How the ISO4730: 2017 Standard Helps Identify Fraudulent Tea Tree Oil

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Pure Australian Tea Tree Oil (TTO) steam distilled from *Melaleuca alternifolia* should always conform to the International Standard **ISO 4730: 2017 "*Oil of Melaleuca, Terpinen-4-ol type*"**.

Species

There are only two named species in the Standard from which TTO can be steam distilled:

1. *Melaleuca alternifolia* (Maiden et Betche)
Cheel 2. *Melaleuca linariifolia* Smith

ISO 4730: 2017 specifies that for *Melaleuca alternifolia,* only the terpinen-4-ol chemotype is to be used; this ensures the terpinen-4-ol level in the TTO exceeds the minimum of 35.00%.

Chromatographic Profile

ISO 4730: 2017 specifies the levels of 15 of the 113+ components of pure Australian TTO:

Component	ISO 4730:2007 Range	
	Minimum %	Maximum %
α-Pinene	1.00	4.00
Sabinene	traces[a]	3.50
α-Terpinene	6.00	12.00
Limonene	0.50	1.50
p-Cymene	0.50	8.00
1,8-Cineole	traces[a]	10.00
γ-terpinene	14.00	28.00
terpinolene	1.50	5.00
terpinen-4-ol	35.00	48.00
α-terpineol	2.00	5.00
aromadendrene	0.20	3.00
ledene	0.10	3.00
δ-cadinene	0.20	3.00
globulol	traces[a]	1.00
viridiflorol	traces[a]	1.00
[a]traces: <0.01%		

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Physical Requirements

ISO 4730: 2017 specifies several physical parameters of pure Australian TTO

Physical Properties	ISO 4730: 2007 Range	
	Minimum %	Maximum %
Appearance	Clear, mobile liquid	
Colour	Colourless to pale yellow	
Odour	Characteristic, Myrtistic	
Relative Density (20°C)	0.885	0.906
Refractive Index (20°C)	1.475	1.482
Optical rotation (20°C)	+ 7.00 °	+ 12.00 °
Miscibility in 85% (v/v) ethanol (20^0C)	Less than 2 volumes	

ISO 4730: 2017 provides a range (maximum and minimum) for all objective parameters to allow for the variation that occurs in natural products such as pure Australian TTO. This natural variability is usually caused by slight differences in growing conditions from year to year, minor genetic variation from plantation to plantation and differing distillation techniques used.

Note: Minor variations can also occur with identical samples. This is usually due to differing conditions (temperature, humidity, atmospheric pressure etc) when an analysis is done, as well as the specified tolerances of the testing equipment. When a sample of TTO is sent to a laboratory for analysis a minimum of 15 ml is usually required; ISO 4730: 2017 specifies a sample size of 50 ml.

Enantiomeric Distribution

In addition to the physical and chromatographic requirements, ISO 4730: 2017 also stipulates a range for the enantiomeric (chiral) ratio of terpinen-4-ol to provide an extra measure of authenticity as shown below:

terpinen-4-ol	ISO 4730: 2017 Enantiomeric Distribution	
	Minimum %	Maximum %
(R) or (+) or (D)	67.00	71.00
(S) or (-) or (L)	29.00	33.00

Parameters Explained

Chromatographic Profile

Pure TTO contains 113+ compounds which can be identified and quantified using a gas chromatograph (CG). It is impractical to base a Standard on all 113+, so Standards committees look very carefully at the GC profile for a substance and choose a limited number of components based on both their importance and uniqueness. This is similar to a fingerprint search: is not possible to compare the entire print, so reference points are used. If any sample does not contain all 15 listed substances that are within the ranges given, then it cannot be sold, used or described as pure TTO.
The absence of a compound is just as telling as having too much, especially the four compounds where the minimum percentage is 'traces' or less than 0.01%.

Physical Properties

Because cheating (extending, adulterating, etc.) can occur, Standards committees also provide a list of physical properties to help an analyst to determine the likelihood of a sample being pure TTO. Some of these are advisory while others are compulsory. They are a mix of subjective and objective properties.
Note: On very rare occasions, pure Australian TTO steam distilled from Melaleuca alternifolia doesn't fully conform to the Standard (e.g. high p-cymene or low terpinen-4-ol). This is usually due to either the incorrect chemotype being selected and grown or incorrect distillation, storage and handling procedures.

Subjective properties

The appearance, colour and odour are subjective (not measured precisely). The Standard describes these so an analyst can, based on both experience and observation, state that a sample either conforms or fails.

Objective properties

These are measured using calibrated precision instruments. The results are compared to the ranges given in the Standard to either conform or fail.

Relative Density

Relative density (RD) is the ratio of the density (mass of a unit volume) of a substance to the density of a given reference material, usually water. If the RD = 1 then it is equivalent to pure water. If the RD is less than 1.00 it is less dense than water and will float. A good example is ice which has an RD of 0.91.

For pure TTO the RD at 20°C must be between 0.885 and 0.906.

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Refractive Index

Refractive index (RI) is the measurement of how hard it is for light to travel through a medium. The higher the number the harder it is. It is compared to air with an RI of 1.00. Examples of the RI for liquids at 20 °C:

Water	1.333
Ethanol	1.360
Benzene	1.501

For pure TTO the RI at 20°C must be between 1.475 and 1.482.

Optical Rotation

When plane-polarised light is passed through a sample of known chiral (enantiomeric) content, the plane of the polarised light is rotated by a very specific and measurable quantity. This is known as the optical rotation (OR) of a substance. This technique is used to measure the purity and concentration of familiar chemicals:

Sucrose	+66.47°
Cholesterol	–31.5°
Camphor	+44.26°
Penicillin V	+223°

For pure TTO the OR at 20°C must be between +7.00° and +12.00°.

Miscibility in 85% (v/v) ethanol

It should be possible to completely dissolve a measured volume of pure TTO in less than twice that volume of 85% ethanol at 20°C to obtain a clear solution.

Enantiomeric Distribution

In the latest (2017) version of ISO 4730, the ranges for the enantiomeric (chiral) distribution of terpinen-4-ol, the most abundant compound in TTO, is included to provide an extra measure of authenticity for pure TTO.

Some components found in TTO (e.g. terpinen-4-ol, α-terpineol, limonene and α-pinene) exist in two enantiomeric forms designated as (R) and (S), D and L or (+) and (-), respectively, to indicate they rotate plane polarised light either to the right (+) or the left (-).

Many enantiomers have distinctly different properties so their presence in the correct form and ratio is critical. Also 100% pure natural essential oils such as TTO contain these enantiomers in known and characteristic ratios. This is upset by the addition of adulterants which may be industrial waste from normalising other essential oils (eg Eucalyptus, Pine and White Camphor) or compounds synthesised either from other essential oil components (eg terpinen-4-ol synthesised from sabinene) or from fossil fuels.

Hence the measurement of the enantiomeric (chiral) ratio is given in an informative annex (Annex C) of ISO 4730: 2017 to provide an inexpensive but very accurate measure of extra authenticity for 100% pure TTO.

For pure TTO, the enantiomeric distribution for terpinen-4-ol must be within the ranges given:
For the Dextro (D) or (+) enantiomer: 67.00% to 71.00%
For the Levo (L) or (-) enantiomer: 29.00% to 33.00%

An Example of a conforming and two non-conforming samples

The following table lists the results of three samples analysed at the Southern Cross University Plant Science's Analytical Research Laboratory. One is 100% pure Australian TTO that has been grown under strict quality assurance while the other two show clear evidence of adulteration:

Component	ISO 4730: 2017 Range		Pure Australian TTO		Adulterated "TTO" #1		Adulterated "TTO" #2	
	Minimum %	Maximum %	Result	Outcome	Result	Outcome	Result	Outcome
α-pinene	1.00	4.00	2.35	conforms	3.25	conforms	3.23	conforms
sabinene	traces	3.50	0.20	conforms	0.14	conforms	0.03	conforms
α-terpinene	6.00	12.00	9.72	conforms	7.92	conforms	9.49	conforms
limonene	0.50	1.50	0.81	conforms	**2.13**	**fail**	**2.66**	**fail**
p-cymene	0.50	8.00	2.20	conforms	2.39	conforms	2.38	conforms
1,8-cineole	traces	10.00	1.91	conforms	3.23	conforms	1.72	conforms
γ-terpinene	14.00	28.00	20.78	conforms	21.35	conforms	21.08	conforms
terpinolene	1.50	5.00	3.50	conforms	2.99	conforms	3.08	conforms
terpinen-4-ol	35.00	48.00	43.76	conforms	40.60	conforms	41.46	conforms
α-terpineol	2.00	5.00	2.94	conforms	4.53	conforms	**5.57**	**fail**
aromadendrene	0.20	3.00	1.02	conforms	1.46	conforms	1.71	conforms
ledene	0.10	3.00	0.89	conforms	0.55	conforms	0.61	conforms
δ-cadinene	0.20	3.00	0.78	conforms	0.39	conforms	**0.07**	**fail**
globulol	traces	1.00	0.12	conforms	0.44	conforms	0.57	conforms
viridiflorol	traces	1.00	0.10	conforms	0.14	conforms	0.11	conforms
Physical Properties	**ISO 4730: 2017 Range**		**Pure Australian TTO**		**Adulterated TTO**		**Adulterated TTO**	
	Minimum %	Maximum %	Result	Outcome	Result	Outcome	Result	Outcome
Appearance	Clear, mobile liquid		conforms	conforms	conforms	conforms	conforms	conforms
Colour	Colourless to pale yellow		conforms	conforms	conforms	conforms	conforms	conforms
Odour	Characteristic		conforms	conforms	**Pine like**	**fail**	**Pine like**	**fail**
Relative Density (20°C)	0.885	0.0906	0.897	conforms	0.897	conforms	0.893	conforms
Refractive Index (20°C)	1.475	1.482	1.4788	conforms	1.477	conforms	1.4773	conforms
Optical rotation (20°C)	+ 7.00°	+ 12.00°	+10.32°	conforms	**+6.99°**	**fail**	**+5.80**	**fail**
Terpinen-4-ol (R) or (+)	67.00	71.00	68.30	conforms	**54.94**	**fail**	**45.76**	**fail**
Terpinen-4-ol (s) or (+)	29.00	33.00	31.70	conforms	**45.06**	**fail**	**54.24**	**fail**
Miscibility in 85% (v/v) ethanol (20°C)	< 2 volumes		0.60	conforms	0.50	conforms	**> 2 volumes**	**fail**

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The 100% pure Australian TTO conforms to all parameters in the Standard and can therefore be sold, used and described as 100% pure TTO steam distilled from *Melaleuca alternifolia*.

Both adulterated samples failed: **Sample #1** failed 5 of the total 24 parameters while **Sample #2** failed 8 of the total 24 parameters.

Adulterated Sample #1

This is a very cleverly constructed example of adulteration and is an excellent demonstration of how a 'good' laboratory can dilute a 100% pure natural product with only one aim: increasing profitability at the expense of the producer and of course the consumer. This comparative analysis of the sample against the ISO 4730: 2017 Standard clearly demonstrates this.

This sample likely contains a high percentage of TTO. It has however been diluted, probably with terpinen-4-ol to boost it above 40.00%; limonene (which is strongly optically positive) has also been added to readjust the optical rotation.
In fact this sample conforms to the 2002 British Pharmacopeia (BP) Standard. It is worth noting that the BP Standard has not been updated since 2002. After more than 15 years both the BP and the *Ph. Eur.* Standard are urgently in need of updating to reflect advancements in modern analytical techniques; ATTIA Ltd therefore considers both BP and *Ph. Eur.* Standards worthless in their current form.

Note that while sample #1 conforms to most of the parameters for ISO 4730: 2017 it fails the enantiomeric (chiral) distribution for terpinen-4-ol, a clear and indisputable indicator that this has been boosted (likely with terpinen-4-ol derived from pine oil based on the odour detected). Despite having 42%+ more limonene than the maximum allowed this sample still does not quite make the minimum range for the optical rotation (OR) in ISO 4730: 2017.

Most tellingly the enantiomeric ratio for terpinen-4-ol was **18% below** the lower range for the R (+) and **36.5% over** the upper range for the D (-) enantiomers of terpenen-4-ol given for 100% pure TTO in ISO 4730: 2017.

It is likely this started out as a low terpinen-4-ol TTO which was purchased cheaply from a producer with the incorrect chemotype (remember it should be the terpinen-4-ol chemotype) and adulterated to make it fit the BP Standard. This does not make it TTO and it is worrying to note that the following compounds were also detected in the sample, none of which occur naturally in 100% pure TTO steam distilled from *M. alternifolia*:

- ρ-menth-3-ene 0.08%
- ρ-menth-1-ene 0.09%
- *trans*-pinocarveol 0.49%

These compounds are most commonly found in pine oil, indicating that this was the source of the terpinen-4-ol used. It is impossible to know what else is present from batch to batch because the adulteration uses uncontrolled waste from a diverse range of industrial fractionation processes to boost the quality of pine oil. It is entirely possible that pesticides, phthalates or other harmful compounds are present in this or other adulterated batches.

Adulterated Sample #2

It is likely that the product was originally an essential oil of some sort but it would have been of very poor quality (eg low terpinen-4-ol and maybe high ρ-cymene). In an attempt to make the product conform, the perpetrator would again have added terpinen-4-ol (often made from sabinene derived from pine oil), aiming for 40%+ which is what many buyers are after. The terpinen-4-ol added to this product has a different optical rotation to that found naturally in TTO so they would then also add synthetic limonene to balance this out but once again they had to overdo the limonene (77% more than the maximum) to achieve the OR of +5.80; once again this passes the BP Standard but the 2017 version of ISO 4730 requires a minimum optical rotation of +7.00 which they were likely unaware of at the time.

If you look at the results, they got the terpinen-4-ol level right so it conforms; but this attempt to construct 'tea tree oil' failed in many other very obvious ways:

1. The α-terpineol level is too high because the terpinen-4-ol used to get to the desired 40%+ level likely also contained a significant portion of α-terpineol due to poor fractionation control.
2. The limonene level is 77% too high because they needed to add it to the product to try to balance the optical rotation.
3. The δ-cadinene level is below the minimum required. This is precisely why minor components are in the Standard: they are always in a pure, natural sample of TTO in the ranges given in ISO 4730: 2017; if they are not present at these levels, then something is wrong with the purity of the sample.
4. The optical rotation is plus (+) 5.58°. Despite overdoing the limonene they still didn't get the optical rotation right although they tried hard to get it to conform to the BP Standard.
5. Most tellingly the enantiomeric ratio for terpinen-4-ol was **31.7 % below** the lower range for the D (+) and **64% over** the upper range for the L (-) enantiomers of terpenen-4-ol given for 100% pure TTO in ISO 4730: 2017.
6. The product failed to meet the miscibility test – it took more than 2 volumes of 85% ethanol to produce a clear solution.

It is again likely this started out as a low terpinen-4-ol TTO which was purchased cheaply from a producer with the incorrect chemotype and adulterated to make it fit the BP Standard. This does not make it TTO and, of much greater concern, the following compounds were also detected in the sample, none of which occur naturally in 100% pure TTO steam distilled from *M. alternifolia.* These additional compounds, never found in 100% pure natural TTO indicate that the industrial waste used to adulterate this product was of even poorer 'quality' than that used in sample #1:

- p-menth-3-ene 0.09%
- p-menth-1-ene 0.30%
- trans pinocarveol 0.33%
- plinol-D 0.09%
- cis-β-terpineol 0.26%

Once again pesticides, phthalates or other harmful compounds may be present in this or other adulterated batches. Plinol-D is likely to have been sourced from fractionating white camphor oil.

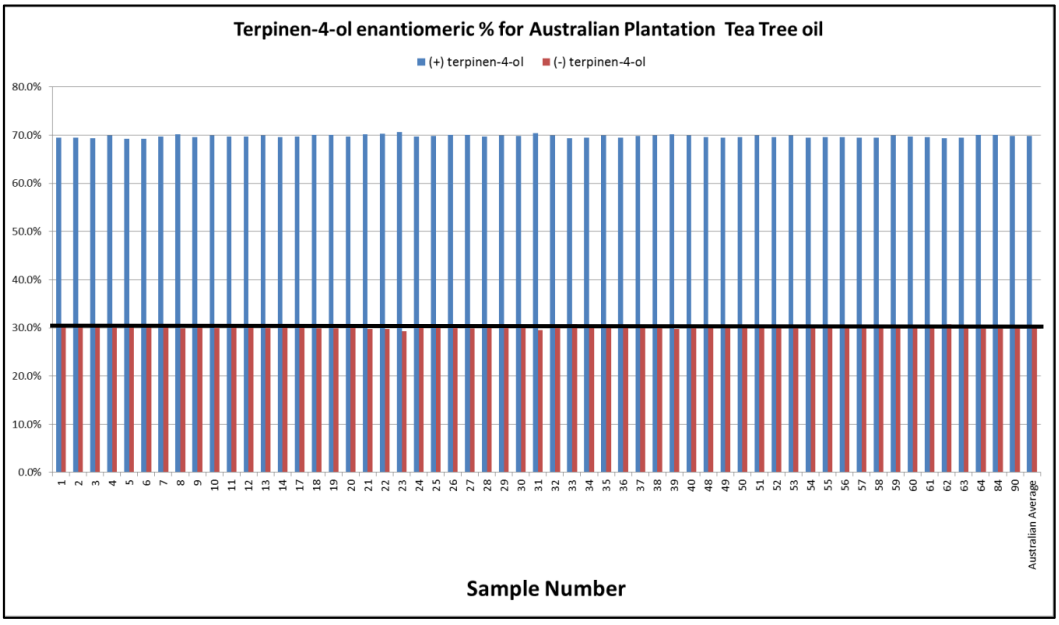
Enantiomeric (chiral) data

While ISO 4730: 2017 only specifies the enantiomeric distribution for terpinen-4-ol, ATTIA routinely requests that chiral data on a further two optically active compounds (limonene and α-terpineol) are also measured as this not only increases the measure of extra authenticity from one to three compounds but also provides a telling 'at a glance' first test for authenticity.

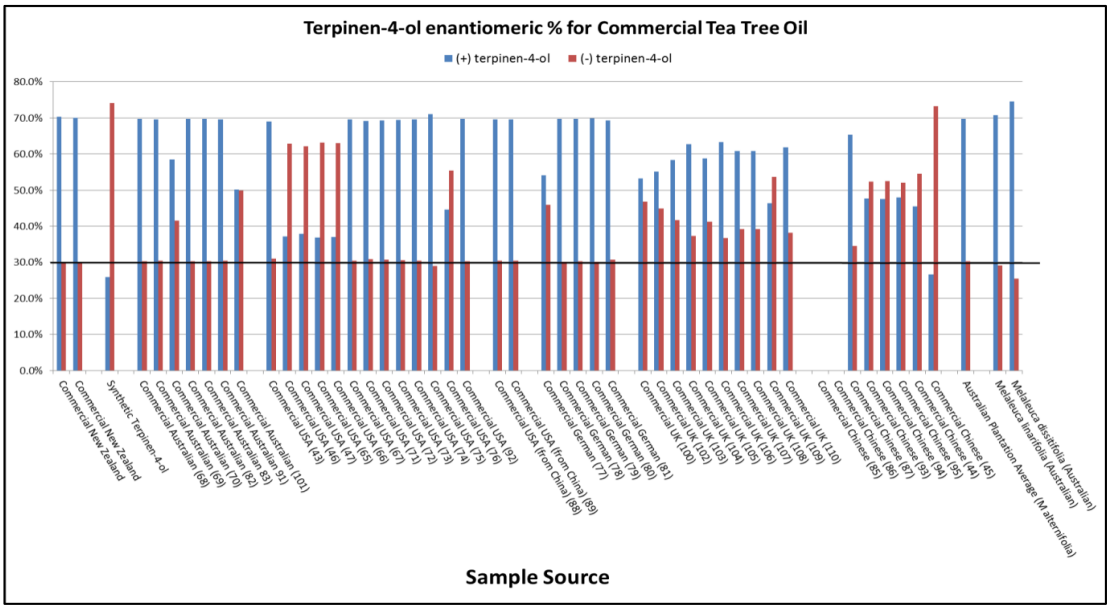
Sample	(-) limonene	(+) limonene	(+) terpinen-4-ol	(-) terpinen-4-ol	(-) α-terpineol	(+) α-terpineol
Pure Australian TTO	37.30	62.70	68.30	31.70	23.70	76.30
Adulterated Sample # 1	**16.85**	**83.15**	**54.94**	**45.06**	**73.73**	**26.28**
Adulterated Sample #2	**4.25**	**95.75**	**45.76**	**54.24**	**84.57**	**15.43**

It is immediately obvious from this that the Pure Australian TTO is as claimed while adulteration is equally obvious for all three compounds measured in the other two samples.

The enantiomeric distribution for terpinen-4-ol, α-terpineol and limonene was accurately measured for 57 samples of 100% pure Australian TTO sourced directly from plantations with samples representing production over a period of five years. The graph for terpinen-4-ol below shows how accurate chiral ratios are in 100% pure TTO; the data for the other two compounds is equally compelling:



When the same was done for a total of 48 commercial samples sourced from around the world a graph immediately and clearly shows which samples are authentic and which have been adulterated:



The following data on 100% pure Australian TTO of known provenance has been collected over the past three years to provide a basis for setting upper and lower ranges (the mean + 3 x the standard deviation (SD) from 131 samples) with a high degree of confidence for these three compounds:

	(-) limonene	(+) limonene	(+) terpinen-4-ol	(-) terpinen-4-ol	(-) α-terpineol	(+) α-terpineol
Average (n=131)	39.81%	60.20%	69.12%	30.89%	25.53%	74.45%
Median (n=131)	39.85%	60.16%	69.47%	30.54%	25.23%	74.74%
Lowest	35.09%	58.19%	66.41%	28.96%	23.23%	68.86%
Highest	41.81%	64.91%	71.04%	33.59%	31.14%	76.77%
Standard Deviation (SD)	0.98%	0.98%	0.86%	0.85%	1.27%	1.28%
3x SD	2.94%	2.95%	2.57%	2.56%	3.80%	3.84%
Lower Range (Average - 3x SD)	**36.87%**	**57.24%**	**66.55%**	**28.33%**	**21.73%**	**70.61%**
Upper Range (Average + 3x SD)	**42.76%**	**63.15%**	**71.68%**	**33.45%**	**29.33%**	**78.29%**

Conclusion

Adulterated material such as those shown in the table on page 4 is often described and offered as 'tea tree oil' or even as '100% pure Australian tea tree oil steam distilled from *Melaleuca alternifolia*' to world markets on a daily basis. It is not TTO, rather a mix of unknown chemicals from unidentified sources that have been put together with a single objective: profit.

No testing for safety and efficacy has ever been done on any of these concoctions.

By claiming this fabrication as TTO the perpetrators of this fraud rely entirely on the decades of research, good-will and the excellent reputation for safety and efficacy that pure Australian TTO enjoys.