
TUTIN TESTING **TECHNICAL NOTE**

Background

Tutin is a very potent toxin found in some NZ honey. The toxin comes from the native Tutu plant (*Coriaria arborea*), which is common in the North Island and the upper South Island. During the summer and autumn the Passion Vine Hopper (*Scolypopa australis*) insect feeds on the sap of the Tutu plant, producing a honeydew which is high in sugars and also contains Tutin from the sap of the plant. Bees collect the honeydew and take it back to the hive, where it is made into honey and stored. Honey made from Tutu honeydew will contain Tutin, and if this is at too high a concentration in extracted honey it poses a risk to human health (up to and including death).

There are MPI regulations which require people selling or providing honey for human consumption to assess the risk of Tutin being present in the honey. While beekeepers in the southern part of the South Island are generally not required to test (because the Tutu plant does not grow there) in the remainder of the country testing is the most common way of meeting MPI requirements.

In extracted honey the MPI regulatory limit for Tutin is a concentration of 0.7 mg/kg in the honey (as of 2016). A much lower limit of 0.01 mg/kg applies to comb honey – specific comments about comb honey are provided later in this Technical Note.

Testing is provided by laboratories who have been approved by IANZ and MPI. Because of the very low limit, sophisticated LC-MS/MS instruments are needed to carry out the testing. There are 2 options available for testing extracted honey samples for Tutin:

- Individual Sample Analysis
- Composite Sample Analysis

This Technical Note has been written to provide information on Tutin testing options, along with some guidelines about how use them and interpret results from them.

Extracted Honey

Individual Sample Analysis

This option involves the laboratory individually preparing and analysing each sample submitted by a client. The result gives the concentration of Tutin in the sample, and this can be directly compared with the MPI limit of 0.7 mg/kg.

Composite Sample Analysis

This option involves the laboratory taking an accurate sub-sample of up to 10 samples, and then combining them into one composite sample for analysis. Please note that MPI Regulations state that the laboratory must carry out the compositing step.

The result is an average of the Tutin concentration of the samples included in the composite. This average result cannot be directly compared with the MPI limit, because it is an average of multiple samples. To interpret the composite test result:

- Divide the MPI limit for an individual sample (0.7 mg/kg) by the number of samples contained in the composite. This gives a maximum amount of Tutin which can be present in the composite sample (see Maximum Test Value in the table below).
- Compare the test result against this calculated Maximum Test Value.

Table 1: Maximum amount of Tutin in Composite samples to meet MPI guidelines

# Samples In Composite	1	2	3	4	5
Maximum Test Value	0.70	0.35	0.23	0.16	0.14
# Samples In Composite	6	7	8	9	10
Maximum Test Value	0.11	0.10	0.09	0.08	0.07

If the Composite test result is lower than this calculated Maximum Test Value, then none of the samples included in the composite can have an individual result that is higher than 0.7 mg/kg. All samples in the composite are able to be regarded as complying with MPI requirements.

If the Composite test result is higher than this calculated Maximum Test Value, it is possible that at least one of the samples included in the composite will be above the MPI limit of 0.7 mg/kg. More testing is needed to identify which of the individual samples are above the MPI limit - either by carrying out Individual Sample Analysis of all the samples included in the failed composite, or by Composite Sample Analysis of smaller groups of the samples included in the failed composite.

Example:

A 10 sample Composite is tested and gives a result of 0.1 mg/kg. This is higher than the Maximum Test Value for a 10 sample Composite of 0.07 (see Table 1). The high Composite test result could be because:

- there is one sample above the MPI limit of 0.7 mg/kg, with other samples having little or no Tutin in them; or
- a number of samples contain small concentrations of Tutin, though none are above the MPI limit of 0.7 mg/kg.

Since there is a chance that one of the individual samples is above the MPI limit of 0.7 mg/kg, this should be followed up using Individual Sample Analysis.

What Is The Best Option To Choose For Testing Extracted Honey?

Composite Sample Analysis is the most cost effective testing option. It is best used when:

- You have a number of samples needing testing at the same time; and
- There is a low risk that the samples will contain more than 0.7 mg/kg of Tutin.

Please note that just because up to 10 samples can be analysed as a Composite sample, this may not be the most efficient number of samples to include in situations where there is a moderate risk of some samples containing Tutin.

- Since the MPI limit was reduced from 2.0 mg/kg to 0.7 mg/kg in early 2016, there have been more examples of composite samples failing and requiring further analysis. The more samples that are included a composite, the more that will require follow up testing in the case of a failed composite.
- In our experience, when honey is harvested after the start of February from regions where Tutin is found it can be less expensive overall to test 5 sample Composites unless the producer feels confident that there will be low/no Tutin present in the honey.

Individual Sample Analysis is best used in situations where:

- A final production batch of honey for human consumption is being produced, and the processor wants a test result for that batch that will satisfy customers the honey meets MPI regulations.
- A composite Tutin test has failed, and you need to confirm which samples are above or below the MPI limit.
- There is a reasonable chance that a honey sample has a high Tutin concentration.

Comb Honey

Comb honey is regarded as being a particularly high risk product for Tutin, and because of this there are specific requirements for testing it. The risk arises because there is no mixing of honey in comb honey, and honey with high Tutin concentration can be present in a few cells. These high Tutin cells pose a major health risk to people who may eat them.

Comb honey samples are taken from the drippings of honey and left over comb collected during the manufacturing process (see Section 3.3 of the Tutin Food Standard 2016). These should be well mixed, and a sub-sample sent to a laboratory for testing.

The maximum amount of Tutin allowed in a comb honey sample is 0.01 mg/kg – 70 times lower than the extracted honey limit.

Comb honey samples must be analysed individually – Composite analysis is not allowed due to the very low limit.

Test Results Which ‘May Not Comply’

Analytica test reports include an assessment of whether an individual or composite test result will Pass or Fail when compared with MPI regulations. Sometimes, instead of saying Pass or Fail, our reports state that the result ‘May Not Comply’.

To understand this, an explanation of Uncertainty Of Measurement in laboratory testing is needed.

Ideally a laboratory test result will be exactly the same every time. However, in practice test results vary because of variation across the steps in the testing process. If you test the same sample multiple times, you will get a (narrow) range of results. The degree to which results vary in a test is called Uncertainty Of Measurement (UoM).

Laboratories are required to measure UoM when they first set up a method, and then to monitor it in an ongoing way. The UoM tells you the range you would expect 95% of results to fall within if the same sample was tested many times.

For Tutin, Analytica has a UoM of plus or minus 16%. In other words, a sample giving a result of 0.70 mg/kg could re-test within a range of 0.59 mg/kg (16% less than 0.70 mg/kg) and 0.81 (16% more than 0.7 mg/kg).

Taking this a little further, if we measure a result of 0.61 mg/kg for an individual sample, this result is below the MPI limit of 0.7 mg/kg and so it does not Fail. However, if retested, there is a chance that sample could return a result of up to 0.71 mg/kg because of the method UoM. If this happened, the result would not comply with the MPI limit.

Hence, Analytica’s test reports let users know that the result May Not Comply for test results that are below but are within 16% of the Individual or relevant Composite limit.

It is up to individuals to work with their MPI auditors to decide on what (if any) action to take for test results which May Not Comply. In Analytica’s experience, auditors are more concerned about Composite results that May Not Comply than Individual results. But this will be a decision reached by an auditor based on their understanding of the way that a particular beekeeping or processing organisation is operated.

Further Reading

Food (Tutin in Honey) Standard 2016 -
<http://www.foodsafety.govt.nz/elibrary/industry/dv11137.htm>