of Science. Additional data from international sources such as FAO, FDA, and WHO have been used.

Due to the lack of consumption data on apple must, the following assumption was made in the Exposure assessment:

Consumers drink apple must instead of apple juice during the apple must season. Thereby the apple must consumption is assumed to be equivalent to that of Swedish data on apple juice consumption.

When estimating degree of exposure, consumption combined with occurrence data of the compound should be obtained. At present, there is little data on occurrence of patulin in apple must, as well as insufficient data on patulin occurrence in Swedish apple cultivars. Therefore, contamination concentrations used in the present study were derived from a study by Kadakal and others from 2005 in which *Golden Delicious* apples with four varying degrees of naturally decayed portions were collected for the preparation of raw apple juice in a hydraulic press. The method was described:

> "Eight different samplings for each decay group were carried out to obtain sound and 30%, 60% and 100% decayed apples. On each sampling day (8 days): 20 kg of apples were obtained randomly for every decay group."
>
> (Kadakal et al. 2005)

Mean detected levels in two out of four contamination degrees (0% and 30%) were used for the present exposure assessment. The results of patulin detection in study by Kadakal (mean, minimum and maximum concentrations) are shown in **Table C,** Appendix.

Motivation for using two degrees of rot is to illustrate two possible scenarios. The 0% decay simulates a most likely, as well as a best case scenario, being that rotten fruit material is discarded. A worst case scenario is portrayed by 30 % rot, if solely decayed fruit is used.

## 2.2 Hazard Identification

Patulin is an unsaturated heterocyclic lactone and classified as a natural contaminant. It is known that apple products represent the most common contaminated food. (Majerus and Kapp 2002) Previous findings in commercial products are mentioned in section *1.4.5*.

## 2.3 Hazard Characterization

Results from toxicological studies on patulin are compiled in "Toxicological evaluation of certain food additives and contaminants" which outlines the latest evaluation made by JECFA. The toxicity of the compound is undeniable but the results are, as a whole, inconclusive. (JECFA, 1995) In recent years additional reviews on patulin toxicity have been presented.

Acute effects reported include gastrointestinal distension, edema, ulceration and nausea. (Puel et al. 2010, Marin et al. 2013) Long-term studies in animals have shown patulin to cause weightloss, and to show neurotoxic and immunotoxic, as well as immunosuppressive effects. (Puel et al. 2010)

It is suggested that patulin's affinity for sulfhydryl groups explains its ability to inhibit enzyme activity. (Celli et al. 2009, Puel et al. 2010) In addition, patulin's structure is electrophilic and may cause interactions with nucleophilic groups, such as DNA and proteins, and may explain the mechanism of DNA damage. (Wu et al. 2008) In fact, JECFA concludes patulin to be genotoxic and it is classified as *in vivo* mutagenic by The UK Committee on Mutagenicity. (Hopkins 1993, JECFA 1995) However, no evaluation of the carcinogenic effect on humans has been made. International Agency for Research on Cancer (IARC) classifies patulin in Group 3: *the agent is not classifiable as to its carcinogenicity to humans*. (IARC 1986)

The established NOEL (No effect level) for patulin (0.43 μg/kg bw/day) is derived from a reproductive toxicity study on rats given gastric intubation doses of patulin. Male rats given the highest dose suffered from premature death by 19 months while 19% of female rats survived until the end of study. (JECFA 1995)

## 2.4 Exposure Assessment

The scientific cooperation study (SCOOP) assessed the dietary intake of patulin by the population of EU member states in 2001. For the Swedish adult population the estimated mean intake was then 1.21 and high intake 1.24 ng/kg bw/day, while the intake among consumers was estimated at 4.20 ng/kg bw/day and high at 11.5 ng/kg bw/day. (Majerus and Kapp 2002) Whereas in a survey from 2008, the mean and 95$^{th}$ percentile intake among 4-year olds in Sweden was estimated at 0.3 and 1.4 ng/kg bw/day, respectively. (Nadjimi, 2008)

Facts regarding the current exposure assessment:

- Consumption data is obtained from *Riksmaten barn 2003* and *Riksmaten vuxna 2010-2011*. See **Table D**, Appendix.
- Obtained consumption data concerns consumers, not the general population, due to the exposure assessment being relevant to people making and presumably also consuming must.
- Consumers are represented by two age groups. (Usually, two or three age groups are considered. However, the choice is justified by the consumption of apple juice among 8-year olds and adults not differing significantly).
- Both age groups are represented by average consumers (mean) and high consumers (95th percentile).
- The estimated intake among adults should be considered as overestimations for male consumers, due to calculations being based on an average body weight of 69 kg.

The estimated patulin intake via apple must from current exposure assessment are presented in **Tables 2-3**.

**Table 2**. *Estimated intake of patulin via apple must among 4-year olds.*

| 4-year olds (18 kg) | | | | |
|---|---|---|---|---|
| Fruit Decay | **0%** | | **30%** | |
| Conc. in juice (µg/l)[1] | 1.9 | | 179 | |
| | **Mean** | **P95** | **Mean** | **P95** |
| Consumption juice (kg/day)[2] | 0.081 | 0.205 | 0.081 | 0.205 |
| Est. intake (µg/kg bw/day) | 0.009 | 0.022 | 0.81 | 2.04 |
| % TDI | 2 | 6 | 203 | 510 |

1. (Kadakal et al. 2005) 2. Livsmedelsverket 2003

**Table 3**. *Estimated intake of patulin via apple must among adults.*

| Adults (69 kg) | | | | |
|---|---|---|---|---|
| Fruit Decay | **0%** | | **30%** | |
| Conc. in juice (µg /l)[1] | 1.9 | | 179 | |
| | **Mean** | **P95** | **Mean** | **P95** |
| Consumption juice (kg/day)[2] | 0.097 | 0.250 | 0.097 | 0.250 |
| Est. intake (µg /kg bw/day) | 0.003 | 0.007 | 0.25 | 0.65 |
| % TDI | <1 | 2 | 63 | 162 |

1. (Kadakal et al. 2005) 2. Livsmedelsverket 2010-2011

With the assumption that Swedish apple must, made from 0% and 30% decayed fruit, would contain the same levels of patulin as Kadakal's samples, the estimated daily intakes through apple must would be 0.009-2.04 µg/kg/bw/day for children and 0.003-0.65 µg/kg bw/day for adults.

## 2.5 Risk Characterization

The established Provisional Maximum Tolerable Daily Intake (PMTDI) at 0.4 µg/kg bw/day is derived from the NOEL at 0.043 mg/kg bw per day divided by a safety factor of 100. (JECFA 1995)

The present estimates come with many uncertainties, which will be discussed further in section *3*. The exposure levels are in five out of eight scenarios within the guidelines for tolerable daily intake. In a best case scenario, using solely sound fruit, patulin levels are very low. However, it is note worthy that despite the fruit appearing healthy, patulin may still be present. (VKM 2014)

The estimates show that an adult high consumer who drinks apple must from 30 % rotten apples could in the worst case reach a daily intake of >160% of PMTDI. The intake of a 4-year old mean consumer, drinking the same apple must, could be 201% of PMTDI, or for a 4-year old high consumer, 509% of the PMTDI. The latter is the worst case scenario among all consumers considered. The big difference in intake of children compared to adults is explained by childrens' high consumption in relation to their lesser body weight. In particular, 8-year olds consume as much apple juice as adults, which entail a much higher potential intake kg $bw^{-1}$. See **Table D**, Appendix.

The estimated exposure calculated above is not in accordance with previously estimated intakes among Swedish consumers. (Majerus and Kapp 2002, Nadjimi, 2008) This is simply explained by those assessments being based on commercial products available on the market. Even if contamination does occur in commercial apple products, the concentrations vary greatly, with the majority of samples containing insignificant levels, since processors in general apply GMP and other routines to monitor and reduce the risk of patulin.

When considering the simulated exposure to patulin from home-made apple must, variations in consumption should be kept in mind, as the consumption of juice may be more different from apple must than what is assumed in this study. Private growers (and their children) getting must from their own harvest may be deemed as relatively few among the Swedish population, but they are presumed to consume large volumes of home-made must during certain periods of the year, which implies a potential underestimation in the amount ingested.

# 3 Discussion

The current work was initiated based on a conjecture that contamination of patulin in home-made apple must could reach increased levels, due to less rigorous sorting and suboptimal handling in the production chain. Considering the frequency of patulin contamination previously reported in apple products (*See section 1.4.5*), there were reasons to investigate home-made apple must in Sweden. (*See section 1.4.5*)

Routines among actors have been studied and presented together with an exposure assessment. The latter yielded results indicating the risks of using bad quality fruit. In accordance to previous predictions, the most exposed group is children due to their large intake of juice relative to their body-weight. (Thuvander et al. 2001, Baert et al. 2007, Piqué et al. 2013) However, results should be read with care. The conditions under which patulin concentration values were derived are probably an inadequate representation of local circumstances. Available data focus on products of other character, i.e. filtered and commercially processed apple juice (instead of raw and cloudy juice) from countries other than Sweden, which leads to them not being directly applicable. Therefore, this assessment does not claim to provide an accurate exposure estimation of patulin in Swedish apple must. The results of patulin intake estimation should not be connected to the overview of routines among processors. The two parts are separate, but together they could illustrate something of the local situation.

Values applied in the current exposure assessment are not fully representative of the Swedish situation, because sampling in the original study by Kadakal et al. (2005) was made with commercial apples, presumably harvested and stored under controlled conditions. Circumstances in Swedish private gardens differ with regard to the varied practices among growers along the chain from fruit to apple must. As conditions in the original study potentially slowed mould growth, the derived concentrations could underestimate values in Swedish private growers' fruit. Practices among private growers that could entail higher risk for mould invasion in fruits include: omission of fungicides, entailing potential harbour of insects; groundharvesting apples; and incorrect storage. (Codex 2002, Elhariry et al. 2011, Piqué et al. 2013, VKM 2014) An additional discrepancy is that the sampling in the study of Kadakal et al. (2005) was made on a homogenous group of equally decayed apples. This implies a large overestimation compared with the Swedish situation, since this degree of decayed fruit in apple must production is not likely to occur. (pers. comm.)

Additionally, to achieve higher certainty of the applicability of patulin concentrations from the original study, the appearance and severity of the decay on the specific cultivar (Golden Delicious) would have to be known (it was not stated in the study). Without this fact, it is difficult to link their findings to potential patulin concentrations in apples of varied appearance delivered by private Swedish growers to juice producers. In particular, since mould growth and toxin production is dependent on environmental conditions, patulin production and concentration is dependent on the character of mould invasion. (Moake et al. 2005, Celli et al. 2009, Bandoh et al. 2008, Elhariry et al. 2011, Garcia et al. 2011, Morales et al. 2005, Puel et al. 2010, Ahmadi-Afzadi 2013) Thus, no correlation between size of lesions and concentration of patulin present can be assumed and the concentration of patulin can be very different depending on internal dispersion. This uneven factor affects the exposure assessment due to difficulties in predicting contamination based on visual assessment.

Additional aspects leading to increased or reduced concentrations of patulin in Swedish apples, compared with the study of Kadakal et al. (2005) are listed in **Table 4**.

**Table 4**. *Factors affecting patulin concentrations in Swedish conditions*

| Factors implying increased concentration of patulin | Factors implying reduced concentration of patulin |
|---|---|
| -Fruit handling before delivery may favour mould growth:<br>1) Swedish private growers are assumed not to use fungicides, which could potentially lead to higher risk for insect damage.<br>2) Ground harvested fruit is collected and stored.<br>3) If overripened fruit is picked.<br>4) Storage of unsound fruit with sound fruit can lead to patulin migration.<br>5) Storage in too high temperatures, in wet conditions and along with debris.<br>6) If conditions prevent visual rot but not patulin, it is impossible to discard rot when sorting. | - Patulin producing fungi may not be as wide spread in Swedish nature.<br>- Swedish cultivars are possibly more resistant to mould infection.<br>-Swedish apple season implies lower temperatures, thus not as favourable for mould growth as summertime. (Moake et al. 2005)<br>- If mould growth is successfully inhibited. (Morales et al. 2005) |
| -In case of no external decay but presence of internal rot or migrated patulin.<br>- If quality assessment and sorting is less regulated than in large scale production.<br>- If rotten parts are removed but fruit may still contain patulin residues in healthy tissues.<br>- Even if quality assessed: fruit with minor bruises is considered OK but contains patulin. | -Short storage times at private growers as well as manufacturers.<br>- Swedish must manufacturers perform adequate quality assessment. |

Despite the uncertainties noted above in applying patulin concentration data from Kadakal et al. (2005) to apples harvested from Swedish gardens, the data from Kadakal's study was still considered the most relevant to use, due to the juicing method applied. It was similar to Swedish small scale must manufacturers: handcrafted press with cloths yielding cloudy, raw apple juice.

Advantageous for data from Kadakal et al. was also the amplitude of samples, as well as the natural initiation of mould invasion. Previous studies on patulin development and concentration tended to conduct experiments under controlled inoculation and growth conditions. (e.g. Bandoh et al. 2008, Celli et al. 2009)

The estimated intake levels being based on a single study introduces further uncertainty into the exposure assessment. The detected mean values in 0% and 30% decayed apple juice (1.9 and 179 µg/l) are meant to simulate concentrations when using solely sound fruit or if using exclusively fallen and bruised fruit, respectively. Kadakal's maximum detected values were not included in the assessment, because it is not likely one consumes apple must with extreme concentrations every day. The best way to simulate realistic long-term exposure is to use mean values. The mean concentrations found when pressing sound fruit should ideally be similar to the mean concentration in the commercial juices – since juice should ideally be made from high quality fruit.

It is unlikely that processors use bad fruit to the same extent as 30% decayed fruit simulated in the study. Even so, the reported concentrations are not unreasonably high, considering a number of previous studies have found concentrations reaching over 1000 μg/l. However, a mean value of 179 μg/l in apple juice products is considered exceptionally high compared to most studies with mean concentrations between 0-50 μg/l. (Murillo-Arbizu 2009, Baert et al. 2012, Brandon 2012, Piemontese 2012, Piqué 2013) A study with results approaching Kadakal's findings is one from Turkey analysing 45 juice samples with 100% positive samples, yielding a mean of 139.9 μg/l. (Murillo-Arbizu 2009) Findings in previous studies are discussed in section *1.4.5*.

Since insight of handling and storage in private homes was not included in the present risk assessment, the potential risks at these stages are not known. It is likely that there are factors and practices that can contribute to increased patulin levels. For example, the fact that some manufacturers demand a minimum volume of delivered fruit may induce clients to harvest the fruit in smaller batches over an extended period, and store it until a sufficient volume is collected. If the conditions are incorrect, such as too warm, fruit being wet, mixed with bad fruit and debris (poorly sorted), or without cover in their basements or outdoors, the risk for fungal proliferation is increased. (Codex 2002)

Quality assessment after storage is performed by most must manufacturers, but this procedure still may be insufficient since rotten fruit is discarded but "bruised or fallen fruit" is approved. This implies that the quality of the must is dependent on individual definitions of bruised *vs* rotten fruit.

In conclusion, the home production and consumption of apple must in Sweden is outside those products currently controlled by patulin regulations (apple juice and other beverages) and furthermore, does not appear to be in accordance with commercial apple juice production with regard to monitoring and consistent handling. Mirroring the risk factors for patulin contamination established in literature to conditions and handling applied during apple must production, there is clearly a risk for increased patulin contamination, since the conditions are occasionally conducive to mould growth. However, the apple musts are not likely to contain the high amounts of patulin modelled in this risk assessment. To minimise the risk for patulin exposure, awareness and knowledge of patulin needs to be increased among private growers and must manufacturers, and could even be implemented in the work of food inspectors.

## Gaps in Knowledge

To achieve a more accurate exposure assessment, data are needed to fill the following gaps in knowledge:

- Updated data on occurrence levels and intake of patulin in Swedish apple products, incl. must.
- Consumption data on apple must alone, not together with apple juice.
- Concentrations of patulin in products of similar character (cloudy, raw and from organic apples).

Additionally:

- The occurrence of patulin-producing moulds in home gardens and the Swedish natural environment.
- More studies on factors that influence patulin concentration in Swedish conditions as well as in Swedish apple cultivars.
- Study on the tendency of using fallen fruit for apple must production, through interviews and/or assessment of fruits arriving at the manufacturers.
- Define and distinguish damage, spots and rot in apples.
- Valuable data would be to observe rotten lesions, measure lesion size, quantify patulin; depending on number of lesions, estimate an average concentration per apple, from which the amount per kilo apples (and subsequently per litre apple must) could be somewhat estimated.
- Bound/non-bound state of patulin in cloudy/raw apple beverages.

## Risk reducing measures

As not all must manufacturers are classified as food sale organisations, responsibility for patulin safety is difficult to assign, and official monitoring is complex. Subsequently, patulin in home-made apple must cannot be effectively regulated by limits, but only through preventive work. It is suggested that local food inspectors gain awareness of risks for patulin contamination in this type of product and that their assessment covers this.

Seeing that there is an increasing interest in making apple must among private growers, it is necessary to communicate the potential risks during harvest and storing:

- Firstly, by preventing mould growth, patulin contamination is prevented.
- Minimizing wounds early will certainly reduce the patulin concentration in the fruit.
- Avoid collecting fallen fruit. If so; wash and dry thoroughly before storing.
- Store in dry and cool space, without branches and debris.
- Wash fruit at some point, preferably before storing; dry well before storage.
- Due to the risk of patulin migrating through fruit tissue, it is recommended to not store bruised fruit with healthy fruit.
- Trimming is proven to be efficient, remove rotten parts prior to storage and delivery.
- Perform careful quality assessment after storage and before delivery. Check for internal rot by cutting fruit open.

For manufacturers, in addition to the above mentioned preventive methods:

- Perform quality assessment at delivery, discard unsound fruit or
- Remove rotten *and* bruised portions of fruit.
- If storing fruit; keep cool, dry and without debris.
- Check for internal rot by cutting fruit open.
- Always wash the fruit before pressing. If not possible, ensure that the customer has washed and dried fruit, as described above.
- Analyse patulin in finished apple must, to see if concentrations comply with legislated maximum limits (for larger manufactuers selling must).

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Information obtained through personal communcation with personnel from:

Värmdö Musteri

Roslagens Musteri

Hå Saftfabrik

Skånehill

Kullamust

Borås Musteri

Gärdets Musteri

Viforsens Musteri

Information obtained through personal communcation with food inspectors from:

Sundsvall Kommun

Borås Kommun

Värmdö Kommun

Stockholms Kommun

# Appendix

**Table A**. *Stages in production of apple must.*

| | |
|---|---|
| 1 Quality assessment | Manufacturers perform quality check to avoid debris, soil or insects. Bruised fruit is in most cases approved while rotten fruits (or parts thereof) are discarded. |
| 2 Water bath | Fruits are washed before pressing. Not all manufacturers provide this. |
| 3 Grating/chopping | Coarse grating to not destroy kernels. |
| 4 Pressing | Specific method varies between the manufacturers. |
| 5 (Transferred to tank) | The must is either bottled at this stage or transferred to a tank to be stored. |
| 6 (Filtration) | Not very common among the small scale manufacturers, but if clear must is made, the treatment is done before pasteurization. |
| 7 Pasteurization | Routines ranging from 75 to 85°C, 10 to 30 min. |
| 8 Bottling | In glass or plastic bottles, large plastic containers, or in a bag in box. Smallest manufacturers do not provide bottles. |

**Table B**. *EU guidance values on patulin*

| **Product** | **Patulin - Maximum Level (μg/kg)** |
|---|---|
| Fruit juices, concentrated fruit juices as reconstituted and fruit nectars | 50 |
| Spirit drinks, cider and other fermented drinks derived from apples or containing apple juice | 50 |
| Solid apple products, including apple compote, apple puree intended for human consumption | 25 |
| Apple juice and solid apple products, including apple compote and apple puree, for infants and young children and labelled and sold as such baby foods other than processed cereal-based foods for infants and young children | 10.0 |

**Table C.** Resulting range of concentrations of Patulin in apple juice, depending on degree of rot

| | **min (µg/l)** | **max (µg/l)** | **mean (µg/l)** |
|---|---|---|---|
| **0%** | 0.3 | 2 | 1.9 +/-0.6 |
| **30%** | 139 | 23* | 179 +/-32.4 |

** The article of Kadakal et al. (2005) seems to have a typing error.*

**Table D**. *Consumption data on apple juice*

| | | | N | min (g/day) | mean (g/day) | p50 (g/day) | p95 (g/day) |
|---|---|---|---|---|---|---|---|
| Apple juice, ready to drink | 4-year olds | Population | 590 | 0 | 25 | 0 | 131 |
| | | Consumers | 181 | 6 | 81 | 53 | 205 |
| | 8-9 year olds | Population | 889 | 0 | 21 | 0 | 126 |
| | | Consumers | 194 | 13 | 97 | 66 | 276 |
| | 11-12 year olds | Population | 1016 | 0 | 18 | 0 | 105 |
| | | Consumers | 166 | 13 | 109 | 59 | 285 |
| | Adults | Population | 1797 | | 15 | 0 | 100 |
| | | Consumers | 285 | | 97 | 75 | 250 |

Source: Livsmedelsverket 2003, 2010-2011