

## **OPINION of the French Agency for Environmental and Occupational Health & Safety**

**concerning the implementation of an experimental protocol for the analysis  
of pine nuts**

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### **1. CONTEXT OF THE REQUEST**

On 13 November 2009, the French Food Safety Agency (AFSSA) received a request from the Directorate General for Competition Policy, Consumer Affairs and Fraud Control (DGCCRF) for an opinion regarding the implementation of an experimental protocol for the analysis of pine nuts.

### **2. BACKGROUND**

Since the summer of 2008, the DGCCRF and the French Poison Control and Monitoring Centres<sup>1</sup> (CAPTV) have recorded a significant number of reports mentioning a bitter taste following the ingestion of pine nuts. Similar cases have also been reported in other countries, the first having been observed in 2001. The symptoms appear after a slight delay, occurring from one to 3 days after ingestion, and the bitter taste is often exacerbated by the consumption of foods; the bitter taste lasts for between a few days and a week.

On 8 September 2009, in response to an initial request from the DGCCRF, AFSSA concluded that there was no scientifically supported hypothesis capable of explaining the reported bitter taste or of suggesting any health risk (AFSSA 2009). The BfR (German Federal Institute for Risk Assessment) also mentions the absence of any acute health risk (BfR 2010). The Food Safety Authority of Ireland stresses that it has not been informed of any adverse effects associated with this dysgeusia<sup>2</sup>. Studies by Mostin in 2001 and the analyses carried out on this type of product during inspections in France and Europe did not reveal the presence of any chemical contaminants in the suspected batches. Questions were raised as to the use of nuts from certain species of pine (particularly *Pinus armandii*).

The DGCCRF wishes to perform a thorough analysis of batches of nuts from several varieties of pine and requests AFSSA's opinion on the implementation of a suitable experimental protocol.

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<sup>1</sup> The CAPTV carried out a survey in 2009

<sup>2</sup> <http://www.fsai.ie/details.aspx?id=8648&LangType=1033&terms=pine+nuts>

### 3. EXPERT ASSESSMENT METHOD

The collective expert assessment was carried out by the expert committee (CES) on "Chemical and Physical Residues and Contaminants" (RCCP) which met on 7 July 2010.

### 4. DISCUSSION

#### Development of the phenomenon

The survey carried out in 2009/2010 by the CAPTV of Strasbourg to assess the importance of the phenomenon revealed a peak of reports in August 2009, followed by a reduction from December 2009. The rise in reports observed in August 2009 may partly be attributable to the opening of several Internet forums on this subject. The reduction in the number of reports observed since December 2009 may be explained by changes in the practices reported by certain distributors (different sources of supply, sifting the nuts, etc.).

#### Pine trees and pine nuts

Pine nuts are seeds that are rich in oil, have a hard shell, and grow under each scale of the pine cone (genus *Pinus*).

There are more than 100 species in the genus *Pinus*. About 30 produce edible nuts, according to the Food and Agriculture Organization of the United Nations (FAO)<sup>3</sup>. The most widely sold nuts come from the following species: *P. pinea* (a European species), *P. gerardiana* (Pakistan, India), *P. koraiensis* (China, Korea, Japan) or *P. sibirica* (Russia, China, Mongolia) (Destailats et al. 2010).

It appears however, that certain Asian species of pine nut that are not listed as edible by the FAO are imported into Europe, in particular the following Chinese species:

- *P. armandii* (Chinese white pine or Huashan pine or Armand pine), from the provinces of Shanxi and Gansu; small calibre nuts (1200-1700 nuts/100g) and **locally considered to be bitter**; this species is common in China and has been acclimatised as an ornamental plant in North America
- *P. massoniana* (Chinese red pine), found only in China and used particularly by the pulp and paper industry
- *P. yunnanensis* (Yunnan pine)
- *P. tabuliformis* (Chinese pine).

It seems that some pine nuts from these Chinese species are marketed as foods. In the absence of any long-term view of their use as food or of any listing in the Codex Alimentarius, and considering the non-European origin of these nuts, marketing them intentionally in Europe should come under the regulations for novel foods.

<sup>3</sup> FAO. Non-wood forest products from conifers, 1998.

### **Attempting to identify the agent responsible**

The symptom associated with the ingestion of pine nuts is dysgeusia characterised by a strong bitter sensation on the absorption of food occurring from one to 3 days after ingesting the nuts. No other symptom has been described. These symptoms are reversible and disappear on average after a period of a few days to a week. The method of preparation (raw/cooked, consumed alone/in association with other ingredients) does not seem to affect the onset of symptoms. It should be noted that the sensation of bitterness does not occur when the pine nuts are first consumed, which can lead to the assumption that the compound sought is not necessarily bitter itself. In addition, there seems to be considerable variability in the way consumers respond.

In order to confirm the hypothesis that these problems are due to a compound intrinsic to the nuts, it would be necessary to develop a method for distinguishing between them based on a phytochemical study consisting in producing successive dichotomous fractions and using volunteers to taste the different fractions obtained. A chemical analysis of each fraction would then make it possible to identify the causal agent more precisely.

If it were not possible to identify bitterness markers, the ideal solution would be to adopt a holistic strategy (known as a “without *a priori*” approach) to detect a specific marker for batches suspected of bitterness. This approach would consist in fingerprinting (by the use of mass spectrometry or nuclear magnetic resonance) a population of control nuts and comparing them with those from a population of nuts known to induce a sensation of bitterness.

Under the current circumstances however, the length of time necessary to set up these protocols is clearly incompatible with the rapid deployment required for this management tool.

Since it is impossible to provide a precise identification of the causal agent in the short term, ANSES recommends to the DGCCRF a protocol based on the identification of the species of *Pinus*. In fact, it has been noted that the surveys carried out between 2008 and 2010 cast suspicion on nuts from China (the geographical origin is indicated on the packets). One of these species, *Pinus armandii*, is not listed by the FAO as being edible. Excluding this species would therefore be a first step in reducing the risk of exposure for consumers.

### **A suggested method for analysing batches of pine nuts**

Several methods could be considered, with widely varying accuracy:

- visual sorting that could potentially exclude *P. armandii* nuts, characterised by their small size (10 to 15 mm long);
- chemical analysis based on the analysis of the composition profiles in  $\Delta$ -5 fatty acids, typical of the genus *Pinus*;
- genetic analysis, the only certain way of identifying the different species of *Pinus*.

No data has yet been published providing genetic criteria for the implementation of an identification protocol.

Since visual sorting and sifting are insufficiently specific, ANSES recommends supplementing this approach with the use of the only method capable of being implemented immediately, the chemical analysis of fatty acid profiles.

The genus *Pinus* develops typical  $\Delta$ -5 unsaturated fatty acids, in particular taxoleic (5,9-18:2), pinolenic (5,9,12-18:3) and sciadonic (5,11,14-20:3) acids. These can be used as taxonomic markers for conifers, as their relative concentration differs from species to species. Destailats (2010) developed a method based on the determination of an index for identifying species by the assaying of fatty acid methyl esters. Table 1 gives these indices calculated for the nuts of various species of *Pinus*.

**Table 1: Destailats indices for the nuts of various species of *Pinus***

Species of <i>Pinus</i>	Calculated index
<i>P. pinea</i>	0.34
<i>P. geradiana</i>	0.17
<i>P. massoniana</i>	3.55
<i>P. sibirica</i>	3.03
<i>P. armandii</i>	2.92
<i>P. koraiensis</i>	2.38

According to the author, this method has proved reliable for identifying the species found in commercial batches when those batches were homogenous. When batches comprise several different species, analysis can be more difficult.

Furthermore, the Norwegian Food Safety Authority recommends that importers of pine nuts use this method to exclude any nuts of species not listed by the FAO.

Since the batches marketed in France may contain mixtures of nuts from different species, ANSES suggests:

- sorting the nuts according to morphological criteria to optimise the homogeneity of batches prior to analysis,
- and then determining the Destailats index on samples of seeds with an identical morphology.

This method, combining morphological and chemical criteria, provides a relatively easy way of identifying batches contaminated by pine nuts from species not recognised as being edible, particularly *P. armandii*.

However, this method has certain limits. It is not particularly discriminatory (especially for heterogeneous batches, made up of different species) and the Destailats index has only been determined for the most common species. Consequently, detecting nuts from a species that has not been studied and is potentially hazardous is much more difficult.

## 5. CONCLUSION

The French Agency for Environmental and Occupational Health & Safety (ANSES) notes that the number of cases reported is declining.

In light of currently available data, the Agency therefore considers that the agent responsible for the bitterness cannot be identified in the short term, and that despite strong suspicions it is not possible to confirm that species from China, including *P. armandii*, are responsible. According to the Food and Agriculture Organization of the United Nations (FAO), the species *P. armandii* is not listed as a species of pine producing edible nuts.

For this reason, in view of current uncertainty, ANSES recommends:

- verifying that only species listed as edible by the FAO are imported,
- monitoring for any change in the number and nature of complaints by consumers,
- making a first visual inspection (seeking out nuts of small size),
- evaluating the usefulness of the Destailats index for verifying the species of pine nuts present in imported batches, with regards to the species listed by the FAO.

Furthermore, ANSES recommends further research to develop a genetic method for identifying the different species of *Pinus* with certainty.

**The Director General**

**Marc MORTUREUX**

#### **KEYWORDS**

PINE NUTS, BITTERNESS, EXPERIMENTAL PROTOCOL

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