codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS | WORLD HEALTH ORGANIZATION

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

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POSITION PAPER ON PATULIN
(Prepared by France)

INTRODUCTION

1. At the 28th session of the CCFAC, France was asked to prepare a position paper on patulin with a view to setting maximum limits of contamination in food. This position paper takes into account existing toxicological data, analytical results supplied Germany, the United Kingdom, the United States and France, and data on exposure of the population. At the 29th session of the CCFAC, France was invited to revise the document in the light of comments submitted. The 30th session of the CCFAC endorsed the advance of the document of France to Step 3, subject to amendment of the proposed maximum patulin limit in apple juice and ready made soft drinks containing apple juice.

2. Patulin is a mycotoxin produced by a large number of different moulds of the genera *Aspergillus*, *Penicillium* and *Byssochlamys*. It can be detected in mouldy fruit, vegetables and cereals as well as in fodder; it can also be detected in cheeses. Patulin is found in many types of fresh or processed fruit and vegetables (juices, sauces, compotes, jellies), after natural infection by *Penicillium*, but the most frequent contamination is that by *Penicillium expansum* which is encountered under certain types of apple spoilage such as “blue mould” on fruit with surface damage. However the presence of patulin in seemingly wholesome fruit cannot be excluded. The degree of contamination correlates with the degree of spoilage and patulin does not spread much from the spoilt tissues; human contamination can therefore only be envisaged from processed fruit. Many patulin analyses were conducted from 1972 to 1979 on apple juices, revealing widely varying contamination levels of between 5 and 2 500 Tg/kg.

PHYSICO-CHEMICAL PROPERTIES

3. Patulin is a lactone (4-hydroxy-4h-furo[3,2-c] pyran-2-(6h)-one) with a molecular weight of 150.12. It forms colourless crystals, has a melting point of 111^{o}C, is soluble in water, ethanol, acetone, ethyl acetate, ether and chloroform, but insoluble in benzene and petroleum ether. Patulin is not destroyed by heat and is stable at an acid pH. Its content is reduced by prolonged storage, the action of sulphites and high temperature, the addition of ascorbic acid, alcoholic fermentation and activated carbon treatment.Patulin loses its biological activity in an alkaline medium and in the presence of molecules comprising sulfhydryl groups such as cysteine and glutathion.

METHODS OF ANALYSIS

4. The patulin determination methods generally follow the sequence of:

a) Extraction
b) Purification or elimination of unwanted compounds

c) Concentration

d) Qualitative and quantitative determination by chromatographic techniques

5. There are standardized methods to determine patulin levels in apple juices. The ISO has published two standards: ISO 8128-1:1993 and ISO 8128-2:1993, one involving thin-layer chromatography and the other High Performance Liquid Chromatography (HPLC). These methods are very similar to those published in the AOAC International Handbook on official methods of analysis: AOAC 974-18 and AOAC 995-10. Other methods are also used, for example the AFNOR NF V76-116 method of November of 1985, which is the official method in France. Its field of application includes apple juices, apple juice concentrates and ciders. Switzerland also has its official methods which were published in Mitt. Gebiete Lebensm, Hyg. 75, 506-513 (1984). These methods are presented in appended Table 1 together with their technical characteristics and levels of repeatability and reproducibility. Also of note is a European Union project coordinated by the United Kingdom, launched on 30 September 1996 and directed towards selecting and standardizing mycotoxin analysis methods for the EU countries and presenting them as CEN standards. For patulin, the chosen matrixes are apple juice and apple compote.

ABSORPTION, DISTRIBUTION AND EXCRETION

6. Patulin labelled with carbon 14 and administered orally in a single dose of 3mg/kg body weight to Sprague-Dawley rats is largely eliminated in the faeces and urine within 24 hours. After seven days, however, 2 to 3 percent remains in the soft tissues (spleen, kidneys, lung, and liver) and blood.

IDENTIFICATION OF TOXICOLOGICAL PROBLEMS

<u>Acute toxicity</u>

7. For mice, LD 50 is 15 to 35 mg/kg, depending on the mode of administration. Patulin has a cytotoxic effect giving it antibiotic, antifungal and antiprotozoal properties. This cytotoxicity is mediated by a rise in membranous permeability. Patulin disorganizes the cytoplasmic microfilaments. It inhibits *in vitro* several enzymes including RNA polymerase and DNA polymerase. It also affects transcription and translation by a direct effect on DNA.

<u>Mutagenicity - carcinogenicity</u>

8. Carcinogenicity studies have been conducted on mice and rats. However, two of the three studies concerned first generation offspring of animals used for teratogenesis studies. The results of the mutagenicity tests are variable: positive for *Bacillus subtilis* and negative for *E. Coli* and *Salmonella Typhimurium*. Patulin causes breaks in DNA but no non-programmed synthesis of DNA. It causes chromosome aberrations but without an exchange of sister chromatids. There is therefore no clear evidence that patulin is carcinogenic even if toxicologists widely agree on the need for a carcinogenesis study on species other than rats. Finally, patulin has immunotoxic effects but these intervene at intakes far higher than that without effect.

<u>Maximum tolerable intake</u>

9. A first toxicological evaluation made at the 35th JECFA meeting in 1990 set a maximum provisional tolerable <u>weekly</u> intake (PTWI) of 7 Tg/kg body weight. A second evaluation at the 44th JECFA meeting in 1995 took into account the fact that most of the patulin ingested by rats is eliminated within 48 hours and 98% in seven days. This absence of accumulation led the JECFA to establish a maximum provisional tolerable <u>daily</u> intake (PTDI). A study on the combined effects of patulin on reproduction, long-term toxicity and carcinogenicity pointed to a harmless intake of 43 Tg/kg body weight per day. On the basis of this work and using the customary security factor of 100, the JECFA therefore set the PTDI 0.4 Tg/kg.

EVALUATION OF EXPOSURE

10. The evaluation of exposure takes into account available analytical results (appended in Tables 2 and 3) and data on consumption of products that may be contaminated (appended in Table 4). These data have been supplied by the countries listed in Paragraph 1. The aim of the Committee is to determine measures that are compatible with consumer safety and that are therefore based on the maximum tolerable intake set by the JECFA, at 0.4 Tg/kg body weight per day.

11. This corresponds to a daily maximum of 24 Tg of patulin for an adult weighing 60 kg, of 8 Tg for a child weighing 20 kg and 4 Tg for a child of 10 kg.

12. Individual fruit juice packages (whether or not for young children) contain between 125 and 200 ml. The Committee decided when reviewing document CX/FAC 98/17 that the regular consumption of a standard serving or unit of packaging should not pose any risk to the consumer. Thus, if a child weighing 10kg consumes in a day the quantity of apple juice in a 125 ml retail unit, the patulin content of this apple juice must not be more than 32 g/l if the PTDI is not to be exceeded. The patulin content must not exceed 40 g/l in the case of a 20 kg child consuming a retail unit of 200 ml per day.

13. Yet, the precise consumption data (Table 4) indicate that some 200 ml of apple juice (223 ml in France for 1500 individuals of between 2 and 65 years of age, 290 and 150 ml for adults and children respectively in the United Kingdom) are consumed regularly per day by certain consumers (in France 5% of apple juice consumers). Finally, in France over 20% of children below the age of 30 months consume fruit juices including 3% consuming juices not specifically prepared for infants (Study SOFRES/Alliance 7, 1997).

14. While solid apple based foods (Table 3) always show a level of contamination of under 50 g/kg, available data indicate that 10 to 30% of apple juices can have a level of contamination above 50 g/kg (Table 2) and that 55% of fruit juices can have a level fo contamination above 25 g/kg (Source: Union Nationale des producteurs et distributeurs de jus de fruits - France). All these data therefore confirm the risk of exceeding the PTDI.

DISCUSSION

15. Patulin is a toxic substance with suspected carcinogenic properties. Even though there is no formal evidence of this risk, the JECFA set a provisional tolerable daily intake (PTDI) of 0.4 Tg/kg body weight in 1995.

16. Only apples present very high patulin contents among spoilt fruit and vegetables. As such apples are obviously never consumed in their existing state, the patulin risk must derive from the processed products. Other than juices, such products (solid foods, compotes, etc.) generally present contamination levels below 50Tg/kg, either because the technological process destroys the patulin or because highly contaminated fruit are discarded from production for organoleptic reasons. The risk of exceeding the tolerable intake is therefore highly improbable for these foods. Fermentation largely destroys patulin in ciders, which besides are not intended for children, making it highly improbable that the maximum provisional tolerable daily intake will be exceeded.

17. Apple juices can be highly contaminated, although the elimination of spoilt fruit from processing is technically possible, gives good results and is a perfectly normal part of good agricultural and manufacturing practices. This can be done by automation (floating the fruit) or manually, the latter producing better results but more time-consuming.

18. It is important to note that the contamination applies to a soft drink, so daily intake may be much higher than for solid foods. This is particularly important as regards the protection of children.

19. Whether consumption is measured in packaging unit of 200 ml, standard serving of 237 ml or daily intake of 200 ml as reported by main countries, these all point to the need to protect young consumers of these products.

RECOMMENDATIONS

20. Given:

- the common occurrence of patulin contamination of products obtained from fruit, mainly apples;
- the sometimes very high levels of apple juice intake relative to body weight by certain population groups, notably children;
- the not insignificant trade of these products in certain geographical regions;
- the guidelines on maximum patulin limits in apple juice and sometimes other products that already exist in certain countries, like Austria, Finland, France, Greece, Iceland, Sweden and the United Kingdom;

a study is recommended to set a maximum limit for patulin using the procedure described in the Codex General Standard on Contaminants and Toxins in Foods.

21. A maximum patulin limit of 25 g/kg in apple juice could be set to ensure the protection of most children, including those who are regular consumers of large quantities of apple juice.

22. However, apple juice manufacturers may not always be able to comply with a limit of 25 µg/kg which may also cause problems of international trade. A maximum limit of 50 µg/kg of patulin in apple juice and ready-made soft drinks containing apple juice may provide sufficient protection for a large majority of consumers, whose consumption of drinks is sufficiently varied. The 50 µg/kg limit is therefore proposed for Step 3 of the Codex procedure, in accordance with the wishes expressed by the CCFAC at its 30th session. This limit could subsequently be revised, if necessary, in the light of new findings on patulin toxicity.

23. In any case, it is of paramount importance that the apple processing industry should be encouraged to adopt the good manufacturing practices. In particular, they should discard spoilt fruit from their production process, their appearance being a good indicator of level of contamination.

24. The processing industry should also be encouraged to introduce measures to decontaminate the juices when harvesting and storage conditions are unfavourable, besides the preliminary sorting. Such treatment could be prolonged storage, the addition of sulphites and higher temperature, the addition of ascorbic acid or activated carbon treatment, although this also discolours the juices with the concurrent absorption of the polyphenols.

25. Toxicological studies should be encouraged on patulin and particularly its carcinogenic action.

26. It may be necessary to ask the Codex Committee on Methods of Analysis and Sampling to decide on methods of determination of patulin contents.

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Table 1 - Methods

Methods	Patulin (µg/l)	Repeatability (µg/l)	Reproducibility (µg/l)	Extraction	Purification	Detection	Quantity limit (Tg/l)
Official French Method NF V 76-116 Nov. 1985		not stated	not stated	Ethyl acetate	Silica gel column	HPLC confirmation by TCL	20
AOAC Official Method 974.18		not stated	not stated	Ethyl acetate	Silica gel column	TCL	20
AOAC Official Method 995.10	20 Tg/1 50 Tg/l 100 Tg/1 200 Tg/l	12.9 18.5 29.4 66.4	16.5 32.2 56.6 78.4	Ethyl acetate	Washing with sodium carbonate	HPLC UV detection	10
ISO 8128-2 : 1993		33.4	41	Ethyl acetate + chloroform	Silica gel column	TLC	25
ISO 8128-1 : 1993	40 Tg/l 207 Tg /l	8.9 41.9	10.5 47.5	Ethyl acetate	Washing with sodium carbonate	HPLC UV detection	10
Official Swiss method Mitt. Gebiete Lebensm Hyg. 75,506 (1984)		not stated	not stated	Ethyl acetate	Washing with sodium carbonate	HPLC UV detection	5-10
Official Swiss Method Mitt. Gebiete Lebensm Hyg. 75,506 (1984)		not stated	not stated	Extrelut	Silica gel column	HPLC UV detection	5-10

Table 2 - Analyses of Apple Juice

Year	Country	Number	% positives	% > 50µg/kg	min-max
1980	F	27	100	---	10-106
1980	UK	136	16	0	1-38
1980	Pol	46	0	0	---
1981	NZ	20	3	---	106-216
1982	I	58	21	0	5-15
1982	Aust	222	57.5	32	5-1130
1983	F	137	---	17	---
1984	F	112	---	19.6	---
1985	UK	38	26	2.6	5-56
1991	F	7	42	---	---
1992	UK	32	57	16	59-434
1992	F	31	3.2	---	---
1993	UK	62	28	4.8	61-118
1993	F	99	10	---	---
1994	UK	191	57	2	54-497
1994	F	50	8.5	---	---
1995	UK	185	35	6	73-490
1996	UK	174	---	0.5	184
1996	F	66	50	11	10-400
1994/96	GER	---	72	6	---
	USA	102	74	26	>500

Table 3 - Analyses of Solid Apple Based Foods

Year	Country	Number	% positives	% >50µg/kg	min-max
1980	UK	113	0	0	---
1981	GER	105	7	0	11-50
1982	AUST	70	25	0	5-32
1983	I	20	50	0	5-50
1993	UK	85	0	0	<25
1991	F	3	0	0	<30
1992	F	1	0	0	<30
1993	F	15	0	0	<30
1993	F	25	0	0	<25
1994	F	21	1	1	---
1996	F	32	9	0	<10
1996 (baby)	F	41	9.7	0	<10

Table 4 - Consumption of Apple Juices

The use of a standard serving (method developed by the United Kingdom and described in document CCFAC 96/15) gives a maximum consumption of 237 ml of fruit juice per day (97.5th percentile of consumption).

Surveys in France and the United Kingdom indicate the following consumption levels:

Country	Age	Average (ml/day)	97.5th percentile (ml/day)
UK*	2-5	50	150
UK*	Adults	61.5	290
F**	Adults/Children	---	223
G***	4-6	158	---
G***	7-9	160	---
G***	25-50	61	---

* Data to the 97.5th percentile of only consumers among the whole population.

** Data to the 97.5th percentile of only consumers among the whole population; maximum regular consumption over seven days through individual consumption surveys corrected for seasonal variation.

*** Data for average daily consumption of fruit juices.