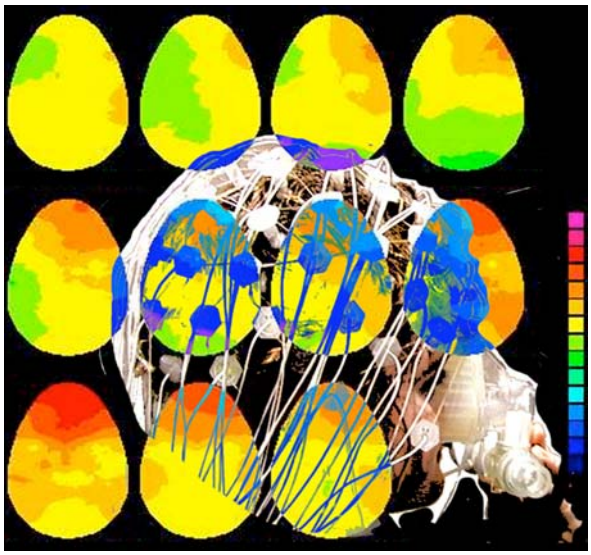
SWINBURNE UNIVERSITY OF TECHNOLOGY

Australian Government
Grape and Wine Research and Development Corporation

A MULTI-DISCIPLINARY ANALYSIS OF SUBJECTIVE & OBJECTIVE RESPONSES TO TCA IN WINE, USING SENSORY, CHEMICAL & ELECTROPHYSIOLOGICAL TECHNIQUES



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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A MULTI-DISCIPLINARY ANALYSIS OF SUBJECTIVE & OBJECTIVE RESPONSES TO TCA IN WINE, USING SENSORY, CHEMICAL & ELECTROPHYSIOLOGICAL TECHNIQUES

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ABSTRACT

This project used subjective and objective techniques to investigate differences in responses of untrained consumers to wine taints – and specifically to TCA, 4-ethyl guaiacol and 4-ethyl phenol. Threshold levels and perceptual responses to these taints were determined. Electrophysiological (brain activity) differences in response to three concentrations of TCA were correlated with reported responses to the TCA odour. The degree of variability in responses to TCA was clearly evident in this study in both subjective and brain activity responses. Even at the low concentrations used, results provide unassailable evidence of a link between brain activity and preference for odours.

EXECUTIVE SUMMARY

Cork taint in wine is a serious problem exacerbated by the difficulty of its assessment. It is reported to adversely affect the sensory properties of wine, but individual responses to tainted wines vary to such a degree that even experienced tasters may have conflicting opinions about the tainting of a wine. Variations in sensitivity to TCA will result in only portions of the population even being aware of its presence, and variations in concentrations of TCA will have different manifestations.

This project investigated differences in responses to taints – and specifically to TCA. Preliminary sensory studies were also undertaken investigating consumer responses to additional taints and flavours: 4-ethyl guaiacol and 4-ethyl phenol. Olfactory threshold responses to the taints were assessed using sensory and EEG responses to different concentrations of TCA, including an investigation of preliminary sensory responses to TCA taints at different concentrations, dosed in wines. These sensory studies were replicated to investigate odour perceptual responses associated with the combinatorial effect of different concentrations of 4-ethyl guaiacol and 4-ethyl phenol dosed in wines.

The **general aim** of the project was to explore the potential of sensory, chemical and electrophysiological techniques to deliver quantitative and qualitative information about the consistency of taint detection responses across the population.

The **specific aims** of the project were:

- To measure TCA odour thresholds and perceptual responses in a population of untrained wine consumers and non-wine drinkers.
- To determine electrophysiological (brain activity) differences in response to the flavours of wines containing different concentrations of TCA, correlated with reported responses to the wine odours, to determine the degree of sub-conscious and conscious responses to TCA and wine flavours.
- To determine threshold levels and perceptual responses to 4-ethyl guaiacol, 4-ethyl phenol and eucalyptus, and the combinatorial effect of these components in odour trials with untrained consumers.

Method

The study was undertaken in two stages of recording and analysis: taint threshold and discrimination information, and brain activity recordings and associated perceptual responses. Participants were screened for olfactory ability, and then attended threshold detection and discrimination sessions for each taint. The threshold tests were conducted using a pen-delivery system with a forced choice ascending-descending staircase method The wine glass discrimination and perception task was then undertaken for each target odour, delivered in either red wine, white wine or ethanol.

Participants also attended two recording sessions during which brain activity (EEG) was recorded while three levels of TCA in ethanol were delivered synchronous with breathing. Participants also provided odour ratings for liking, strength and complexity of the odour stimulus. Changes in EEG responses are analysed (frequency and topography) to determine differences associated. The brain activity was then analysed for changes associated with TCA concentration levels and perceptual responses to the TCA concentrations

Summary: Threshold testing of wine taints

All conditions resulted in reference to the odour being stronger, less pleasant and generally more negatively regarded in the tainted base *cf* the untainted control odours. Detection and discrimination thresholds, and descriptor responses of untrained participants

revealed within- and between- subject differences. Despite being naïve to the descriptions of the target odours, participants provided clearly different descriptors for the different taints in the pens and base solutions. TCA descriptor responses supported industry expectations of some negative attributes when detected above threshold levels; 4-EP and 4-EG responses were also typically associated with "chemical" and negative descriptors but also were reported with differing ranges of attributes. These results demonstrated the variation in consumer responses associated with preferences for the wine taints odours, and with the type of wine or alcohol the taint was presented in, providing the wine industry with preliminary evidence of consumer preference responses to these wine taints.

Summary: Electrophysiological Relative % Power Analysis for Liking, Strength and Complexity effects across concentrations

Differences in brain activity responses for the different rating scales were observed, with significance assessed using the Bonferroni adjustment value of p=0.004.

Liking effects across concentrations: There were differences indicating increased responses to the *Low* concentration of TCA in the theta (4-8 Hz) range associated with increased liking for TCA, evident in the Left temporoparietal, Left and Right Frontal areas. Mid concentration responses revealed a decrease in responses to TCA in the alpha range (9-12 Hz) and at 14 Hz in the Left temporoparietal and Left Frontal areas.

Strength effects across concentrations: In the *Mid* concentration, there were significant Left temporoparietal and approaching significant Left frontal differences associated with increased perceptions of strength – with an increase in 11 Hz (alpha range) responses. There were Left and Right temporoparietal differences in the theta (4-8 Hz) range in response to the *High* concentration of the TCA stimulus, with a decrease in response to an increased perception of strength.

Complexity effects across concentrations: There was an increase in response to the *Low* concentration of TCA associated with perceived increases in *Complexity* at both 6 Hz and 13 Hz in the Left Frontal, and in both the Left and Right temporoparietal areas.

Concluding Summary

Overall, the project has revealed important industry issues.

- Taints are not universally disliked by the inexperienced.
- Taints are, in fact, sometimes described positively by the inexperienced.
- Detection of taints at low concentrations has been confirmed by changes in brain electrical activity to the taint, which can be the only cause of the changes in our paradigm.
- The use of EEG as a method for probing the development and characterisation of hedonic responses has been further supported by the nature of the results.
- It has not been possible to definitively state what sort of brain electrical change is always aligned with 'liking' or 'dislike', but there are strong suggestions of a pattern.
- Inexpert consumers are very diverse and the need to totally eliminate TCA is not recommended, because only 10% of inexperienced consumers may find it unpleasant.
- Inexpert consumers may include 10% of people who cannot detect the TCA even at 1 $ng.l^{-1}$.
- Of the population in our study, the majority could detect the TCA at 1 $ng.l^{-1}$, and, they were more likely to state a liking for it than a dislike, based on our 100-point scale with 'neutral' set at 50.

- The level of inconsistency in our sub-population was marked: TCA responses ranged from like to dislike, but the trend was for the rating of the dislike to be less extreme than the like rating.
- Even at low concentrations there was no evidence of an absence of EEG response to the stimulus.
- Some of the EEG data suggest – but not universally so – that those who expressed liking for the TCA trended towards activity patterns which are often associated with relaxation.
- Some of the EEG data suggest – but not universally so – that those who expressed dislike for the TCA trended towards activity patterns which are often associated with increased activation and a movement away from relaxation.
- Importantly, the EEG responses were obtained when the participants were breathing normally. There was no enhancement of olfactory performance by sniffing involved.
- Even at the lowest concentration used, the non-expert participants all expressed a subjective preference for the TCA when compared with the baseline control carrier.

1. BACKGROUND

1.1 Introduction

Acceptance of wine is dependent on sensory appreciation, but this is strongly influenced by emotional and experiential aspects. Techniques are needed which can determine differences between and within individuals to flavour responses at different concentrations (sub- and supra-threshold) to determine levels of discrimination and threshold detection for wine flavours to assist the wine industry in gaining an understanding of the factors, both chemical and sensory, affecting wine flavours and consumer perceptions.

The perceptual experience of a flavour is the combination of a cascade of physiological responses involving odour, taste, texture, temperature, with the odour component providing approximately 75% of the flavour experience. In attempting to gain a greater understanding of the factors contributing to the success or failure of a flavour, it is therefore of great relevance to flavour research to further investigate differences in responses to odours and flavours and their interactions with emotional or hedonic responses which may ultimately contribute to the enjoyment of and subsequent success of a product.

Our sense of smell provides us with a great deal of information, most of which can be transparent to our consciousness. Personal experience shows us that we ignore smells after a short period of time, unless we choose, or, are obliged, to notice them. Changes in our electrical brainwaves relate to what our brain is doing. It is clear that some aspects of brain electrical activity changes must relate to processing of a flavour or odour. It has been established that electro-encephalographic (EEG) recordings alter when an odour is present or when the concentration of an odour is altered (Owen, 1998; Patterson *et al*., 1998; Owen *et al*., 2002a), and even when the individual is not consciously aware of the presence of the odour (Owen, 1998). There is also evidence that the nature of some components of the EEG differ between those who like a flavour compared with those who dislike the flavour (Owen & Patterson, 2002). The diversity of human preferences and the wide variety of experiences within and between individuals combine so that any individual is almost unique in terms of their attitude to a flavour or odour.

The complexity of human preferences and variety of experiences combine to make studies of flavour responses complicated and their results need further verification. While there is unassailable evidence of a link between EEG structure and preference for odours, the precise nature of the link has not yet been revealed in these studies. In addition, previous studies have focussed on EEG responses to single components only and the complex interactions associated with different mixtures and with the extreme variations in the range of influencing parameters of the human volunteers (threshold, preference, experience, age, gender, ethnic background, olfactory performance) were not controlled for completely. In other words, the results may only apply to a small group and cannot yet be generalised over the population.

A major influence on whether an odour is subliminal or above-conscious detection levels is the individual's threshold level for detecting that odour. This "detection threshold" is derived from studies relying on identification. It does not necessarily mean the actual concentration at which the odour is detectable by the olfactory receptors, as this may be much lower than the concentration at which we become consciously aware of an odour. This is an additional aspect where the independent measurement of the response to an odour by means other than relying on language is needed. The use of changes in brain electrical activity to monitor changes caused by odours not subjectively detected was incorporated into this project.

While advances have been made in chemical analysis, the extent and variety of responses to wine flavours is still largely reliant on subjective reports and awareness of the odour. Individuals vary greatly in sensitivity to odours, both positive (enhancers) or negative (taints). This is in part due to physiological differences and to differences in experiences. In addition, subjective responses to odours and taints are strongly influenced by the degree and quality of experience with the odour, and its associated emotional and memory responses. Electrophysiological (EEG) or brain electrical activity responses have been found to change in association with reported preference, providing an objective measure of responses, independent of language and experience.

1.1.1 Previous research: EEG responses to TCA

Previous research conducted at Swinburne University has demonstrated the potential for combining traditional sensory analysis with objective physiological techniques to monitor responses to flavours, in both complex mixes and single components, as a language- and experience-free method for monitoring preference responses. This technique has been combined with traditional sensory panel work, and gas chromatography analysis of flavours to demonstrate the varying influences of components in sub- or supra-threshold concentrations which can influence flavour responses.

Pilot work was conducted to study the relationship between EEG activity and the presence of TCA in alcohol. Preliminary recordings of EEG responses to TCA in 100% Ethanol versus 100% Ethanol were conducted on 12 subjects (7 males, 5 females; mean age 24.5 years). TCA was delivered into the face mask synchronous with inspiration at a concentration of 40 ppb (subjected to a 1:25 dilution within the delivery mask).

Significant differences were evident in both time series (amplitude and latency) and frequency data. Amplitude changes at frontal and temporal electrode sites were significantly different (for TCA *vs* Ethanol) in the 300–340ms, 420-460ms and 520–560ms post-stimulus delivery (see Figure 1.1), with TCA responses more positive than Ethanol responses at both left and right hemisphere locations, although the strongest differences were evident in the right hemisphere. These periods are associated with the basic sensory response, prior to cognitive awareness and decision making responses.

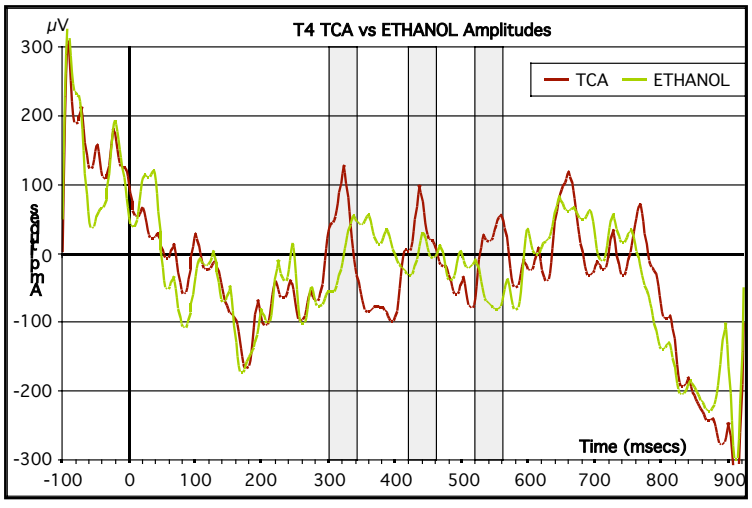


Figure 1.1: Right temporal (T4) amplitude responses to TCA and Ethanol over a 1s period (100ms pre-stimulus, 900 ms post-stimulus). The three time periods subjected to statistical analysis are highlighted, showing the significantly greater amplitudes for TCA in comparison to Ethanol.

Significant differences were observed between TCA and Ethanol frequency responses in the right temporoparietal region for the 4-8 Hz and 12-16 Hz bands (see Figure 1.2), and in particular at the right temporal (T4) site for the 4-8 Hz response. There was a [non-significant] trend for an increase in relative % power in the 8-12 Hz (alpha) band evident across all electrodes except in the right frontal region (RF, F4, F8). These preliminary results indicated significant differences in brain electrical activity associated with the presence of TCA in alcohol, even when the odour was undetected.

Anecdotal evidence of apparent TCA thresholds in this study appeared to conflict with the sensitivities reported in the wine industry literature. TCA recognition thresholds have been reported to be as low as 6 ppt (ETS Laboratories, Cork Quality Council, USA). The estimate of TCA concentrations delivered in the pilot study was in the range of 10-20 ppt, varying with respiratory cycles, at which levels the participants were able to detect an odour but were unable to recognise or label the odour (detection threshold - a more sensitive threshold, rather than recognition threshold which is typically higher or less sensitive than detection threshold). In addition, analysis of the subjective reports and odour descriptors provided during the recording trials suggest that the presence of TCA may have enhanced the perception of alcohol in the odour samples.

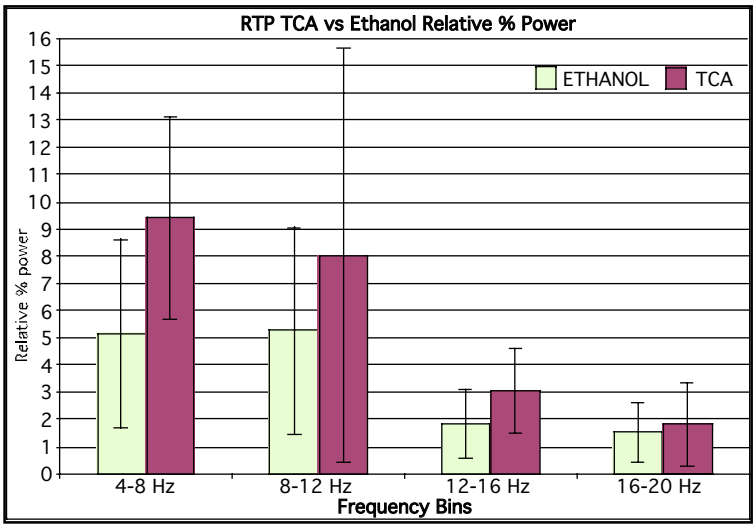


Figure 1.2: Right temporoparietal (RTP) grouped electrode relative % frequency responses to TCA and Ethanol for 4 frequency ranges. There was a significant difference in the relative % power in both the 4-8 Hz and 12-16 Hz bands.

This physiological evidence of changes induced by TCA independent of subjective awareness supported the need for further research to determine the consistency of these responses across a larger subject group, and correlation with measured sensitivity and subjective reports of responses to the odour. The alcohol and flavour interaction with TCA and the variability of responses in consumers is of great interest for future research, strongly supported by wine and cork producers.

1.2 Project Aim and Output

Cork taint in wine is a serious problem exacerbated by the difficulty of its assessment. Research by the Australian Wine Industry has suggested that the incidence of cork taint in Australian wines is 2 - 5 % (Pollnitz *et al*., 1996). It is recognised that cork taint adversely affects the sensory properties of wine, but individual responses to tainted wines vary to such a degree that even experienced tasters may have conflicting opinions about the tainting of a wine. Variations in sensitivity to TCA will result in only portions of the population even being aware of its presence, and variations in concentrations of TCA will have different manifestations. Even when not consciously detected, TCA is reported to suppress the aroma and flavour of wine (Butzke *et al*., 1998). It is also reported that we quickly become adapted to the smell of TCA, which in turn affects the subjective assessment of its presence or effect on a wine. Cork producers are continuing to address the problem of cork taint in an attempt to develop new cork manufacturing techniques to reduce the incidence of taint detected in corks supplied to the wine industry. This may result in changes to the degree to which TCA is present in corks, but is not contributing to a greater understanding of the degree to which consumers perceive and respond to TCA.

Consultation with the Industry Reference Group in planning this project revealed significant interest in preliminary studies to determine consumer responses to taints, and in particular to 4-ethyl guaiacol, 4-ethyl phenol, and eucalyptus which were reported by the Group to be difficult taints with varying repercussions in the wine making process.

This project proposed to investigate differences in responses to taints – and specifically to TCA. In addition and in response to the Industry Reference Group's concerns, preliminary sensory studies were also to be undertaken investigating consumer responses to additional taints and flavours: 4-ethyl guaiacol, 4-ethyl phenol, and eucalyptus.

Olfactory threshold responses to the taints were assessed using sensory and EEG responses to different concentrations of TCA. Additional studies were therefore incorporated to investigate preliminary sensory responses to these taints at different concentrations, dosed in wines. Additional sensory studies were also to investigate odour perceptual responses associated with the combinatorial effect of different concentrations of 4-ethyl guaiacol and 4-ethyl phenol dosed in wines.

The **general aim** of the project was to explore the potential of sensory, chemical and electrophysiological techniques to deliver quantitative and qualitative information about the consistency of taint detection responses across the population.

The **specific aims** of the project were:

- To measure TCA odour thresholds and perceptual responses in a population of untrained wine consumers and non-wine drinkers.
- To determine electrophysiological differences in response to the flavours of wines containing different concentrations of TCA, correlated with reported responses to the wine odours, to determine the degree of sub-conscious and conscious responses to TCA and wine flavours.
- To determine threshold levels and perceptual responses to 4-ethyl guaiacol, 4-ethyl phenol and eucalyptus, and the combinatorial effect of these components in odour trials with untrained consumers.

This comprehensive, pre-competitive, multi-disciplinary research approach was designed to provide the Australian wine industry with a greater understanding of the interaction between chemical components and perceptual responses to TCA in consumers. Such an approach has the potential to provide a greater understanding of the consistency of taint detection responses across the population, and ultimately the extent to which wines responses may be affected by TCA, particularly for the majority who are not experts in understanding wine flavour.

2. PROJECT AIMS AND PERFORMANCE TARGETS

The planned outputs and performance targets as they appeared in the original approved application are outlined below in Targets 1 – 7.

Outputs	Performance Targets
1. Screen participating subjects for olfactory performance, and assess individual TCA detection threshold levels.	1. A group of 40 subjects will be established and tested in year 1, with an additional 40 subjects incorporated into the study in each of Years 2 & 3.
2. Assess TCA thresholds using sensory techniques (odours), incorporating doses of TCA different wine varieties.	2. Conduct sensory panel odour sessions for all subjects (3 sessions per subject per year), using different wines with different concentrations of TCA.
3. Assessment of electrophysiological responses to TCA odour.	3. Repeated EEG recording sessions to sub-, near- and supra-threshold concentrations of TCA as odour and taste stimuli. (2 sessions per subject per year).
4. Correlation of sensory and electrophysiological responses to TCA	4. Statistical analysis and comparison of EEG and rating responses to TCA. Ongoing in years 1 – 3.
5. Assess wine taint thresholds and perceptual responses using sensory techniques (odours), for taints dosed in wines.	5. Conduct sensory panel odour sessions (3 sessions per subject per year), using 4-ethyl guaiacol, 4-ethyl phenol, eucalyptus dosed in wines. 20 subjects in each Year 1 and 2.
6. Assess combinatorial effect of taints in wines using sensory techniques (odours).	6. Conduct sensory panel odour sessions (3 sessions per subject per year), using 4-ethyl guaiacol, 4-ethyl phenol in different concentrations and combinations dosed in wines. 20 subjects in each Year 1 and 2.
7. Dissemination of information to industry.	7. Reporting of general project results to industry at conferences and/or industry seminars in Years 1 and 2, and through journal publications in Year 3.

3. BASIC METHODOLOGY

The basic methodological approaches used in different parts of the study are outlined in the following section. Section 4 will provide further details of specific methodological factors and then report the results and discussions associated with each part of the study.

3.1 Code of Conduct and Ethics Assurance

All research was conducted within the parameters and procedures established by the Swinburne University of Technology's policies on Code of Conduct, Human Experimental Ethics Clearances and Intellectual Property.

3.2 Preliminary Screening Questionnaire

Prior to participation, all volunteers completed a basic screening questionnaire, designed to screen for general health issues that may impact on olfactory performance and participation in this study. Questions included age, gender, handedness, upper respiratory acute or chronic conditions, self-assessment of smell and taste ability, information about known allergies or irritations etc. All information was recorded under code numbers. No information was found that required any participants to be excluded from the current study.

3.3 Olfactory Performance Testing

All participants are screened for general olfactory ability using the *Sniffin' Sticks* Test of Olfactory Performance (Burghart, Wedel, Germany) that tests threshold, identification and discrimination abilities. This provides a screening system as a measure of an individual's olfactory performance for comparison and consideration when interpreting sensory and EEG performance.

This is a portable, commercially available test used internationally to assess olfactory performance, particularly in clinical and research settings (Kobal *et al*., 2000). This test of olfactory performance consists of a basic screening test and an advanced test. The screening test includes a basic smell identification task. The advanced test is composed of three sub-tests: the threshold test for the odorant *n-butanol* (the "just noticeable difference" detection threshold; a forced choice triangle staircase test), discrimination test which assesses the ability to discriminate between two odorants at one time (a forced choice triangle test), and the odour identification test which assesses identification ability using a choice of four descriptors for each odour stimulus (Hummel *et al*., 1997; see Figure 3.1). The scores from the Threshold, Discrimination and Identification tests are summed to form the TDI score, with a total maximum score out of 48.



Figure 3.1. Subject performing the *Sniffin' Sticks* threshold test.

In addition to using these procedures as screening tests to ensure participants have a sense of smell within a normal range, we are also interested in determining olfactory performance differences within the consumer population in general, and therefore use these

performance tests across a range of projects to assess olfactory performance of the community and differences in ability associated with demographic differences: age (ranging from children to the elderly), ethnic background (with Australia's vast range of ethnic groups, including large European and Asian communities), gender etc. Results from these studies have been published in a collaborative project with Griffith University to establish a database of the olfactory performance of Australia's population (Mackay-Sim *et al*., 2004; Mackay-Sim *et al*., 2006).

3.4 Sensory Panel Odour Tests

Sensory panel responses were used to measure the odour thresholds and perceptual responses to TCA, 4-ethyl guaiacol and 4-ethyl phenol in the sample population of untrained wine consumers. Rating scales in response to TCA stimuli were also used for comparison with the objective EEG responses. The threshold and perceptual response processes are described in Section 3.5.

During the EEG recordings, each participant completed a psychometric rating of the odour (hedonic or emotional response and perceived strength) after each odour delivery trial. These ratings were based on simple 100 mm Likert scales: *Liking* (strong like = 0; strong dislike = 100) and *Strength* (very weak = 0; very strong = 100). Subjects also provided descriptions of the odour and any associated responses or reactions. Liking ratings are then grouped according to these rating levels: *Like* (< 40), *Neutral* (40 – 60) and *Dislike* (> 60).

3.5 Sensory threshold and perceptual response procedure

3.5.1 Wine taint threshold testing

Threshold tests for TCA, 4-ethyl guaiacol and 4-ethyl phenol were designed, based on the Sniffin' Sticks pen-delivery procedure described in section 3.2. Concentrations of each odour solution were developed for presentation in felt-tipped pen dispensers, with 7 concentration steps for TCA and 11 concentration steps for both 4-ethyl guaiacol and 4-ethyl phenol. The cap of the pen is removed to release the odour. The participant smells the released odour while blindfolded – and is required to identify which of three pens (target plus two blanks, presented in random order) contains the odour. See Table 3.1 for the concentration steps for each taint threshold test.

The threshold testing procedure used a forced choice ascending-descending triangle test, commonly used in olfactory threshold testing, with the reversal of the staircase triggered by the correct/incorrect identification of the target pen. The procedure continues for a total of 7 reversals of the staircase method, with the threshold calculated as the average of the final four reversal points.



Figure 3.2. Threshold pen-delivered test kits for TCA, 4-ethyl guaiacol and 4-ethyl phenol.

Any comments made by participants as they were exposed to the odour pens and made their decisions were recorded, to assist in identifying qualitative responses to the detected odours.

Table 3.1: Taint threshold testing: Concentration steps of wine taint delivered using the pen-devices.

	TCA concentration steps (ppb)	4-ethyl guaiacol concentration steps (ppm)	4-ethyl phenol concentration steps (ppm)
1	200	1000	1000
2	100	200	200
3	40	100	100
4	20	40	40
5	10	20	20
6	4	8	8
7	0.4	4	4
8		0.4	0.4
9		0.08	0.08
10		0.016	0.016
11		0.0032	0.0032

3.5.2 Wine taint discrimination and perception ratings

A forced choice ascending-descending staircase method was also adopted to assess discrimination ability for taints presented in wine or ethanol.

For this assessment, 36 wine glasses were arranged into 3 sets of 12 glasses, labelled as glass samples 1 – 8 and blank pairs (B1, B1, B2, B2). The glasses in each set were filled with 50 ml of red wine (commercially available shiraz cabernet), white wine (commercially available colombard chardonnay) or diluted ethanol (at a dilution of 100 ml ethanol in 1 litre of distilled water). Each glass was then covered with a petri dish. All glasses were located in a fume hood, and assessment conducted adjacent to the fume hood throughout the proceedings. Participants were blindfolded and seated adjacent to the fume hood for the testing procedure.

Quantities of wine taint at increasing concentrations were added to the 50 ml of solution in each of the wine glasses labelled 1 – 8 (the blank glasses remained with only 50 ml of red or white wine or ethanol). Table 3.2 provides details of the concentration of taint in 50 ml of alcohol for each of the sets of glasses.

Starting with the weakest concentration (glass 1), the target glass and 2 blank glasses were presented in random order to the participant by briefly removing the protective petri-dish while the participant sniffed the odour arising from the glass. The participant was then required to identify the one that was different, and state why they thought it was different. This process was repeated until the participant correctly identified the target glass 3 times in succession, or until the strongest solution was tested (glass 8). After a brief recovery period, the process was repeated, beginning with a concentration step just below that where the participant first detected the taint. The above process was completed for all 3 sets of glasses (red wine, white wine, ethanol) for the same taint.

Participants then completed a red/white/ethanol comparison trial, where they were required to discriminate between the blank (untainted) samples of each of these solutions. One “target” solution and two contrast solutions were presented in random order, to provide the following combinations: ethanol/ethanol/white, ethanol/ethanol/red, white/white/red, white/white/ethanol, red/red/ethanol, red/red/white. The order of presentation of these combinations of solutions was randomly varied across participants. The participant was required to identify the one that was different, to provide a description of that solution and also a description of the remaining 2 samples.

Table 3.2: Wine taint discrimination and perception assessment: Concentrations of wine taint in 50 ml of alcohol for each of the red wine, white wine and ethanol test sets.

	TCA concentration in 50 ml of alcohol ($ng.l^{-1}$ or ppt)	4-ethyl guaiacol concentration in 50 ml of alcohol ($ng.l^{-1}$ or ppt)	4-ethyl phenol concentration in 50 ml of alcohol ($ng.l^{-1}$ or ppt)
1	0.4	20	20
2	1.2	40	40
3	2	80	80
4	4	120	120
5	10	200	200
6	20	400	400
7	40	800	800
8	80	1600	1600



Figure 3.3. Experimenter adding taint to wine glasses in the fume hood as part of the wine taint discrimination and perception assessment task.



Figure 3.4. Blind-folded participant sniffing the tainted wine odour during the wine taint discrimination and perception assessment task.

3.6 Odour delivery system: the Continuous Respiration Olfactometer (CRO)

A 2-channel odour delivery system with removable syringes used in the studies was developed at Swinburne University of Technology. The continuous respiration olfactometer (CRO) system was devised for use with brain electrical recording techniques to monitor changes in brain electrical activity associated with odour responses induced during natural respiration (Owen, 1998). The odour syringe is filled with 50 ml of the odorous gas sample, with a similar volume of a room air (or a control odour) in the control air syringe. The CRO delivers odour during normal respiration by closely monitoring the subject's natural respiratory cycle using a pneumotachograph mounted on a facemask with a two-way non-rebreathing valve. The computer-controlled delivery syringes use a pseudo-random sequence (usually at a ratio of 3:1 control:odour) to deliver odour or air/control stimuli, timed to the subject's inspiration, taking approximately 500 ms to deliver 1 ml of air/control or odour through ports in the facemask. This synchronous method of controlling delivery of the odorant permits the isolation of the periods of electrical activity associated with the odour response for comparison with electrical activity associated with breathing specific odour-free air or a specific control odour. The apparatus is described in detail elsewhere (Owen *et al.,* 1997; Patterson *et al.,* 1998; Owen *et al.,* 1999, 2002b).

Use of the CRO system, delivering known quantities of odour synchronous with the inspiratory phase of the natural breathing cycle, avoids the problems associated with sniff or blast techniques used elsewhere and provides the timing information required for analysis of the EEG response.

3.7 Electrophysiological Recordings

Brain electrical activity is recorded with a 64-channel ElectroGeodesic Inc (EGI) EEG sensor system (saline electrodes), referenced at Cz channel, with a sample rate of 500 samples/sec, and high and low-pass filter settings of 200 Hz and 0.1 Hz respectively. Stimulus presentation records are achieved by triggering the EGI through its interface port between the acquisition and task computers. The source of the trigger is a pair of specific outputs indicating syringe drive from the CRO. In this way the EGI-EEG record is marked when odour or air are delivered. A 2-button response box is used when subject responses during the EEG trial are required, marking the EEG file with event codes for odour detection.



Figure 3.5. Subject wearing the 64-channel EEG net and holding in place the CRO odour delivery and respiratory monitoring mask.

The use of EEG in monitoring olfactory responses requires strict control of odour delivery, to allow analysis of changes in brain activity associated with the odour sensation. The system used in this project combines an odour delivery technique timed with respiration (see description in section 3.5) with the above EEG system (see Figure 3.5). Using this system, differences caused by the odour in comparison to air without odour are recorded and analysed. Analysis involves correlating the odour nature with the psychometric rating responses, obtained with ratings carried out between trials at the time of recording. Using this system, three-dimensional reconstructions of brain activity can be mapped onto digitised representations of an individual subject's head for more complete visualisation of the regional brain responses to stimuli. This provides a method of utilising different techniques to better quantify the electrophysiological effects of odours, reducing the problem of the subjectivity of responses found in much of the research into hedonic odour responses.

3.8 Experimental paradigm summary

PARTICIPANT SCREENING MEASURES

- Participants completed the screening questionnaire and Sniffin' Sticks Olfactory Performance Test prior to undertaking all other procedures. No participants were identified with olfactory difficulties as a result of this olfactory screening process.

TAINT THRESHOLD AND DISCRIMINATION TEST INFO

- Participants attended Threshold discrimination test sessions for each taint.
- The threshold test was conducted using the pen-delivery system with a forced choice ascending-descending staircase method (see Section 3.5).
- The wine glass discrimination and perception task was then undertaken for the target odour, as described in Section 3.5.
- The red/white/ethanol comparison trial was then undertaken using the blank (untainted) solutions.
- Participants attended separate sessions for testing of different wine taints.

BRAIN ACTIVITY RECORDINGS:

- Brain recording sessions are conducted for 5-minute recording trials, with up to 6 trials conducted per session.
- Equivalent volumes of a control sample or a target odour are delivered synchronous with breathing. The control deliveries occur in a pseudo-random sequence so the subject is not able to predict the presence of the target.
- The number of control deliveries exceed the targets so that no target stimuli are presented consecutively (to avoid any potential adaptation effects). A control:target ratio of 3:1 is commonly used.
- EEG is recorded from 64 channels using a light electrode assembly (EGI Sensor Net: saline electrodes held in a geodesic frame of nylon lines).
- The EEG record is marked for stimulus delivery (target, control) and/or subject responses.
- Psychometric ratings to the odour (liking and strength/intensity) are completed following each trial. Responses are averaged across trials to determine mean Liking and Strength responses to each odour concentration for each participant.
- Changes in EEG responses are analysed (frequency and topography) to determine differences associated with:
    - TCA concentration levels.
    - Perceptual responses to TCA concentrations.

3.9 EEG analytical techniques

Initial analysis involves the extraction of the control EEG signals and the odour-related EEG signals from the continuous stream of 64 channels. After averaging the control and odour signals separately using the time of delivery event markers, these data can be processed by subtraction of the control from the odour signals leaving just the effects of the odour. As the individual is not aware of delivery sequence they treat every breath as novel. The perceptive subjects will realise that a long sequence of no smell must increase the likelihood of a smell being presented. To overcome this likely "expectancy" effect, we use low concentrations of odour which are around the threshold for the subject. In other words, the smells are rarely very obvious to the participant.

Subsequent analysis involves traditional Fourier spectral analysis, examination of the averaged EEG time-series and frequency analyses, the individual electrodes, and the animated topographic maps. Subjects are asked to complete a simple questionnaire which, using scales, determines their hedonic and preference response to the odour. These are then correlated with the electrophysiological data. A final comparison is made with the subject responses acquired during the delivery run. All these techniques are performed regularly in our laboratory.

3.10 EEG frequency responses

Normal EEG shows activity in the range of 1-30 Hz, with amplitudes in the range of 20-100 ≤V (Westbrook, 2000). Traditionally, EEG frequency has been separated into frequency bands: Delta from 0.1 to 4 Hz, Theta from 4 to 8 Hz, Alpha from 8 to 12 Hz and Beta from 13 Hz up. The EEG spectrum can be obtained when processed by Fourier analysis which separates the various rhythms and performs frequency estimations to quantify the amount of activity within a frequency band. Alpha and beta wave bands are commonly interpreted to be associated with changes in activation or arousal. Alpha and theta waves are typical of relaxed wakefulness and drowsiness - inversely related to cortical activity (Coan *et al*., 2001). Beta bands consist of fast waves of low amplitude and are more prominent during intense mental activity (alert or anxious). It is generally regarded as a normal rhythm, the dominant rhythm when eyes are open, and is the typical EEG of the awake adult.

Suppression of alpha activity and an increase in beta has been equated to an increase in activation in response to stimulation (Westbrook, 2000). Decreases in alpha are common in EEG research, and are taken to be suggestive of cognitive activity, but are more commonly associated with cortical arousal due to sensory stimulation (Lorig *et al*., 1990). This has been demonstrated in response to visual and auditory stimulation (Brauchli *et al.*, 1995) and has more recently been reported by EEG studies in response to olfactory stimulation, particularly in frontal brain regions (Lorig *et al*., 1990, 1991; Klemm *et al*., 1992; Kobal *et al*., 1992; Schwartz *et al*., 1992; Van Toller *et al.*, 1993; Lorig, 1994; Brauchli *et al.*, 1995; Martin, 1998; Kline *et al*., 2000; Kobal & Kettenmann, 2000). Stimulation with an unpleasant odour may lead to stronger cortical deactivation than stimulation with a pleasant odour, as also suggested by previous research (Ehrlichman & Bastone, 1992; Miltner *et al.*, 1994). The trend for reduced left frontal activation in response to odours in the 4-8Hz or theta range has been reported, with findings of reduced theta activity in response to pleasant food odours such as chocolate (synthetic and real), spearmint (synthetic) and spiced apple (Lorig & Schwartz, 1988; Lorig *et al.,* 1990; Martin, 1998). It has also been proposed that the olfactory effects on theta may be specific to different odours possessing similar psychological properties (Martin, 1998). Recent studies have demonstrated changes in alpha and theta activity in the left frontal region associated with liking responses to odour (Owen & Patterson, 2002; Patterson *et al*., 2004) that may reflect the shift in attention associated with liking an odour.

3.11 Chemical Analysis Techniques

Analysis of flavours is conducted using Solid Phase Microextraction (SPME), gas chromatography mass spectrometry (GC-MS) and gas chromatography olfactometry (GC-O). These techniques are used to optimise aroma profiling, using chemical ionisation, and MS–MS to identify key flavour components.

Chemical Analysis Gas Chromatograph/Mass Spectrometer

Gas chromatography: Varian Chrompack 3800 with 1079 temperature programmable split/splitless injector, cryogenic oven cooling to –10˚C, Electronic Flow Control, flame ionisation detector (FID) and a 'Merlin Microseal' septum-less injector-port.

Mass spectrometry: Varian Saturn ion trap mass spectrometer, electronic and methanol chemical ionisation and ms/ms analysis.

Olfactory Analysis

GC-FID-Olfactometry: 1:10 FID:olfactory-port split. GC-MS is conducted separately. Odour compounds are matched up with mass spectra using a retention mix of straight chain alkanes. Almost 100% of compounds pass through the olfactory-port, with no loss in intensity.

GC-MS-Olfactometry with the ODO II (SGE Australia): 1:1 split MS:olfactory-port. Both MS and olfactory analysis are conducted simultaneously, so odours can be identified in real time. This is less sensitive than the above method.

Solid Phase Microextraction Analysis of Aroma

Solid Phase Microextraction (SPME) of aroma volatiles. Most olfactometry and general volatile analyses in the laboratory have been performed using SPME. In most cases the most suitable fibre is the 75 μm PDMS-Carboxen phase. Techniques for the olfactory profiling of wine, beer and cheese have been successfully developed. A polar WAX column is invariably used for chromatographic separation of odour volatile compounds.

4. RESULTS

This section will outline the two studies undertaken as part of this project. An additional preliminary section is included describing chemical analysis of wines undertaken in the early stages of this project. Each section will outline the methods specific to the study, followed by the results and a brief discussion of these results. These sections will then be followed by a general discussion, bringing together the results of all these studies and providing a more general discussion relating to the wine industry's interests.

4.1 Preliminary analysis of wine aroma and 2,4,6-trichloroanisole *via* SPME-GC-MS-MS olfactometry

As previously stated, the laboratory has developed techniques for the analysis and characterisation of important wine aroma components using SPME and a combination of gas chromatography (GC), mass spectrometry (MS) and olfactometry (O). The instrumentation currently used includes a Varian Chrompack 3800 gas chromatograph, Varian Saturn 2000 ion trap mass spectrometer with MS-MS and chemical ionisation (CI) capability. Olfactometry (ODO-II, SGE Australia) is routinely performed simultaneously with the mass spectrometer attached, allowing accurate matches of odours and mass spectra. The ratio of GC effluent flow was approximately 4: 1, mass spectrometer: sniffing port line. All wine and component analytical protocols have been developed on a WAX-type column (30 m, 0.32 i.d, 0.25 μm film).

Figures 4.1.1a - 4.1.1d shows a typical chemical/olfactory profile of a Cabernet Sauvignon wine. More than 80 peaks could be identified on the basis of electron impact and methanol chemical ionisation mass spectra, many trace peaks were not readily identifiable. At least 28 compounds could be perceived at the olfactory port, most identifiable from mass spectra and odour quality. Most of the odour active compounds have been described previously in the literature.

A number of compounds potentially connected to wine taints were picked up by the fibres: 2,4,6-trichloroanisole (TCA), and 4-ethyl guaiacol and 4-ethyl phenol, the latter compounds being often associated with "*Brettanomyces* flavour" in wines. In addition a number of other strong smelling compounds such as methoxy phenol, and 4-methyl guaiacol were measured. Some trace sulphur compounds, such as methanethiol, methional, dimethyl trisulphide and methionol also potentially contributed a savoury dimension to the wine aroma.

A number of analytical approaches to the analysis of TCA in wine and other matrices have been refined. TCA has been successfully detected in wine, after 2 hours or overnight sampling, with Carboxen 75 μm SPME fibres via full scan electron-impact mass spectrometry and simultaneously via olfactometry at the sniffing port. In general, however, the most sensitive and most satisfactory methodology utilises an MS-MS approach, with TCA parent ion isolation and collision induced dissociation (adapted from the approach reported by Moneti and Pieraccini at the University of Florence, Italy).

Figure 4.1.2 shows the SPME profile of a Merlot wine (Cask-wine), which was subjectively assessed to have a mild musty taint by a number of laboratory staff (who are familiar with the qualities of TCA and can readily identify it). Corresponding with the musty TCA odour detected at the sniffing port, there was an EI mass spectrum, readily identifiable as 2,4,6-trichloro-anisole in the NIST 98 spectral database (Figure 4.1.3).

TCA was also analysed using an MS-MS approach. A parent ion mass of 211.9 and a window of 5 amu in non-resonant mode was used, with an excitation storage level of 80.0 v and excitation amplitude of 70 v. A 10 ml aliquot of 10% ethanol with 1 g NaCL and a stir bar

was spiked with TCA to give a final volume of 5-100 ng. The solutions were stirred at ambient temperature and a PDMS (100 μm) was incubated for 15 minutes above the solution and desorbed onto the column. Figure 4.1.4 shows typical peak area counts for the TCA samples from 5 ng.l^{-1} to 100 ng.l^{-1}.

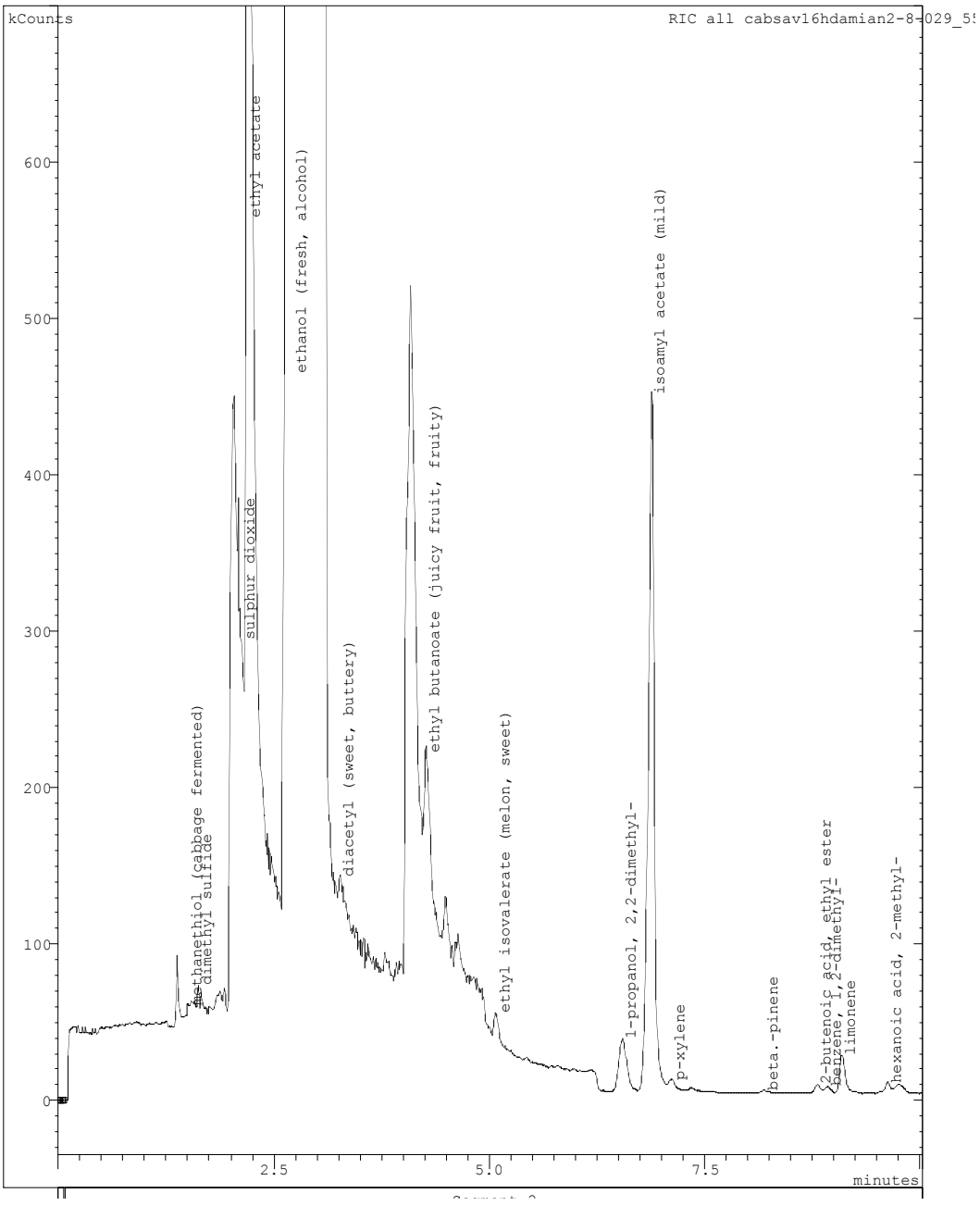


Figure 4.1.1a. Chemical and odour profile of Cabernet Sauvignon wine. SPME sampling 16 hours with Carboxen fibre (0-10 minutes)

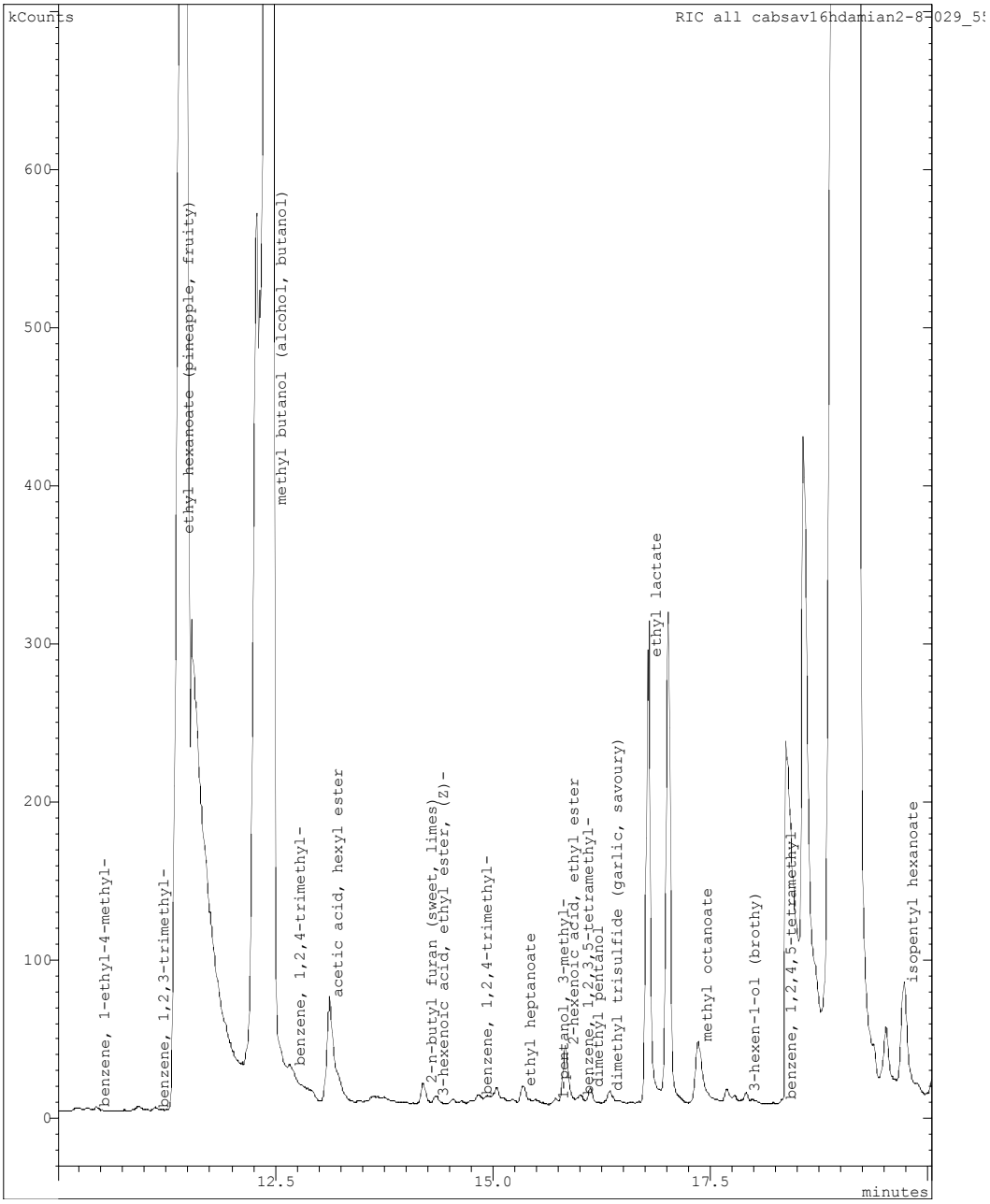


Figure 4.1.1b. Chemical and odour profile of Cabernet Sauvignon wine. SPME sampling 16 hours with Carboxen fibre (0-20 minutes)

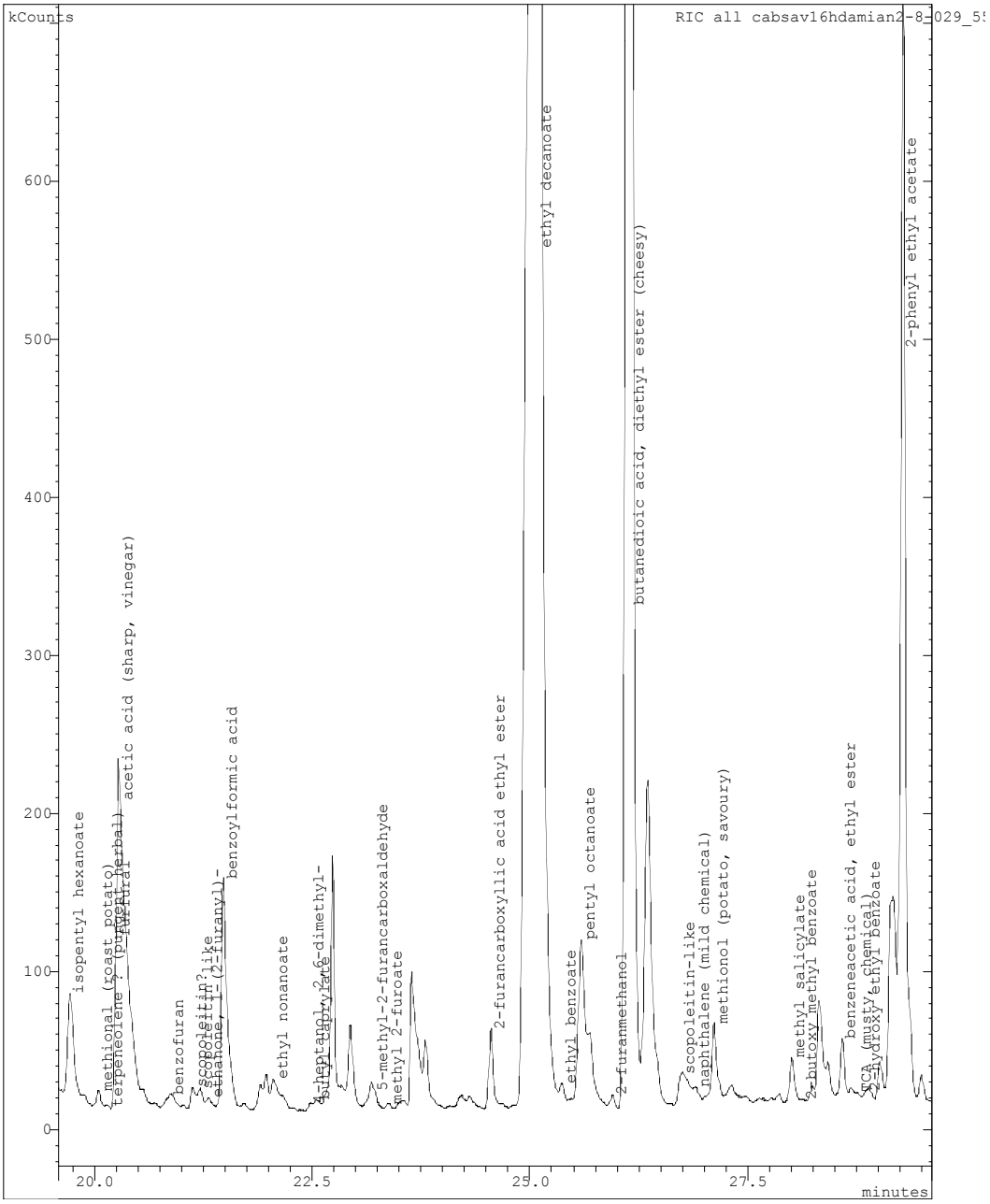


Figure 4.1.1c. Chemical and odour profile of Cabernet Sauvignon wine. SPME sampling 16 hours with Carboxen fibre (20-30 minutes)

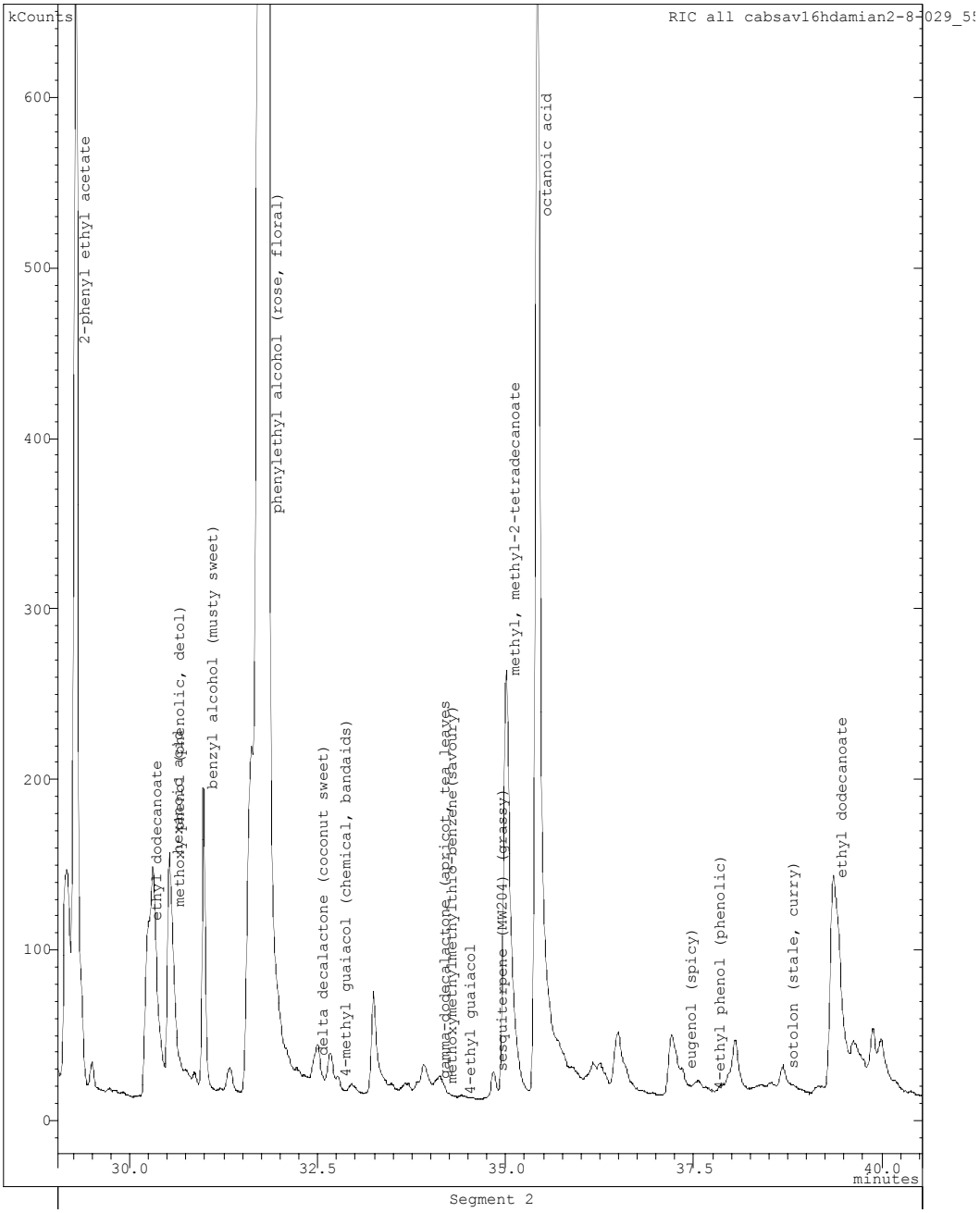


Figure 4.1.1d. Chemical and odour profile of Cabernet Sauvignon wine. SPME sampling 16 hours with Carboxen fibre (30-40 minutes).

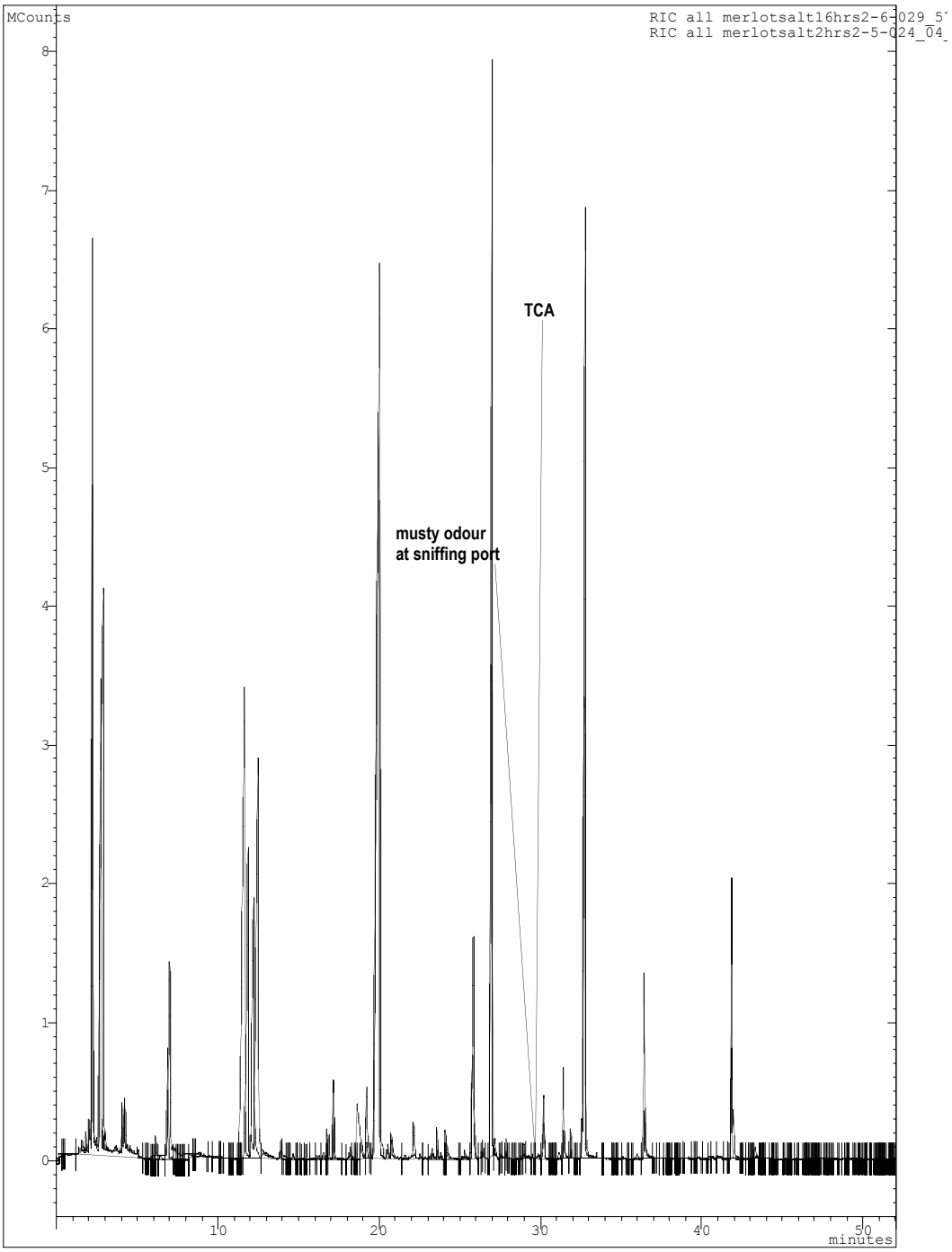


Figure 4.1.2: Total ion chromatogram of SPME sampled headspace (Merlot) after 16 and 2 hours (superimposed), showing elution time of TCA.

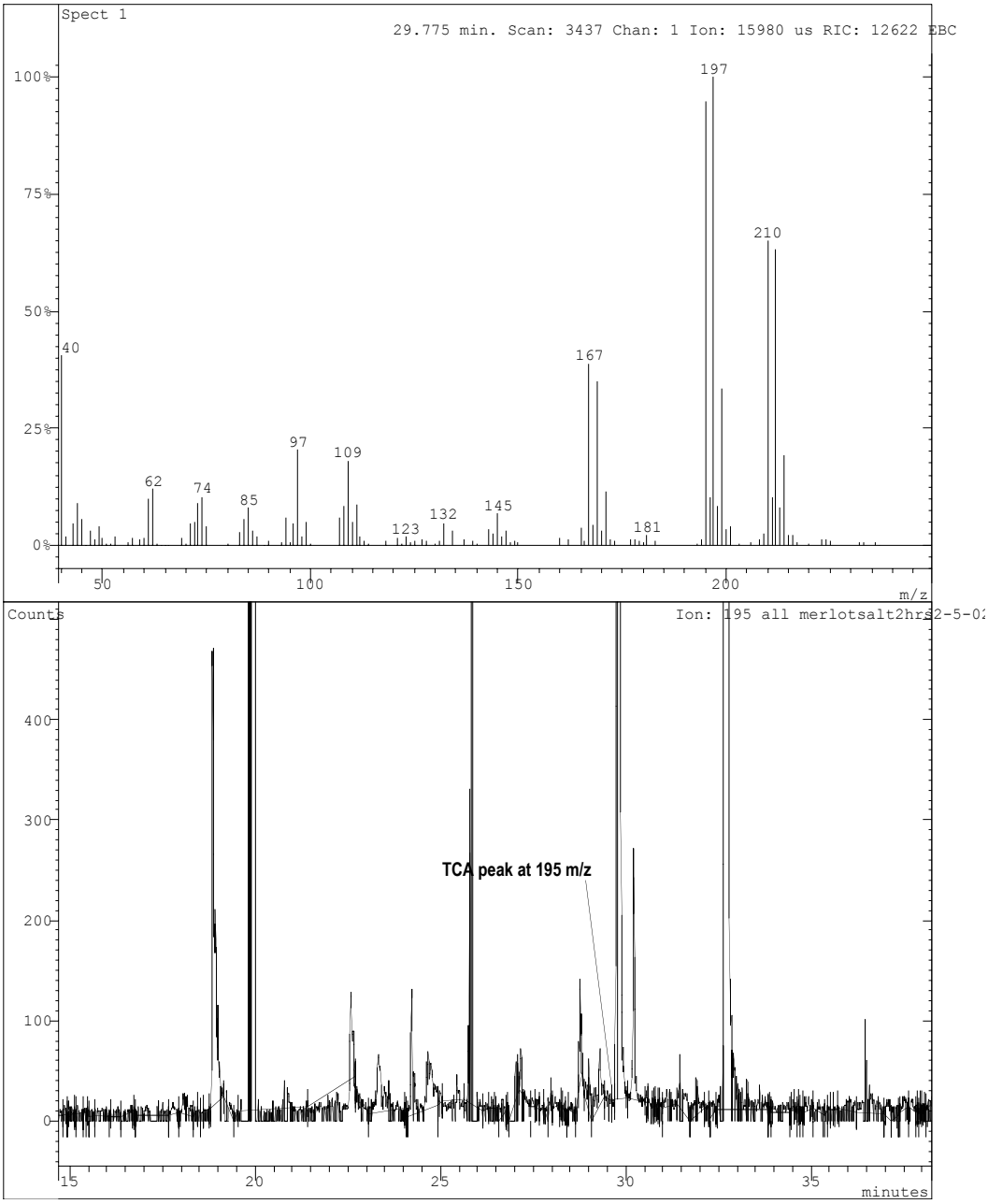


Figure 4.1.3: Plot of ion current at m/z 195 and full scan electron impact mass spectrum of TCA.

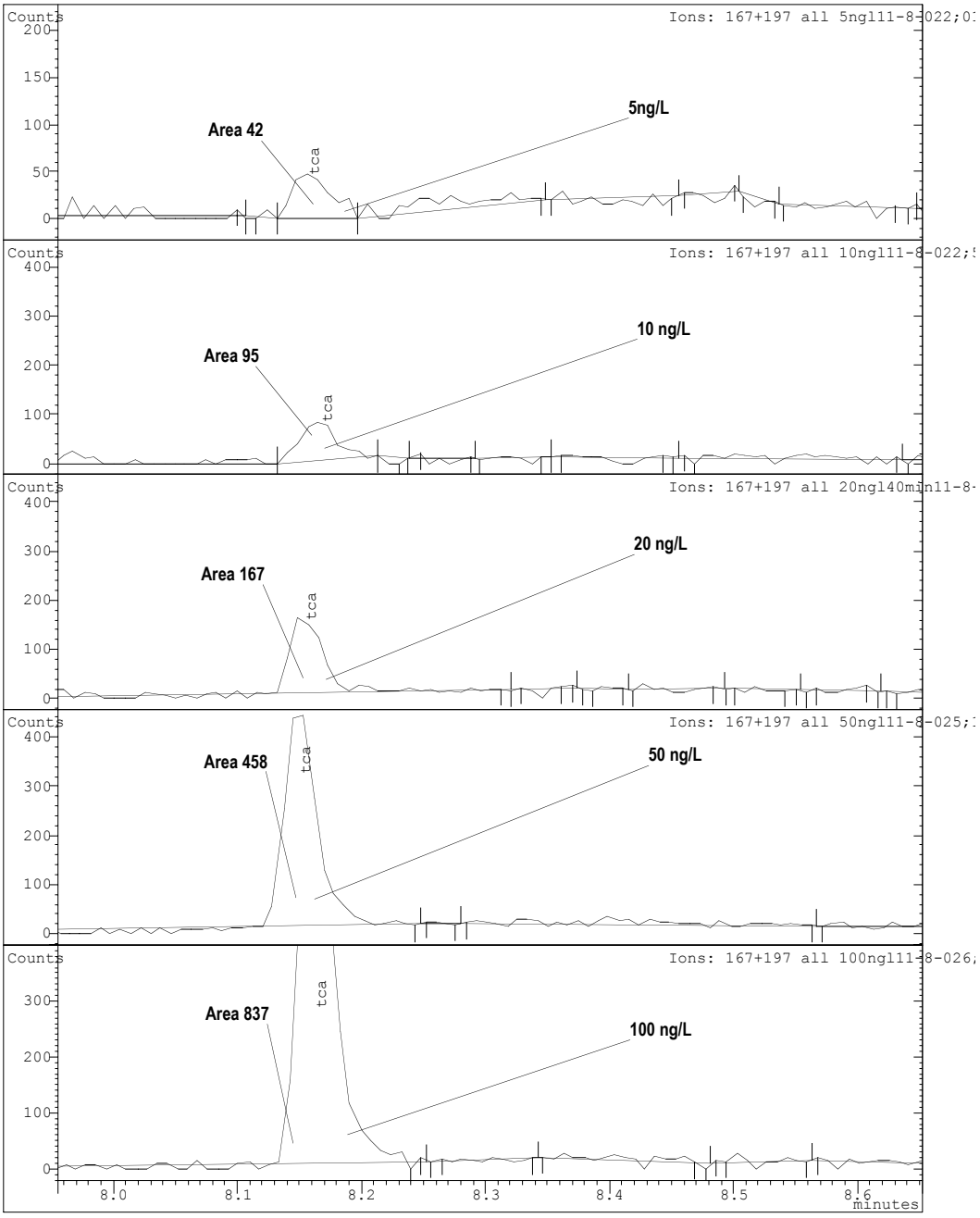


Figure 4.1.4.Total ion chromatograms (plot of m/z 167+197) showing 5-100 ng.l^{-1} TCA measured in headspace of 10% ethanol solution with olfactory port attached.

4.1.1 Preliminary Chemical Analysis Conclusion

The purposes behind these chemical analysis studies were to:

- Provide a method for quality assurance of the sources of TCA (several batches were of very inferior quality having much less TCA than quoted).
- Gain experience in processing and handling TCA, including use of SPME fibres for sampling.
- Evaluate techniques for reliable preparation of known TCA concentrations.
- Determine the reliability of our analytical techniques for the assessment of TCA.
- Ensure there were suitable techniques for determining the concentration of TCA in the delivery syringes used for the EEG study.
- Quality assurance of all of the samples containing TCA to ensure that all batches were prepared to maintain required concentrations for sensory testing.

The processes and techniques we developed in-house are, or course, available in other centres where such analyses are very common. Unfortunately, access to these was limited by geography and the need for us to be able to quickly determine if a process underdevelopment was heading in the right direction. The critical nature of the actual amount of TCA being used as a stimulus required that we could reliably and confidently assess how much TCA was present. By using the GC-O port on the GC-MS we were also in a position to assess how much TCA was needed in the analysis to ensure that its character (mustiness) was perceived. This became a subjective confirmation of the TCA in addition to the mass spectrometry detection and identification.

It was also necessary for us to be able to determine if the wine to be used as the carrier for dosing with TCA and other taints was free of these contaminants. The development of analytical techniques for TCA was a primary requirement to QA the inexpensive bulk wine used in large quantities for the assessment of the preference for the addition of TCA.

Another important consideration, mentioned above, was the assessment of the delivery of the TCA in ethanol for the EEG responses. While obtaining headspace gas may be a suitable method for delivery of known odour samples, with the TCA we experienced some difficulties with the nature of its interactions with our gas-tight (Teflon plunger) syringes. We found that the TCA was sticking to the syringes in certain circumstances and there was a reduction in the TCA available for delivery in the gas-phase. If the efforts on QA of the whole process had been less rigorous, the loss of TCA would have been ignored and the levels of exposure would have been even less precise.

The last issue in which the analysis of TCA was vital is the creation of the odour pens. While the concept of the pens had been developed for an earlier project, funded by another research corporation, the special conditions represented by TCA imposed an extensive re-evaluation of the use of the pens as a delivery method for the taint. A large part of this, not including the simple analysis, was the evaluation of the delivery of TCA from the porous tip of the pen. We undertook a large number of measures, over time with different concentrations of TCA of the yield of the taint from the pen tips by obtaining the headspace from the pens which were put into glass vials of known size. After standing for various periods the ‘yield’ of the TCA was measured by using the validated SPME sampling method.

While the project was based on the use of well-characterised taints for which there is a lot of chemical information, the actual processes involved in the study were novel and required considerable effort to ensure that the delivery was reliable and effective. The project was at risk very early on because of a faulty batch of TCA obtained from a commercial source. Our emphasis on independent analysis was able to demonstrate this faulty batch to the suppliers and avoid experiments being based on sub-standard concentrations calculated by weight of material which was not as pure as quoted.

4.2 Threshold testing of 2,4,6-trichloroanisole, 4-ethyl phenol and 4-ethyl guaiacol

Taints or off-flavours can adversely affect the sensory properties of wine, but individual responses to tainted wines are so variable that even experienced tasters may disagree about the tainting of a wine. Individual differences in sensitivity are partly due to physiology – that is, variations in sensitivity or discrimination abilities (Owen & Patterson, 2002). Responses to wine odours are also strongly influenced by the degree and quality of odour experience, and associated emotional and memory responses (Patterson *et al*., 2004). Variations in sensitivity can result in only portions of the population being aware of the presence of a taint. However, even when not consciously detected, wine taints can alter the aroma and flavour of wine.

Industry experts have identified taint or off-flavours which are perceived as detrimental to the wine experience. The phenolic compounds 4-ethyl phenol (4-EP) and 4-ethyl guaiacol (4-EG) have been identified as associated with the "Bretty" off-odour in wine, typically associated with descriptors such as Band-aid, burnt plastic, and wet leather. 2,4,6-trichloroanisole (TCA) is typically associated with negative descriptors such as mouldy, musty, damp wood. The degree to which untrained consumers are able to perceive these qualities and negative associations has rarely been determined. This study investigated differences in consumer responses to wine taint odours.

4.2.1 Method

A panel of 40 university student volunteers (age: M = 21.45 years; range 18 – 32 years; 7 males and 31 females) completed sensory recording trials to establish odour threshold levels and perceptual responses to 2,4,6-trichloroanisole (TCA), 4-ethyl guaiacol (4-EG) and 4-ethyl phenol (4-EP). All participants were untrained in sensory analysis, were aware they were assessing a wine-related odours but were naïve to the identity and "taint" associations of the odours.

For each test odour, the following tests were completed:

- Detection threshold test using forced choice triangle ascending staircase method, developed at Swinburne University of Technology using a pen delivery technique.
- Discrimination threshold forced-choice triangle test for different concentrations of each taint delivered in Ethanol, White wine (commercially available colombard chardonnay), and Red wine (commercially available shiraz cabernet).
- Descriptors were recorded for each discrimination decision.
- The abilities of participants to discriminate between the blank (untainted) samples of ethanol, white wine and red wine were also tested.
- The taint odour samples were delivered to blind-folded participants in 50 ml of liquid (ethanol, white or red wine) using wine glasses with glass covers.

Data were analysed to determine within- and between-subject variations in taint responses (detection threshold, discrimination threshold and descriptor responses) as reported by untrained participants for each of the taints: TCA, 4-EP & 4-EG. Statistical analysis was conducted using SPSS (version 11.0 for Macintosh; Release 11.0.4; SPSS Inc., 2005).

4.2.2 Results

Descriptor responses*:* The untrained participants reported a range of descriptors in response to the different taints delivered in red or white wine or ethanol. The main descriptors reported associated the threshold level responses are summarised in Tables 4.2.1 (a), (b) and (c).

Table 4.2.1: Summary of main descriptors provided during discrimination task for wine taints TCA (a), 4-EP (b) and 4-EG (c) presented in ethanol, white or red wine.

(a) TCA descriptors

TCA odour pen	TCA in white wine	TCA in red wine	TCA in ethanol
Stronger	Stronger alcohol	Stronger alcohol, less wine smell	Stronger
Musty, mouldy	Musty, stale, damp, mouldy	Musty, stale, damp, mouldy	Musty, stale, damp
Chemical	Chemical	Chemical	Chemical, leather
Marker pen, texta, rotten	Off / rotten	Off / rotten	Off
Unpleasant/ negative	Unpleasant/ negative	Unpleasant/ negative	Unpleasant/ negative
Woody	Woody	Woody	Woody
Fruity, citrus	Less sweet/fruit, flat	Less sweet/fruity, flat	Sweeter, bitter

(b) 4-EP descriptors

4-EP odour pen	4-EP in white wine	4-EP in red wine	4-EP in ethanol
Stronger, pungent	Stronger	Stronger, sharper	Stronger, pungent
Woody, musky	Musky	Musky	Rubber
Plastic	Mushroom	Plastic	Plastic
Unpleasant, negative	Unpleasant, negative	Unpleasant, negative	Unpleasant, negative
Chemical	Chemical, paint	Chemical, paint	Chemical, paint
Cow shed, barn yard	Cow shed, farm, barn yard	Cow shed, dairy farm	Barn smell

(c) 4-EG descriptors

4-EG odour pen	4-EG in white wine	4-EG in red wine	4-EG in ethanol
Stronger, more complex	Stronger, sharper, pungent	Stronger	Stronger
Bandaids	Bandaids	Damp, stale	Woody, musty, organic
Bitter	Smoke, charcoal	Smoke	Smoke, charcoal
Bacon, BBQ	Bacon, BBQ, ham	Bacon, BBQ, ham	Bacon, BBQ
Unpleasant/ negative	Unpleasant/ negative	Unpleasant/ negative	Unpleasant/ negative
Less sweet, more sour/ bitter/ spicy	More sweet/ sour/ bitter	More sweet/ sour/ bitter	More sweet/ sour/ bitter

Detection threshold responses: Although 40 participants were tested, only results for 37 can be reported due to issues related to incomplete test procedures and temporary health problems that may have confounded the results of three of the participants.

Pen detection threshold responses (determined on a scale with the larger number as the weakest and 1 as the strongest odour pen) exhibited variation among individuals, although the mean for each taint was approximately in the pen 4-5 range. However, it should be noted that each taint was at a different level in this concentration range: TCA – M = 3.86 (SD = 1.62; range 20-40 ppt); 4-EP – M = 4.75 (SD = 2.86; range 20-40 ppm); 4-EG – M = 5.14 (SD = 2.22; range = 8-20 ppm). The mean threshold responses and equivalent concentration

ranges for the wine taints presented as odour pens and presented in 50 ml of alcohol (white wine, red wine or ethanol) are summarised in Table 4.2.2.

Table 4.2.2: Summary of mean detection thresholds and corresponding concentration ranges for the wine taints TCA, 4-EP and 4-EG presented as odour pens and in white wine, red wine or ethanol. Note: The concentration levels provided for White wine, Red wine and Ethanol are calculated in relation to the 50ml of the alcohol delivery medium.

Taint	Odour pens		White wine		Red wine		Ethanol	
	M (SD)	Concentration range	M (SD)	Concentration range	M (SD)	Concentration range	M (SD)	Concentration range
TCA	3.86 (1.62)	20-40 ppt	5.60 (2.09)	10-20 ppt	5.53 (1.69)	10-20 ppt	6.49 (1.86)	20-40 ppt
4-EP	4.75 (2.86)	20-40 ppm	6.20 (1.71)	400-800 ppt	6.14 (1.66)	400-800 ppt	6.54 (1.78)	400-800 ppt
4-EG	5.14 (2.22)	8-20 ppm	5.76 (2.04)	200-400 ppt	6.00 (2.17)	400 ppt	6.16 (2.08)	400-800 ppt

Figure 4.2.1 illustrates the differences in threshold responses for the taints delivered in red or white wine or ethanol. Note the different concentration scales for the different taints; the threshold concentration values correspond with those listed above in Table 4.2.2.

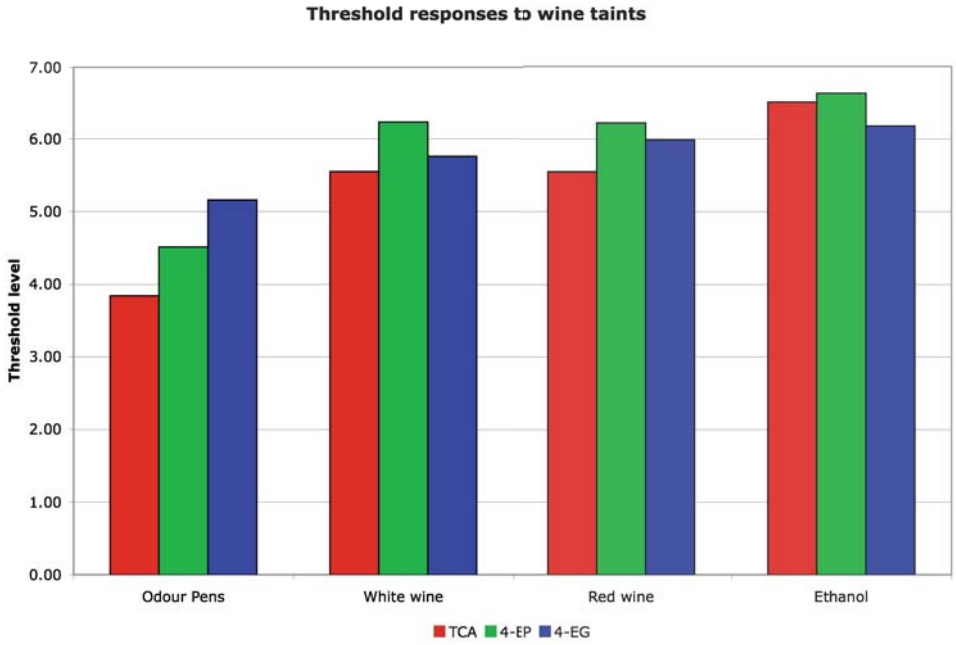


Figure 4.2.1. Summary graphs of mean threshold responses to TCA, 4-EP & 4-EG when delivered in odour pens, or in 50ml of white wine, red wine or ethanol. These results reveal differences in discrimination sensitivity associated with the type of wine or alcohol in which the taint was presented. The higher threshold step number (left axis) equates to weaker solutions and the lower numbers to stronger solutions.

Threshold responses to the odour pens varied across odours type: there was greater sensitivity to 4-EG than to 4-EP and TCA. One-way repeated measures ANOVA analyses were conducted to compare threshold responses for each odour threshold test condition (pens, white wine, red wine and ethanol) for each odour, with a significance level of 0.05, revealing the following results:

- TCA: There was a strong significant effect for odour threshold test condition [Wilks' Lambda = 0.44, $F(3,34) = 14.529$, $p<0.001$, multivariate partial eta squared = 0.56].

That is, there was a difference in threshold levels for the TCA delivered in pens *cf* delivered in white wine or red wine. The threshold levels for white and red wine were at similar concentration ranges, whereas the threshold levels for TCA in the odour pens and in ethanol were at similar concentration ranges.

- 4-EP: There was a weak significant effect for odour threshold test condition [Wilks' Lambda = 0.79, $F(3,34) = 2.972$, $p<0.05$, multivariate partial eta squared = 0.21]. There was greater sensitivity to 4-EP when delivered in the odour pens *cf* delivered in white wine, red wine or ethanol, but there was little difference in the sensitivity
- 4-EG: There was no significant effect for odour threshold test condition [$p>0.05$]. The threshold levels for 4-EG in white wine, red wine or ethanol were at a comparable concentration level (approximately 400 ppt).

Discrimination responses*:* The untrained participants were also assessed with forced choice triangle tests of discrimination ability for the untainted white wine, red wine and ethanol, presented in 6 combinations of blank bases. The range of possible responses was from 0 for no correct discrimination to 6 for all correctly discriminated. The mean discrimination responses for the blank comparisons were: TCA – M 5.11 (SD = 1.76; range 3-6); 4-EP – M = 5.08 (SD = 1.00; range 2-6); 4-EG – M = 4.86 (SD = 1.13; range 1-6). These data suggest that the participants were consistent in their abilities to discriminate the blank bases, and differences in detection responses for tainted bases were therefore related to the effect of the taints.

4.2.3 Discussion and Summary

The occurrence of taints or off-flavours in wine that cause unpleasant flavours or odours are a significant economic problem to the wine industry (Taylor *et al*., 2000; Silva Periera *et al*., 2000). While there are different sources of taints in wines, the most commonly reported is the compound 2,4,6-trichloroanisole (TCA) (Fuller, 1995; Taylor *et al*., 2000; Silva Periera *et a*l., 2000).

The effect of TCA on consumer responses to wines is a serious problem exacerbated by the difficulty of its assessment. It is generally recognised that TCA can adversely affect the sensory properties of wine, although recent research has indicated that wines exhibiting cork taint may have low or chemically undetectable concentrations of TCA (Soleas *et al*., 2002). Individual responses to wines vary to such a degree that even experienced tasters may have conflicting opinions about the tainting of a wine (Simpson and Veitch, 1993; Butzke *et al*., 1999; Casey, 1999). Variations in sensitivity to TCA will result in only portions of the population even being aware of its presence in a wine, and variations in concentrations of TCA will have different manifestations. Even when not consciously detected, TCA is reported to suppress and alter the aroma and flavour of wine (Casey, 1999).

Analysis of results in this study demonstrated variations in sensitivity to the different wine taints – including TCA – and also highlighted the extent to which these taints can affect the perception of a wine-related odour, even when detected at low concentrations and by participants naive to the recognised qualities of the taints. It is important to keep in mind the fact that participants were blind folded during the test procedure, and therefore remained unaware of the base in which the odour was delivered, and remained naïve to the taints and their associated qualities – with just a general understanding that they were sniffing wine-related odours, and no prior information about taints.

There were within- and between- subject differences in sensory responses associated with responses to the wine odours, and with the type of wine or alcohol the taint was presented in. This preliminary data suggests the following trends:

- TCA detection threshold responses were more sensitive when presented in the white wine and red wine delivery mediums, with TCA detected or discriminated in the 10-20 ppt range, whereas detection threshold responses to the odour pens and ethanol were in the 20-40 ppt range.

- There was a decrease in sensitivity to 4-EP when delivered in wine (red or white) and ethanol *cf* odour pen delivery, but threshold levels were similar when delivered in white wine, red wine, or ethanol.
- Greater sensitivity to 4-EG alone (as seen in the "higher" threshold level with the pens), and a reduction in sensitivity (but similar response) with white wine, red wine and ethanol.

There were differences in descriptors applied to the different wine taints:

- TCA responses across all conditions included reference to musty, mouldy, stale, damp and woody. TCA in the wine/ethanol conditions was associated with increased perception of alcohol, and a generally flatter, less fruity quality – in particular, a reduction in "wine" quality was reported for the white and red wine bases.
- 4-EP responses across all conditions included reference to cow shed, dairy farm, barn smells, and also reference to mushroom and musky smells.
- 4-EG responses across all conditions included reference to bandaids, bacon, smoke and barbecue smells, in addition to damp and stale or organic smells.

All conditions resulted in reference to the odour being stronger, less pleasant and generally more negatively regarded in the tainted base *cf* the untainted control odours.

In conclusion, detection and discrimination thresholds, and descriptor responses of untrained participants revealed within- and between- subject differences. Despite being naïve to the descriptions of the target odours, participants provided clearly different descriptors for the different taints in the pens and base solutions. TCA descriptor responses supported industry expectations of some negative attributes when detected above threshold levels; 4-EP and 4-EG responses were also typically associated with "chemical" and negative descriptors but also were reported with differing ranges of attributes.

This study has demonstrated the variation in consumer responses associated with preferences for the wine taints odours, and with the type of wine or alcohol the taint was presented in, providing the wine industry with preliminary evidence of consumer preference responses to these wine taints.

4.3 TCA Concentration Study: Subjective and Objective Responses

The degree to which consumers vary in responses to TCA has rarely been investigated using objective physiological techniques. This formed the basis of a previous study conducted for the GWRDC that provided preliminary information concerning the degree to which consumers perceive and respond to TCA, even when not consciously aware of its presence. Responses to TCA and ethanol were assessed using subjective and objective techniques to monitor perceived and physiological responses in an attempt to assess differences in perceptual responses to TCA in a population of wine consumers, untrained in taint (TCA) detection.

The approach-withdrawal theory (as described by Davidson 1987, 1992; Davidson & Sutton, 1995) proposed that positive responses to a stimulus would be evident in changes in anterior EEG activation in the left frontal region, seen as decreased alpha activation (increased arousal or attention) in response to the positive stimuli and a corresponding increased alpha activation in response to negative stimuli. The results of the TCA and Ethanol responses associated with liking group (*Like vs Neutral*) in the preliminary study provided evidence of alpha changes associated with stronger like responses, with a decrease in alpha associated with *Liking cf Neutral*. As cortical alpha power has reported to be inversely related to cortical activity (Coan *et al*., 2001), suppression or decrease of alpha activity has been equated to an increase in activation in response to stimulation, supporting the assumption that these decreases are related to the *Liking* response for the odour stimulus.

This study was undertaken to provide preliminary information concerning the degree to which consumers perceive and respond to one concentration of TCA, even when not consciously aware of its presence. These results provided preliminary evidence of changes in brain activity associated with stimulation by TCA (delivered in ethanol) versus stimulation by ethanol alone. However, results of sensory ratings reported in the literature to a range of odours suggest that responses to odours can vary with the concentration of the odour stimulus – with many odours becoming more negative as the concentration increases.

The current TCA concentration study was designed to assess changes in brain electrical activity and in perception associated with TCA concentration level, and in particular to determine if brain electrical activity correlates with changes in TCA perceptual responses by participants naïve to the odour and its qualities.

4.3.1 Method

Participants

From a total of 120 participants, a panel of 72 university student volunteers (untrained in sensory analysis) completed both recording sessions of TCA brain electrical activity (18 males and 54 females; mean age 20.8 years; age range 18–32 years; 87% non-smokers). As with the pen study, all participants were aware they were assessing wine-related odours but were naïve to the identity and "taint" associations of the odours. In the final analysis of the brain recording responses, additional participants were excluded from the final analysis due to problems with the data files in one or more concentrations. This resulted in a final group of a total of 62 participants with usable data from all four TCA concentration sessions.

All volunteer participants were screened for olfactory performance using the *Sniffin' Sticks* Test of Olfactory Performance (Burghart, Wedel, Germany), with test results indicating all had total olfactory performance scores (TDI score: the sum of Threshold, Discrimination and Identification scores) within a normal range for this age group (Hummel *et al*., 1997; Kobal *et al*., 2000): TDI = 36.71 ±4.02.

Odour Stimuli

The TCA odour samples were prepared at three concentrations: Low (0.1 ng.l^{-1}), Mid (0.5 ng.l^{-1}) and High (1.0 ng.l^{-1}).

The base TCA solution was prepared by dissolving the weighted dry powder TCA (Sigma-Aldrich, Castle Hill, Australia) in 100% ethanol. One ≤L of the TCA solution was then placed in the 50 ml glass odour syringe, and 50 ml of air with ethanol (prepared by placing 20≤L in a1 L bag) was drawn from the ethanol bag to fill the 50 ml glass control syringe incorporated into the syringe delivery system of the CRO.

In the initial process of determining the concentration of ethanol and TCA in ethanol for use in the EEG recordings, SPME-GC analysis of the generated TCA and Ethanol odour samples was conducted using a Chrompack 3800 gas chromatograph (Varian, Australia) fitted with a 1079 injector and flame ionisation detector. These odour samples were separated on an EC-WAX capillary column (Altech, Australia) (30 m, id. 0.32, film 0.25 μm), and concentrated using solid phase micro extraction (SPME) fibres (Supelco, Bellafonte, PA, USA). This analysis was conducted to determine concentration and consistency of odour sample preparation throughout the experimental period. The concentration of the ethanol was calculated to be 31.6 mg.l^{-1}, present in both the TCA and ethanol odour syringes.

Procedure

All subjects had already completed the TCA odour pen and the TCA wine discrimination sessions before attending the EEG sessions. Participants then attended two EEG recording sessions (summarised in Table 4.3.1) consisting of 6 x 5-minute trial recording periods (Session 1: 3 x TCA *Low* and 3 x TCA *Mid 1* trials; Session 2: 3 x TCA *Mid 2* and 3 x TCA *High* trials) resulting in a total of 15 minutes of EEG recorded for each test odour at each session. The *Mid 1* and *Mid 2* concentrations used were the same, but occurred at different times and were used as an internal control.

Table 4.3.1: Summary of EEG recording protocol with different TCA concentrations. Sessions 1 and 2 occurred on different days.

Session 1											
Low Concentration						*Mid 1* Concentration					
Trial 1	Rating scales	Trial 2	Rating scales	Trial 3	Rating scales	Trial 4	Rating scales	Trial 5	Rating scales	Trial 6	Rating scales
Session 2											
Mid 2 Concentration						*High* Concentration					
Trial 1	Rating scales	Trial 2	Rating scales	Trial 3	Rating scales	Trial 4	Rating scales	Trial 5	Rating scales	Trial 6	Rating scales

Following each trial, participants completed a rating sheet, using 10-point Likert scales to indicate their perceived *Liking*, *Strength* (intensity) and *Complexity* ratings, and describing the odour they detected during the recording. Differences caused by the TCA concentrations in comparison to ethanol without TCA were recorded and analysed. The effects of the TCA concentration on EEG responses was obtained by subtracting all the brain electrical activity frequency values during the ethanol sample breathing from the signals obtained when the participant breathed in the TCA in ethanol mixtures.

Data were analysed to determine key descriptors and differences in rating responses associated with the different concentrations of TCA in ethanol.

4.3.2 Results

Descriptor Responses to Concentrations of TCA

The descriptors acquired following each EEG trial were analysed to examine differences in descriptive responses for the three TCA concentrations. Analysis was conducted to assess the key descriptor themes commonly reported for the three TCA concentrations in the EEG trials. The frequency of these key descriptors are summarised in Table 4.3.2. When participants were unable to detect an odour, it was assigned the label "below threshold".

Data for the two sets of *Mid* concentration (*Mid 1* and *Mid 2*) were initially analysed separately, as these were assessed in different sessions (usually occurring on different days, and certainly at different times of day), and may vary due to natural variations in sensory ability on a day-to-day basis. However, analysis of ratings of these odour responses revealed no significant differences ($p>0.05$, as reported in the following section), and so the descriptor responses for the two *Mid* concentrations were combined.

With increasing concentrations of TCA, the following descriptor trends were observed:

- A decrease in the percentage of "chemical" labels as TCA concentrations increased.
- A decrease in the percentage of "alcohol" labels as TCA concentrations increased.
- An increase in the percentage of "fruity" labels provided for the odour as TCA concentrations increased.
- An increase in the percentage of "musty" labels provided for the odour as TCA concentrations increased.
- An increase in the percentage of "negative" terms across increasing concentrations of TCA in ethanol when all clearly "negative" descriptors (bitter, musty, mouldy and rotten) are combined: TCA *Low* = 9.9%, TCA *Mid* = 16.2 %, TCA *High* = 16.6%.
- There was a small increase in the percentage of "below threshold" as TCA concentrations increased; that is, with increasing concentrations, there was a small increase in the number of participants who were unable to report and describe an odour.

Table 4.3.2: Summary of percentage of participants identifying key descriptors during the EEG recording trials in response to the three concentrations of TCA in ethanol. Frequency is reported as the percentage (%) reported for each of the TCA concentrations *Low*, *Mid* and *High*.

Descriptors	TCA Low	TCA Mid	TCA High
Chemical	32.6 %	22.7 %	17.4 %
Alcohol	14.8 %	12.0 %	10.1 %
Sweet	14.0 %	12.5 %	16.9 %
Fruity	7.7 %	10.1 %	12.4 %
Floral	5.1 %	3.9 %	5.8 %
Bitter	3.4 %	3.9 %	3.4 %
Musty	3.2 %	5.0 %	7.1 %
Mouldy	1.3 %	5.1 %	3.3 %
Rotten	2.0 %	2.2 %	2.8 %
Other	10.2 %	9.4 %	9.7 %
Below Threshold	7.7 %	7.4 %	11.1 %

Liking, Strength and Complexity Responses to Concentrations of TCA

The ratings acquired following each EEG trial were analysed to examine differences in descriptive responses and ratings of *Liking* and *Strength* (Intensity) and *Complexity* ratings for the three TCA concentrations. The rating responses for the *Mid 1* and *Mid 2* concentrations were analysed using repeated measures t-tests, revealing that there were no significant differences ($p>0.05$) in ratings for Liking, Strength or Complexity between *Mid 1* and *Mid 2* concentrations. The means of these two *Mid* concentration scores were therefore obtained for each participant and further analysis conducted using repeated measures one-way ANOVA for a comparison of ratings across the *Low*, *Mid* and *High* concentrations of TCA in ethanol.

Although small differences in mean ratings were evident, repeated measures one-way ANOVA revealed no significant differences in mean *Liking*, *Strength* or *Complexity* responses ($p>0.05$) for the three concentrations of TCA delivered in ethanol. The similarity of the means for the ratings across TCA concentrations is evident in the means displayed in Table 4.3.3.

Table 4.3.3: Summary of mean ratings (and standard deviations) for Liking, Strength and Complexity across the trials for the three concentrations of TCA in ethanol.

Rating Scale	TCA *Low*	TCA *Mid*	TCA *High*
Liking	M = 45 SD = 14 Range: 6 - 77	M = 45 SD = 11 Range: 15 - 72	M = 44 SD = 16 Range: 2 - 80
Strength	M = 37 SD = 20 Range: 0 - 83	M = 38 SD = 15 Range: 3 - 70	M = 33 SD = 20 Range: 0 - 82
Complexity	M = 46 SD = 17 Range: 2 - 96	M = 43 SD = 12 Range: 3 - 95	M = 46 SD = 17 Range: 3 – 85

Note: Ratings were measured in mm on a 100 mm scale, with 0 = strong dislike/very weak/lacking complexity, and 100 = strong like/very strong/very complex.
The TCA *Mid* responses are an average across the *Mid* responses from both Sessions 1 and 2.

Correlations of TCA Thresholds with Liking, Strength and Complexity Ratings to Concentrations of TCA

The relationship between TCA threshold levels and the ratings *of Liking* and *Strength* (Intensity) and *Complexity* acquired following each EEG trial at each concentration were investigated using Pearson product-moment correlation coefficient.

There were no significant correlations between TCA threshold levels and any of the rating scales; that is, sensitivity to TCA did not impact on perceptions of *Liking*, *Strength* or *Complexity*.

The following significant correlations were revealed (as summarised in Table 4.3.4):

- A medium positive correlation between *Liking* and *Strength* ratings at the *Low* concentration ($r = 0.39$, $n=59$, $p<0.01$), with greater *Like* responses associated with higher *Strength* ratings.
- A medium positive correlation between *Liking* and *Complexity* ratings at the *Low* concentration ($r = 0.36$, $n=59$, $p<0.01$), with greater *Like* responses associated with higher *Complexity* ratings.
- A medium positive correlation between *Liking* ratings at *Mid* and *High* concentrations ($r = 0.39$, $n=57$, $p<0.01$), with higher *Liking* ratings at the *Mid* concentration associated with higher *Liking* ratings at the *High* concentration.

- A strong positive correlation between *Strength* and *Complexity* ratings at the *Low* concentration (r = 0.49, n=61, p<0.001), with greater *Strength* responses associated with higher *Complexity* ratings.
- A medium positive correlation between *Liking* and *Strength* ratings at the *Mid* concentration (r = 0.33, n=60, p<0.05), with greater *Like* responses associated with higher *Strength* ratings.
- A medium positive correlation between *Strength* and *Complexity* ratings at the *Mid* concentration (r = 0.44, n=60, p<0.001), with greater *Strength* responses associated with higher *Complexity* ratings.
- A strong positive correlation between *Strength* and *Complexity* ratings at the *High* concentration (r = 0.51, n=56, p<0.001), with greater *Strength* responses associated with higher *Complexity* ratings.
- A medium positive correlation between *Strength* ratings at *Low* and *Mid* concentrations (r = 0.35, n=60, p<0.01), with higher *Strength* ratings at the *Low* concentration associated with higher *Strength* ratings at the *Mid* concentration.
- A medium positive correlation between *Strength* ratings at *Mid* and *High* concentrations (r = 0.34, n=60, p<0.05), with higher *Strength* ratings at the *Mid* concentration associated with higher *Strength* ratings at the *High* concentration.
- A weak positive correlation between *Strength* and *Complexity* ratings at the *Mid* concentration (r = 0.26, n=57, p<0.001), with greater *Strength* responses associated with higher *Complexity* ratings.
- A strong positive correlation between *Complexity* ratings at *Low* and *Mid* concentrations (r = 0.59, n=60, p<0.001), with higher *Complexity* ratings at the *Low* concentration associated with higher *Complexity* ratings at the *Mid* concentration.

Table 4.3.4: Significant correlations between comparisons of TCA thresholds, and *Liking*, *Strength* and *Complexity* ratings across the three concentrations.

	Like Low	***Like Mid***	***Like High***	***Strength Low***	***Strength Mid***	***Strength High***	***Complex Low***	***Complex Mid***	***Complex High***
Like Low	1								
Like Mid	0.17	1							
Like High	-0.01	0.39 **	1						
Strength Low	0.39 *	0.04	0.02	1					
Strength Mid	0.36 *	0.33 *	0.27 #	0.35 *	1				
Strength High	0.16	0.01	0.26	0.06	0.34 #	1			
Complex Low	0.93 **	0.09	- 0.6	0.49 **	0.24	0.11	1		
Complex Mid	0.84 **	0.23	0.08	0.19	0.44 **	0.21	0.59 **	1	
Complex High	0.15	0.03	0.25	- 0.20	0.15	0.51 **		0.25	1

** Significant at p<0.001 level
* Significant at p<0.01 level
# Significant at p<0.05 level

Electrophysiological Power Analysis: Concentration effects across frequencies

Relative % power odour–ethanol difference responses for the 4 – 16 Hz frequencies were submitted to statistical analysis to determine the significance of differences in responses associated with electrode location and odour concentration. Anterior electrode locations were selected for this analysis to provide corresponding left and right hemisphere sites: Left and Right frontal (LF and RF) groups, Left and Right temporoparietal (LTP and RTP) groups, and individual electrode sites: F3, F4, F7, and F8 (see Figure 4.3.1).

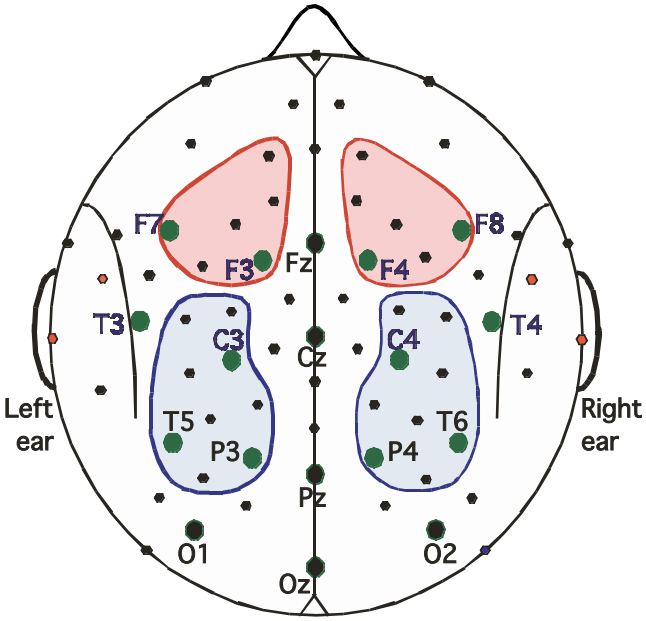


Figure 4.3.1. Illustration of electrode site locations, viewed from above (nose at top, right and left ears as indicated). The EGI electrode sites have been labelled for the sites equalling the International 10/20 system. The shaded areas indicate the Left and Right Frontal areas (overlapping F3 and F7, and F4 and F8 respectively) and the Left and Right Temporoparietal areas (including T5 and T6 respectively).

A one-way repeated measures analysis of variance (ANOVA) was conducted for each frequency to evaluate the impact of odour concentration (*Low vs Mid vs High*) on relative % power odour – ethanol difference responses at the different frontal electrode sites. To reduce the impact of Type 1 error on this series of ANOVAs, a Bonferroni adjustment was used, resulting in the use of an alpha value of 0.004; that is, results are only considered significant if the probability value is less than 0.004

Using this alpha value, there were no significant effects for concentration at any of the electrode groups. However, if we revert to the normal alpha value of 0.05, the following significant effects would be observed:

- Left Frontal electrode group for the 4 Hz frequency: (Wilks Lambda = 0.882; F (2,59) = 3.942, p = 0.025, partial eta squared = 0.118). There was a weak trend for an increase in 4 Hz frequency responses to the TCA odour (*cf* ethanol only) for the *High* concentration in comparison to the *Low* and *Mid* concentrations.
- Right Temporoparietal electrode group for the 11 Hz frequency: (Wilks Lambda = 0.904; F (2,59) = 3.143, p = 0.05, partial eta squared = 0.096). There was a very weak trend for an increase in 11 Hz responses to the TCA odour (*cf* ethanol only) for the *Mid* concentration in comparison to the *Low* and *High* concentrations.
- Further, there was a similar [non-significant] trend in the 11 Hz Left Frontal electrode responses: (Wilks Lambda = 2.558; F (2,59) = 3.143, p = 0.05, partial eta squared = 0.08). There was a very weak trend for an increase in 11 Hz responses to the TCA odour (*cf* ethanol only) for the *Mid* concentration in comparison to the *Low* and *High* concentrations.

The means and standard deviations are presented in Table 4.3.5.

Table 4.3.5: Summary of the close to significant means (and standard deviations) for relative % power differences for the three concentrations of TCA in ethanol.

Rating Scale	Frequency	N	TCA *Low*	TCA *Mid*	TCA *High*
Left Frontal	4 Hz	61	M = - 0.53 SD = 7.24	M = - 0.60 SD = 5.10	M = 2.92 SD = 7.56
Left Frontal	11 Hz	61	M = - 0.07 SD = 4.80	M = 0.77 SD = 4.66	M = - 0.76 SD = 3.79
Right Temporoparietal	11 Hz	61	M = - 0.04 SD = 6.05	M = 1.29 SD = 5.08	M = - 1.67 SD = 7.04

Electrophysiological Relative % Power Analysis: Liking, Strength and Complexity effects across concentrations

A one-way between-groups multivariate analysis of variance (MANOVA) was conducted for the Liking, Strength and Complexity groups for each TCA concentration, using the relative % power test odour – ethanol difference responses across the 4 – 16 Hz frequency range. In each MANOVA, the independent variable was the groupings for *Liking* (*Dislike, Neutral, Like*), Strength (*Weak, Mid, Strong*) or *Complexity* (*Simple, Mid, Complex*). Each of these groupings was calculated from the separation of the rating scales in the following ways: *Like/Weak/Simple*: <40 mm, *Neutral/Mid/Mid* = 40-60 mm; *Like/Strong/Complex* = >60 mm. The dependent variables were the different frequencies in the 4 – 16 Hz range.

Again, a Bonferroni adjustment was used to reduce the impact of Type 1 error on this series of MANOVAs, resulting in the use of an alpha value of 0.004; that is, results are only considered significant if the probability value is less than 0.004. In addition, due to some violations of assumptions of covariance and equal variance across the different frequencies, together with unequal N values, Pillai's trace was used as the statistic to test the significance of differences between groups.

Liking power effects across frequencies

All responses were analysed for significant differences between *Liking* groups in the combined frequencies. Results for the frequencies were then considered separately, using the Bonferroni adjusted alpha level of 0.004. All significant *Liking* responses are summarised in Table 4.3.6.

Left Frontal relative % power difference responses:

There was no statistically significant LF difference between *Liking* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following LF responses in *Low* concentration *Liking* groups were observed to be approaching statistical significance:

- *Low* concentration 6 Hz: There was an [approaching] significant difference between *Liking* groups: F(2, 47) = 3.59, p = 0.035, partial eta squared = 0.13. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Liking* for the odour *cf Dislike* and *Neutral* responses.

There was no statistically significant LF difference between *Liking* groups in the *Mid* concentration on the combined frequencies (p>0.004). Further, there were no significant LF differences in *Mid* concentration *Liking* groups for the separate frequencies.

- *Mid* concentration 9 Hz: There was an [approaching] significant difference between *Liking* groups: F(2, 54) = 3.16, p = 0.037, partial eta squared = 0.10. An inspection of

the mean scores indicated an decreased relative % power TCA response associated with increased *Liking* for the odour *cf Dislike* and *Neutral* responses.

- *Mid* concentration 12 Hz: There was an [approaching] significant difference between *Liking* groups: F(2, 54) = 4.71, p = 0.013, partial eta squared = 0.14. An inspection of the mean scores indicated a decreased relative % power TCA response associated with increased *Liking* for the odour *cf Dislike* responses.

There was no statistically significant LF difference between *Liking* groups in the *High* concentration on the combined frequencies (p>0.004). Further, there were no significant LF differences in *High* concentration *Liking* groups for the separate frequencies.

Right Frontal (RF) relative % power difference responses:

There was no statistically significant RF difference between *Liking* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RF *Low* concentration *Liking* responses were observed to be approaching statistical significance:

- *Low* concentration 5 Hz: There was an [approaching] significant difference between *Liking* groups: F(2, 57) = 2.42, p = 0.098, partial eta squared = 0.08. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Liking* for the odour *cf Neutral* responses.

There was no statistically significant RF difference between *Liking* groups in the *Mid* concentration on the combined frequencies (p>0.004). Further, there were no significant RF differences in *Mid* concentration *Liking* groups for the frequencies when considered separately.

There was no statistically significant RF difference between *Liking* groups in the *High* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RF *High* concentration responses were observed to be approaching statistical significance:

- *High* concentration 5 Hz: There was an [approaching] significant difference between *Liking* groups: F(2, 54) = 3.52, p = 0.037, partial eta squared = 0.12. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased Liking for the odour *cf Dislike* and *Neutral* responses.

Left Temporoparietal (LTP) relative % power difference responses:

There was no statistically significant difference between *Liking* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following LTP *Low* concentration responses reached statistical significance:

- *Low* concentration 6 Hz: There was a statistically significant LTP differences between *Liking* groups: F(2, 57) = 7.22, p = 0.002, partial eta squared = 0.20. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Liking* for the odour *cf Dislike* and *Neutral* responses.

There was no statistically significant LTP difference between *Liking* groups in the *Mid* concentration on the combined frequencies (p>0.004). However, the differences between *Liking* groups were approaching significance: F(26,94) = 1.59, p = 0.06, partial eta squared = 0.305. When the results for the frequencies were considered separately, the following LTP *Mid* concentration responses reached statistical significance:

- *Mid* concentration 14 Hz: There was a statistically significant difference between *Liking* groups: F(2, 58) = 10.13, p = 0.000, partial eta squared = 0.26. An inspection of the mean scores indicated a decreased relative % power TCA response associated with increased *Liking* for the odour *cf Dislike* and *Neutral* responses.

There was no statistically significant LTP difference between *Liking* groups in the *High* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following LTP *High* concentration responses reached statistical significance:

- *High* concentration 11 Hz: There was a statistically significant difference between *Liking* groups: F(16,39) = 4.21, p = 0.02, partial eta squared = 0.18. An inspection of the mean scores indicated a increased relative % power TCA response associated with increased *Liking* for the odour *cf Neutral* responses.

Right Temporoparietal (RTP) relative % power difference responses:

There was no statistically significant RTP difference between *Liking* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RTP responses were observed to be approaching statistical significance:

- *Low* concentration 13 Hz: There was an [approaching] significant difference between Liking groups: F(2, 57) = 3.08, p = 0.05, partial eta squared = .098. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Liking* for the odour *cf Dislike* and *Neutral* responses.

There was no statistically significant RTP difference between *Liking* groups in the *Mid* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RTP *Mid* concentration responses were observed to be approaching statistical significance:

- *Mid* concentration 15 Hz: There was an [approaching] significant difference between Liking groups: F(2, 58) = 4.11, p = 0.02, partial eta squared = 0.26. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Liking* for the odour *cf Dislike* and *Neutral* responses.

There was no statistically significant RTP difference between *Liking* groups in the *High* concentration on the combined frequencies (p>0.004). Further, there were no significant RTP differences in *Liking* groups for the separate frequencies.

Summary of trends in relative % power differences for Liking groups at each TCA concentration

When visual comparisons are made between the various relative percentage differences in the regions of the scalp where EEG recordings were made some general comments can be made. Appendix 5 provides a set of bar graphs which show these relative percentages for each opinion (dislike, neutral and like) and across a range of frequencies (4 Hz to 16 Hz) and a graph for region (left frontal, right frontal, left frontoparietal and right frontoparietal), as well as for concentration of TCA (*Low*, *Mid* and *High*). Such complex interactions are difficult to quantitatively analyse, but can be informative, nevertheless.

Frontal responses: At the *Low* concentrations in both the left and right frontal regions, the tendency is for the *Dislike* state to be reducing the percentage difference (less activity in response to the TCA) in the lower frequency ranges, but not the higher ranges. For those who liked the TCA their tendency was for the increase in relative differences to be in the lower middle range of frequencies in both left and right frontal regions, but overall, decreasing the difference in the upper frequency ranges. A particular frequency which showed a marked change was in the 5 - 6 Hz range, with those who liked the TCA responding with an increase in activity suggesting a more relaxed response.

Temporoparietal responses: In contrast, the temporoparietal regions had a similar increase in 6 Hz but only in the left side in those who liked the TCA. The tendency was for the increase to be in the higher frequencies in the right temporoparietal. In these two scalp regions the “dislike” people had an increase in the right temporoparietal in the lower

frequencies to the TCA. For the high concentrations of TCA, the tendency in all scalp regions was an increase in relative frequency for those who reported disliking the taint. While this was apparent in many of the frequencies studied, it was most obvious in the lower frequencies. Significantly, those who expressed a preference for liking the TCA showed increases in the alpha frequency range (8 Hz to 12 Hz), which was in both sides but was more pronounced in the right temporoparietal. The patterns observed with the responses to the middle concentration of TCA were reminiscent of a mixture of the low and the high concentrations patterns across the frequencies.

Table 4.3.6. *Liking* responses: Summary of the means identified through Post-hoc comparisons (using the Student-Newman-Keuls test) as significantly different relative % power Liking frequency responses for the four electrode groups and three concentrations of TCA in ethanol.

Location	[TCA]	Frequency	Group	N	Means	
Left Frontal	*Low*	6 Hz	*Dislike*	21	- 2.64	
			Neutral	23	0.24	
			Like	6		4.14 *
	Mid	9 Hz	*Dislike*	18	0.54	
			Neutral	38	1.16	
			Like	5		- 1.78 *
	Mid	12 Hz	*Dislike*	18	1.30	
			Neutral	38	- 0.08	- 0.08
			Like	5		- 1.47 *
Right Frontal	*Low*	5 Hz	*Dislike*	25	0.37	0.37
			Neutral	28	- 0.77	
			Like	7		4.53 #
	High	5 Hz	*Dislike*	18	2.33	
			Neutral	31	1.82	
			Like	8		- 2.35 *
Left Temporoparietal	***Low***	**6 Hz**	***Dislike***	**25**	**- 0.18**	
			Neutral	**28**	**- 1.61**	
			Like	**7**		**6.96 ****
	Mid	**14 Hz**	***Dislike***	**25**	**- 0.59**	
			Neutral	**28**	**- 0.13**	
			Like	**7**		**- 2.87 ****
	High	11 Hz	*Dislike*	31	- 0.43	- 0.43
			Neutral	17	- 2.69	
			Like	8		3.76 *
Right Temporoparietal	*Low*	13 Hz	*Dislike*	25	0.31	
			Neutral	28	0.30	
			Like	7		3.26 *
	Mid	15 Hz	*Dislike*	18	- 0.07	
			Neutral	38	0.22	
			Like	5		1.23 *

** **Bolded** significant at Bonferroni adjusted alpha value, p<0.004
* significant only at p≤0.05, but above Bonferroni adjusted value (p>0.004).
# Not significant (p>0.05) but differences evident in Post-hoc comparisons.

Investigation of relative % power difference responses of Liking sub-groups

Responses from *Dislike* and *Like* sub-groups for each concentration were studied to investigate trends in responses associated with clearly defined responses. Only scores in the <26 and >63 range were studied, to clearly separate these responders from those with ratings in the more neutral range. No statistical significance was found – partly due to the small numbers in each group, but some of the differences (p=0.04 for *Low Dislike* versus *Low Like* at 9 Hz) were approaching significance.

Strength power effects across frequencies

All responses were analysed for significant differences between *Strength* groups in the combined frequencies. Results for the frequencies were then considered separately, using the Bonferroni adjusted alpha level of 0.004. All significant *Strength* responses are summarised in Table 4.37.

Left Frontal relative % power difference responses:

There was no statistically significant LF difference between *Strength* groups in the *Low* concentration on the combined frequencies (p>0.004) or for the separate frequencies.

There was no statistically significant RF difference between *Strength* groups in the *Mid* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RF *Mid* concentration responses were observed to be approaching statistical significance:

- *Mid* concentration 11 Hz: There was an [approaching] statistically significant RF differences between *Strength* groups: F(2, 58) = 2.44, p = 0.10, partial eta squared = 0.08. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Strength* responses for the odour *cf Weak* responses.

There was no statistically significant LF difference between *Strength* groups in the *High* concentration on the combined frequencies (p>0.004). Further, there were no significant LF differences in Strength groups for the separate frequencies.

Right Frontal (RF) relative % power difference responses:

There was no statistically significant RF difference between *Strength* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RF *Low* concentration responses were observed to be approaching statistical significance:

- *Low* concentration 5 Hz: There was an [approaching] significant difference between *Strength* groups: F(2, 58) = 4.79, p = 0.01, partial eta squared = 0.14. An inspection of the mean scores indicated an increased relative % power TCA response associated with decreased *Strength* (*Weak*) responses for the odour *cf Strong* responses.

There was no statistically significant RF difference between *Strength* groups in the *Mid* concentration on the combined frequencies (p>0.004) or for the separate frequencies.

There was no statistically significant RF difference between *Strength* groups in the *High* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RF *High* concentration responses were observed to be approaching statistical significance:

- *High* concentration 8 Hz: There was an [approaching] significant difference between *Strength* groups: F(2, 54) = 2.93, p = 0.06, partial eta squared = 0.10. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Strength* for the odour *cf Weak* and *Mid-Strength* responses.

Left Temporoparietal (LTP) relative % power difference responses:

There was no statistically significant difference between *Strength* groups in the *Low* concentration on the combined frequencies (p>0.004) or for the separate frequencies.

There was no statistically significant LTP difference between *Strength* groups in the *Mid* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following LTP *Mid* concentration responses reached statistical significance:

- *Mid* concentration 8 Hz: There was an approaching significant LTP differences between *Strength* groups: F(2, 58) = 2.82, p = 0.07, partial eta squared = 0.09. An inspection of the mean scores indicated an increased relative % power TCA response associated with decreased *Strength* (*Weak*) responses for the odour *cf Strong* responses.
- *Mid* concentration 11 Hz: There was a statistically significant LTP differences between *Strength* groups: F(2, 58) = 5.95, p = 0.004, partial eta squared = 0.17. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Strength* for the odour *cf Weak* and *Mid-Strength* responses.

There was no statistically significant LTP difference between *Strength* groups in the *High* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following LTP *High* concentration responses reached statistical significance:

- *High* concentration 6 Hz: There was a statistically significant LTP differences between *Strength* groups: F(2, 54) = 6.27, p = 0.004, partial eta squared = 0.19. An inspection of the mean scores indicated a decreased relative % power TCA response associated with increased *Strength* for the odour *cf Weak* and *Mid-Strength* responses.

Right Temporoparietal (RTP) relative % power difference responses:

There was no statistically significant RTP differences between *Strength* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RTP *Low* concentration responses were observed to be approaching statistical significance:

- *Low* concentration 14 Hz: There was an [approaching] significant difference between *Strength* groups: F(2, 58) = 2.69, p = 0.08, partial eta squared = 0.09. An inspection of the mean scores indicated an increased relative % power TCA response associated with *Mid-Strength cf Strong* responses.
- *Low* concentration 16 Hz: There was an [approaching] significant difference between *Strength* groups: F(2, 58) = 2.47, p = 0.09, partial eta squared = 0.08. An inspection of the mean scores indicated an increased relative % power TCA response associated with *Mid-Strength cf Strong* responses.

There was no statistically significant RTP differences between *Strength* groups in the *Mid* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RTP *Mid* concentration responses were observed to be approaching statistical significance:

- *Mid* concentration 8 Hz: There was an [approaching] significant difference between *Strength* groups: F(2, 58) = 2.49, p = 0.09, partial eta squared = 0.08. An inspection of the mean scores indicated an increased relative % power TCA response associated with *Mid-Strength* and *Weak cf Strong* responses.

There was no statistically significant RTP differences between *Strength* groups in the *High* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RTP *High* concentration responses were observed to be statistical significance:

- *High* concentration 6 Hz: There was a statistically significant difference between *Strength* groups: F(2, 54) = 6.27, p = 0.004, partial eta squared = 0.19. An inspection of the mean scores indicated an increased relative % power TCA response associated with *Mid-Strength* and *Weak cf Strong* responses.

Table 4.3.7. *Strength* responses: Summary of the means identified through Post-hoc comparisons (using the Student-Newman-Keuls test) as significantly different relative % power Strength frequency responses for the four electrode groups and three concentrations of TCA in ethanol.

Location	[TCA]	Frequency	Group	N	Means	
Left Frontal	*Mid*	11 Hz	*Weak*	26	- 0.01	
			Mid strength	31	0.83	
			Strong	4		5.39 #
Right Frontal	*Low*	5 Hz	*Weak*	21	- 0.70	- 0.70
			Mid strength	23	3.12	
			Strong	6		- 0.41 *
	High	8 Hz	*Weak*	30	0.06	
			Mid strength	23	-1.40	
			Strong	4		4.92 #
Left Temporoparietal	*Mid*	8 Hz	*Weak*	26	1.24	
			Mid strength	31	- 0.25	- 0.25
			Strong	4		- 2.53 #
	Mid	**11 Hz**	***Weak***	**26**	**- 0.46**	
			Mid strength	**31**	**0.80**	
			Strong	**4**		**6.28 ****
	High	**6 Hz**	***Weak***	**30**	**0.49**	
			Mid strength	**23**	**0.26**	
			Strong	**4**		**- 8.09 ****
Right Temporoparietal	*Low*	14 Hz	*Weak*	33	0.21	0.21
			Mid strength	22	0.83	
			Strong	6		- 0.97 #
	Low	16 Hz	*Weak*	33	- 0.06	
			Mid strength	22	0.03	
			Strong	6		- 1.53 #
	Mid	8 Hz	*Weak*	26	0.37	
			Mid strength	31	0.24	
			Strong	4		- 3.72 #
	High	**6 Hz**	***Weak***	**30**	**0.49**	
			Mid strength	**23**	**0.26**	
			Strong	**4**		**- 8.09 ****

**** Bolded**: significant at Bonferroni adjusted alpha value, p<0.004
* significant only at p≤0.05, but above Bonferroni adjusted value (p>0.004).
# Not significant (p>0.05) but differences evident in Post-hoc comparisons.

Complexity power effects across frequencies

All responses were analysed for significant differences between *Complexity* groups in the combined frequencies. Results for the frequencies were then considered separately, using the Bonferroni adjusted alpha level of 0.004. All significant *Complexity* responses are summarised in Table 4.3.8.

Left Frontal relative % power difference responses:

There was no statistically significant LF difference between *Complexity* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following LF responses were observed to be approaching statistical significance:

- *Low* concentration 6 Hz: There was a statistically significant LF differences between *Complexity* groups: F(2, 48) = 3.59, p = 0.04, partial eta squared = 0.13. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Complexity* for the odour *cf Simple* responses.

There was no statistically significant LF difference between *Complexity* groups in the *Mid* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following LF responses were observed to be approaching statistical significance:

- *Mid* concentration 13 Hz: There was a statistically significant LF differences between *Complexity* groups: F(2, 48) = 3.28, p = 0.05, partial eta squared = 0.10. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Complexity* for the odour *cf Simple* responses.

There was no statistically significant LF difference between *Complexity* groups in the *High* concentration on the combined frequencies (p>0.004). Further, there were no significant LF differences in Complexity groups for the frequencies when considered separately.

Right Frontal (RF) relative % power difference responses:

There was no statistically significant RF difference between *Complexity* groups in the *Low*, *Mid* or *High* concentrations on the combined frequencies (p>0.004). Further, there were no significant RF differences in *Complexity* groups for the frequencies at these concentrations when considered separately.

Left Temporoparietal (LTP) relative % power difference responses:

There was no statistically significant difference between *Complexity* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following LTP responses were significant:

- *Low* concentration 6 Hz: There was a statistically significant LTP differences between *Complexity* groups: F(2, 58) = 2.3, p = 0.001, partial eta squared = 0.20. An inspection of the mean scores indicated an increased relative % power TCA response associated with increased *Complexity* of the odour *cf Simple* and *Mid Complex* responses.

There was no statistically significant LTP difference between *Complexity* groups in the *Mid* or *High* concentrations on the combined frequencies (p>0.004). Further, there were no significant LTP differences in *Complexity* groups for the frequencies at these concentrations when considered separately.

Right Temporoparietal (RTP) relative % power difference responses:

There was no statistically significant RTP difference between *Complexity* groups in the *Low* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RTP responses were observed to be approaching statistical significance:

- *Low* concentration 13 Hz: There was an [approaching] significant difference between *Complexity* groups: F(2, 58) = 3.11, p = 0.05, partial eta squared = .10. An inspection of the mean scores indicated an increased relative % power TCA response associated with *Mid Strength cf Strong* responses.

There was no statistically significant RTP difference between *Complexity* groups in the *Mid* concentration on the combined frequencies (p>0.004). When the results for the frequencies were considered separately, the following RTP responses were observed to be approaching statistical significance:

- *Mid* concentration 14 Hz: There was an [approaching] significant difference between *Complexity* groups: F(2, 58) = 3.30, p = 0.04, partial eta squared = 0.10. An inspection of the mean scores indicated an increased relative % power TCA response associated with *Simple* and *Mid Strength cf* Strong responses.

There was no statistically significant RTP difference between *Complexity* groups in the *High* concentration on the combined frequencies (p>0.004) or for the frequencies when considered separately.

Table 4.3.8. *Complexity* responses: Summary of the means identified through Post-hoc comparisons (using the Student-Newman-Keuls test) as significantly different relative % power Strength frequency responses for the four electrode groups and three concentrations of TCA in ethanol.

Location	[TCA]	Frequency	Group	N	Means	
Left Frontal	*Low*	13 Hz	*Simple*	21	- 2.64	
			Mid	24	0.11	0.11
			Complex	6		4.14 *
	Mid	13 Hz	*Simple*	20	- 0.13	
			Mid	37	- 0.16	
			Complex	4		1.21 *
Left Temporoparietal	***Low***	**6 Hz**	***Simple***	**25**	**- 0.18**	
			Mid	**29**	**- 1.65**	
			Complex	**7**		**6.96 ****
Right Temporoparietal	*Low*	13 Hz	*Simple*	25	0.31	
			Mid	29	0.33	
			Complex	7		3.26 *
	Mid	14 Hz	*Simple*	20	0.51	
			Mid	37	0.03	
			Complex	4		- 1.41 #

**** Bolded**: significant at Bonferroni adjusted alpha value, p<0.004
* significant only at p≤0.05, but above Bonferroni adjusted value (p>0.004).
# Not significant (p>0.05) but differences evident in Post-hoc comparisons.

Summary: Electrophysiological Relative % Power Analysis for Liking, Strength and Complexity effects across concentrations

In summary, the following differences in brain activity responses for the different rating scales were observed, with significance assessed using the Bonferroni adjustment value of p=0.004.

Liking effects across concentrations: Differences indicated increased responses to *Low* concentrations of TCA in the theta (4-8 Hz) range associated with increased liking, significant in the Left temporoparietal (LTP) area, and further supported by similar [approaching significance] changes in the Left and Right Frontal areas.

In the *Mid* concentration range, the difference between *Liking* groups for the *Mid* concentration TCA stimulus on the combined frequencies approached significance (p = 0.05). There was an approaching significant decrease in responses to TCA in the alpha range (9-12 Hz) and at 14 Hz, associated with increased liking for the odour - significant for 14 Hz in the Left temporoparietal (LTP) area, and [approaching significance] alpha changes in the Left Frontal area.

Strength effects across concentrations: In the *Mid* concentration, there were significant Left temporoparietal and approaching significant Left frontal differences associated with increased perceptions of strength – with an increase in 11 Hz (alpha range) responses.

There were Left and Right temporoparietal differences in the theta (4-8 Hz) range in response to the *High* concentration of the TCA stimulus, with a decrease in response to an increased perception of strength.

Complexity effects across concentrations: There was an increase in response to the *Low* concentration of TCA associated with perceived increases in complexity at both 6 Hz and 13 Hz in the Left Frontal, and in both the Left and Right temporoparietal areas.

These differences are summarised in Figure 4.3.2.

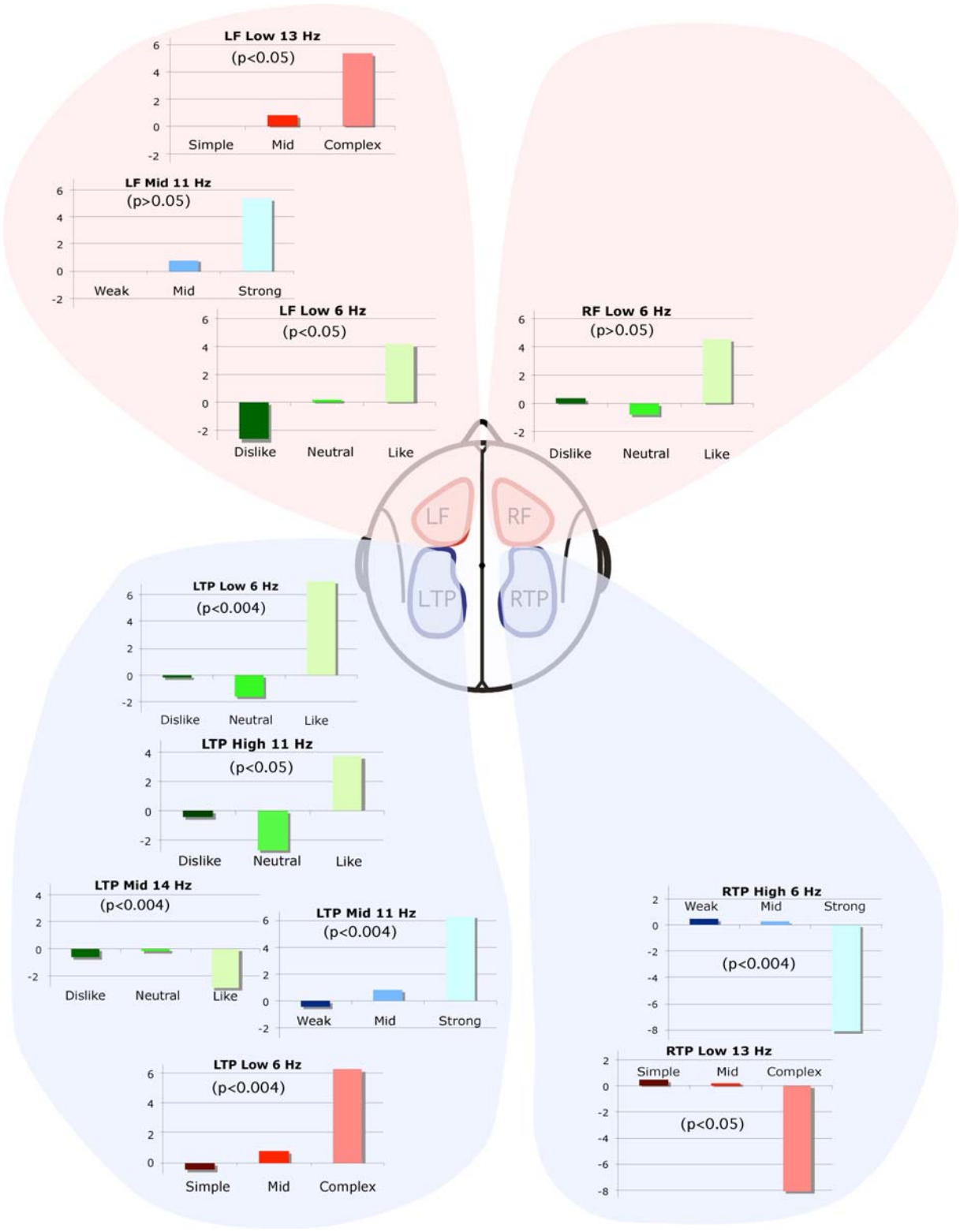


Figure 4.3.2. Illustration of the key differences in brain responses to the three concentrations of TCA stimuli for the Liking, Strength and Complexity ratings groups. The shaded areas indicate the Left and Right Frontal areas (top) and the Left and Right Temporoparietal areas (bottom). *Liking*: Dislike = dark green, Like = light green; *Strength*: Weak – dark blue, Strong = light blue; *Complexity*: Simple = dark red, Complex = light red. Significance levels are indicated.

Gender effect on relative % power differences

A series of independent-samples t-tests were conducted to compare relative % power differences for males and females at each frequency and for each electrode group. Results were again considered using the Bonferroni adjusted alpha level of 0.004.

There were no significant differences in relative % power difference responses ($p>0.004$), but there were some differences that approached significance ($p<0.05$), as summarised in Table 4.3.9.

Table 4.3.9. Gender effects - Summary of the approaching significant differences in relative % power frequency responses for males versus females at the four electrode groups and three concentrations of TCA in ethanol.

Location	[TCA]	Frequency	Significance	Group	N	Means
Left Frontal	*Low*	13 Hz	p = 0.04	Male Female	10 51	0.28 - 0.33
Right Frontal	*Mid*	13 Hz	p = 0.02	Male Female	10 51	- 0.60 0.12
	High	14 Hz	p = 0.04	Male Female	10 51	- 0.39 0.25
Left Temporoparietal	*Mid*	14 Hz	p = 0.05	Male Female	10 51	- 1.11 0.05
	High	16 Hz	p = 0.05	Male Female	10 51	- 0.86 0.20
Right Temporoparietal	*High*	11 Hz	p = 0.02	Male Female	10 51	- 6.55 - 0.71
	High	12 Hz	p = 0.01	Male Female	10 51	4.92 0.81

As can be seen from this table, there were only very small differences at some frequencies – predominantly in the 11 – 14 Hz range. With due consideration of the imbalance in numbers of males versus females, these results do not provide any evidence of consistent differences in brain activity responses related to gender in the sample population.

5 DISCUSSION

A major factor in our sense of smell is the variation in performance between people. Our sensitivity is best when we are young, but our identification is poorest. As we age, our identification improves substantially, and for most of middle age, we have good sensitivity. The variation in olfactory ability, at any age, is large, whether from sensitivity or identification limitations. To obtain acceptable 'population' data large numbers of participants are required to ensure that enough people across the spectrum of abilities are contained within the study. This was an initial goal of the current project. Unfamiliar, or unknown smells pose problems for those interested in using the sense of smell, or, relying on its functionality to get people to appreciate a product. The need for people to know a smell is not mandatory for positive or negative reactions.

It is necessary for an ability to communicate and relate our experiences to others. It is well known that expertise and familiarity can improve the recognition of odours and tastes. In fact, most industries relying on the sense of smell expect that their experts will be 'grown' not borne. This is true with the wine industry where wine judges need to be exposed to taints and then have the taint confirmed, before they can recognise and label these in the future. This process implies that the taint isn't self-evident to those who haven't experienced it previously, even if it is detected; that is, its features need to be learned. Another feature of our sense of smell is that it may lend itself to improvement with experience. The detection threshold may be lowered as one becomes more familiar with the odour. The mechanism behind this is not known, but it may be due to better handling of the signals by the brain, rather than a change to the olfactory epithelium resulting in altered detection by the receptors, or, an increase in the number of the receptors. Notwithstanding the mechanisms, it is quite clear that expertise and familiarity alters the ability to be aware of an odour. The logical extension of this is that experts are going to have different issues with familiar taints than with unfamiliar taints, and, non-experts will be different again in their reactions. What is less clear is how this familiarity alters appreciation. Do we all experience odours in the same way regardless of our familiarity or sensitivity? Part of the current project was to see if there were any relationships between sensitivity and appreciation. What is known is that, generally, as the concentration of an odour increases it usually becomes less pleasant and tolerable. How this is impacted by experience is not clear. The last issue which will impact on the whole response to taints is the decline in odour sensitivity with age. Experience comes with age, but deterioration in olfactory capacity also can increase with age, particularly with men. How experts can ensure their olfactory capacity is at a relevant sensitivity is an issue that the industry may have to address.

In summary, the project aimed to address some fundamental issues concerning olfaction and taints.

- The attitude to TCA in a group of randomly obtained non-expert consumers.
- The sensitivity to TCA of a group of randomly obtained non-expert consumers.
- The effects of TCA on the brain electrical activity (considered to be more objective than asking opinions) in a group of randomly obtained naïve consumers.

The method chosen for addressing these issues was to attract a large number of students to partake in the study where they would be asked to undertake three tasks:

- A smell sensitivity test to determine TCA threshold using specially designed odour pens.
- A smell sensitivity/preference test of TCA offered in 10% ethanol, red wine and white wine glasses.
- Using the determined concentration threshold for TCA the brain electrical activity changes caused by an ethanol/TCA mixture were recorded.

5.1 Methodological issues

It had been planned to obtain 120 participants for all three components of the study. A vigorous advertising campaign was commenced to determine expressions of interest. At first there were no reasons to expect that the goal of 40 students per year would be feasible. Progressively the numbers of students willing to participate declined. Those who did participate did not always complete all sections of the project. Despite two generous time-extensions to the project researchers by the GWRDC, it did not prove possible to obtain the planned numbers. The original project costs were determined assuming a high level of compliance by volunteers. As there was considerable time necessary to dilute the TCA in the ethanol and wine, prepare the EEG instrument and set out the threshold pens, if the volunteer failed to turn up, then the cost of the research assistant's wages for preparation still had to be covered. Additionally, there was the cost of the wasted materials and the cost of cleaning –up. In fact the wages cost was double the expected level for all of these 'failures". Over the initial duration of the project, the non-attendance for all three sessions was a minimum of 5%. Towards the end of the project the failure to attend all sessions climbed to 30%. Because the goal of the project was to analyse across the various protocols, this resulted in very much reduced capacity to fulfil the project's goals. An additional aspect was the slowly reducing number of volunteers over the life of the project. It became increasingly difficult to obtain participants and long periods of time occurred when recordings were not possible. The 'seasonal' nature of students as participants also was an issue. Unexpectedly, it was very difficult and not successful to attract students over the break between years where there is a four-month interval during which the students are not attending university. While this may have been something that should have been expected, the reality was that our previous studies, and the pilot study carried out for the GWRDC didn't suggest this to be as much of a problem as it turned out to be. In fact the consequence was that the students were available when the staff involved in the project had the least available time (teaching periods) and were not as available when the staff were free.

In an attempt to push the project and overcome the declining availability of GWRDC funds because to the unacceptable number of 'no shows', a request was made to the Faculty for support and an amount of $20,000 was provided. It was hoped that this would allow, during the last extension provided by the GWRDC, the employment of more research assistants who could be available when the potential participants were most likely to be around. Sadly, this coincided with the decline in participation. The researchers are quite disappointed in this outcome when a very well designed project which depended on numbers of participants could not achieve the expected target had not been as effective as expected. The consequence of there being only effectively 62 people who have completed all tasks is a much higher attrition rate than was anticipated; in fact, much higher than any attrition in any other studies carried out in the researcher's laboratory where over 500 participants have been processed in a range of projects. It is not clear what the cause of the decline in participation was, except in does not seem to be restricted to this one project, but may be endemic. Possibly the competition for student participants in our university has become counter-productive. Possibly the competition for subjects where other projects were offering compensation which was several times higher than that being provided in the project being reported here, reduced the willingness of students to be involved. Possibly, students have to earn more money to survive and the compensation for their time was considered a 'poor' comparison to part-time work. In other projects carried out in our laboratory, the percentage of international students – who often reside nearby – was higher than in the current project. There is anecdotal evidence that over the period of this study, student attitudes to attending classes and involving themselves in university life may have changed.

Notwithstanding these serious limitations, for which there is no remedial action, the results obtained from the completed participants contain some important - and significant – data. It must be emphasised that all participants were random volunteers, all were young, the majority (75% of the participants in the EEG-TCA concentration study) were female (an

important point), and most were non-smokers (87%). They all had been evaluated as having a normal score in an internationally accepted olfactory performance test (Kobal *et al.,* 2000). Very few assessments of the influence of TCA would be based on such a critically assessed group. With a mean age of 20.8 years (with the oldest 32 years), this participant group would be expected to have a mature and effective sense of smell, which was confirmed by their scores on the evaluation test.

5.2 Descriptor ratings and concentration effects of TCA in ethanol

The first set of results concerns the use of descriptors by these non-expert participants when exposed to TCA in ethanol. The methodology used for this study resulted in a comparison being made between ethanol, and, ethanol plus the designated TCA concentration. Consequently, the difference is due to the presence of TCA only. The concentrations of TCA involved were chosen to be around those levels generally accepted by the industry as around threshold or just below for most individuals. Although there seems to be a wide range in the industry as to what these levels are, those chosen for this study were in line with observations reported in the literature (Evans *et al*., 1997; Casey, 1999; Prescott *et al*., 2005): these were three concentrations from 0.1 $ng.l^{-1}$, 0.5 $ng.l^{-1}$ and 1.0 $ng.l^{-1}$.

There were several significant trends of interest in light of some of the literature based on experts' responses. The first trend in conflict with the literature is the decrease in the use of "*chemical*" labels and alcohol labels as the concentration of TCA increased in the samples. It has been reported in the literature that TCA detracts from the wine flavours as the levels of TCA increase (Butzke *et al*., 1999), and yet our participants lost the chemical and alcohol perceptions. In other words, the nature of what they were smelling was regarded more favourably as the TCA concentration increased. Further, the increased use of "*fruity*" labels is in conflict with the reports from expert-based studies (Butzke *et al*., 1999). The increased use of "*musty*" labels for descriptors as TCA levels increased does support the fact that the TCA was, in fact, more evident, and supports reports from the literature that "*musty*" odour qualities are only present in much higher concentrations of TCA (Casey, 1999). What these non-expert participants are telling us is that the TCA is not necessarily as negative a component as experts would report. While there was a slight increase in the "*negative*" descriptors with exposure to the increased TCA concentrations, the change was not great. These descriptors – "*bitter, musty, mouldy and rotten*" – could be expected to be aligned with a certain amount of consumer dissatisfaction if experienced in a product. Interestingly, the largest step increase (from a total of 9.9% to 16.2%) in these descriptors being reported occurred with the first concentration increase from 0.1 $ng.l^{-1}$ to 0.5 $ng.l^{-1}$, while the next step showed little change (from 16.2% to 16.6%). Yet, the actual percentage of people using these descriptors was small, the maximum being 7.1% for "*musty*" for the strong concentration of TCA. This compares with 16.9 for "*sweet*" and 12.4 for "*fruity*". This strongly suggests that the reactions to the TCA in non-expert consumers, even at the highest concentration used, was not universally negative. It suggests that less than 10% of people may be put off by 1.0 $ng.l^{-1}$ of TCA. The decline in the use of "*chemical*" descriptors with increasing TCA concentration might even be suggestive of an "improvement" in the acceptance of the straight ethanol which was used for the substrate. At the high concentration of TCA, 11.1% of participants could not detect the TCA. Approximately the same percentage of participants (7% - 8%) were unable to detect the TCA at the low and middle concentrations.

5.3 Subjective ratings and concentration effects of TCA in ethanol

The second set of results relate to the rating of the subjective preference and complexity of the TCA in ethanol. Because these resulted in *Likert* scale numbers, statistics could be carried out to determine levels of significance. Interestingly, there were no significance differences in liking across the TCA concentrations with most placing their preference in the middle of the scale between "*strong dislike*" to "*strong like*". This suggests that the majority

of people were neutral to the experience, even at the higher concentration. This could result in the conclusion that the presence of TCA at these levels (up to 1.0 ng.l^{-1}) is neither pleasant nor unpleasant, and therefore may not detract from the experience of the smell of alcohol at these levels.

The results from the reports of *Strength* and *Complexity* were very similar with no significant differences between the concentrations and the means were close to the middle of the allowed range. For the *Strength* report, the means were closer to the "*very weak*" (means of 37, 38 and 33) end of the scale even for the strongest concentration (mean was 33). This is consistent with the results obtained from non-expert participants when their thresholds for TCA were measured which suggested that the mean level of threshold was 3.86 ng.l^{-1} (using odour pens) and so the levels used for the "*strength*" determination were below the mean threshold. It is also interesting that the "*complexity*" rating did not change with concentration, particularly as there was an increase in the reporting of fruitiness and sweetness of the ethanol with more concentrated TCA. Perhaps the concept of "*complexity*" is not clear to the non-expert consumer. Confound this with the fact that ethanol is not "*complex*" and the subtlety of changes in the nature of it with added TCA appears lost on the less experienced. It would be very interesting to compare directly non-expert consumers with a group of "young" and a group of "older" experts using ethanol, rather than the more complex situation occurring with wines. While TCA is not consumed in ethanol, the addition of the wine complexity is likely to blur the ratings from the non-expert panel who lack the lexicon and experience to be able to conclude their opinion.

One of the consistencies in the study on L*iking*, S*trength* and C*omplexity* was the similarity of the ratings and the standard deviations. Individuals clearly were rating their subjective determinations of these three descriptors across the full range (0 – 100) of the scales. The lowest rating was "0" (for *Strength* with the *Low* concentration of TCA) to "96" for *Complexity* (also in the *Low* TCA concentration). It may very well be that these results are merely reflecting a very 'noisy' system and that for such difficult subjective measures, in the absence of experience to guide them, the non-expert panel results reflected their 'ignorance' more than their true opinions. If so, then TCA as a taint is not going to be predictable in its impact on the majority of consumers, and apart from its elimination completely, its negative impact on consumers cannot be predicted at any low level. This conclusion may be supported by the observation that there was no correlation found between a person's threshold level and their subjective report of how much they liked the mixture, how strong they found it, or how complex it was. In other words, no matter how sensitive they were to be able to detect TCA, their rating of it was nearly random. The most likely explanation, other than the population was not representative (it must be noted here the high proportion of women in the trial), is that the lack of experience and potential 'bias' produces a broad range of reports for which there is no overall conclusion possible. That is, the group tested in this study failed to provide an overall conclusive 'choice' about TCA and it presence or near absence was basically irrelevant. It would be very interesting to repeat this with one gender and with a wider range of concentrations.

There were, however, significant correlations obtained for some interactions in this study. '*Liking'* and '*Strength'* were weakly positively correlated. This conflicts with the usual observations in experts that more TCA is less liked. Similarly, '*Liking'* and '*Complexity'* were weakly positively correlated. It must be borne in mind, however, that these observations are based on results in which the means were very similar and that the strength is not a fully open-ended measure, but has a lower limit, and the mean threshold level was above the concentrations used in this part of the study. This will compromise the ability, especially of these inexperienced people, to make interpretative comments of the nature of the TCA. Notwithstanding this limitation, it is unexpected for TCA, as a taint, to be reported as more liked as the concentration increases. Further, the positive correlations were also observed in the middle concentration and the highest concentration, which might be saying that the TCA concentration chosen is not an issue, as the correlations didn't change with concentration.

5.4 Electrophysiological responses: brain activity as an objective measure

As discussed previously, the nature of inexpert responses are potentially limited by this lack of experience. This can be taken further so that as naïve consumers have less basis upon which to judge small imperfections in wines, they are less able to report these. Experts, though, have their discipline dogma which may influence their opinions: few people, expert or otherwise, will fly in the face of the popular opinion. Just as the acceptance of alternative closures to cork will take time for it to be generally accepted, so will any change in general views be slow to be accepted.

One approach to overcome some of these concerns is to use a more objective measure for some of the determinations; that is to record changes in the electrical activity of the brain in responses to the presence of the odour. It is assumed that the early responses recorded in the brain to odours are less subject to alteration by cognitive and emotional processes. These occur very quickly and it is believed that it takes several seconds for emotional and considered opinions to occur. It is also proposed that the brain will respond to odours even if there is no conscious awareness. In other words the basic change in brain activity is independent of the need to identify the odour as identification takes much longer to occur.

For this reason we have engaged EEG recording as a means of independently verifying that people can respond to the odour and are not 'faking', which is much harder to be sure of in oral reporting. The basis of the recording is that the TCA is delivered in air including ethanol, because the TCA must be dissolved first. The problem that could arise with is approach is that we have to choose something with which to compare the responses to odour. In our previous experiments we have always used two states and compared the response from each: we take all the changes in brain activity when people inspire air and compare these to (or subtract these from) responses to air + some odour mixed in the air. Because the air is common to both, as is the activity involved in breathing, then the response left when we subtract the two sets of signals must be caused only by the odour. The delivery is via our unique odour delivery system which delivers a small volume (usually 1 ml) of air, or air+odour from a motorised syringe into the inspired air stream so that the participant is able to take the odour sample into their nose. The odour is exhaled at the next breath. All the delivery, then, is controlled by the individual's breathing which we monitor. This is a very natural way of being exposed to odours. It must be stated that this does not engage the most sensitive way of detecting odours which requires 'sniffing' the odour. Rather what we are doing mimics the effects of someone first detecting a smell as they breath normally. We fully expect that the responses we obtain in this approach are always going to be lower, or less intense, than would occur if we allowed the participants to sniff.

In the current study this procedure had to be changed slightly as the odour – TCA – is not soluble in air. After extensive trials involving much GC-MS analysis we finally settled on dissolving the TCA in alcohol and then putting a very small volume of the mixture – to provide the required concentration of TCA in the inspired gas – into the delivery syringe. Instead of our usual 'air' control, we used a syringe into which the same quantity of ethanol had been added. In these circumstances the only difference between the two conditions is the presence of TCA. So, the results we have reported for the TCA are in fact the result of subtracting the ethanol control observations from those coming from breaths which inspired the TCA. Consequently, changes can be to increase or decrease a measure of brain activity as a response to TCA. The concentrations chosen were intended to be similar to those used in other parts of the study. Unfortunately one of the limitations of the approach we use is that people inspire at different rates (air flow velocity) and different levels (air flow volumes) and these will both effect the actual concentration of the TCA hitting their olfactory epithelium so the absolute amounts impacting on their receptors are unknown and are expected to vary between people. It is therefore more appropriate to look at the differences in response to the three step changes in concentration of TCA, because here we are looking at the effect of a known increase. All that can be calculated is the quantity of TCA which is available to be

delivered each breath. What the concentration of this is when it impacts on the olfactory epithelium is no more capable of being absolutely determined than when people sniff wine!

5.5 Relative % power associated with concentration differences

The amounts of TCA delivered each breath in this study were 20 ng (*Low*), 100 ng (*Mid*) and 200 ng (*High*). Brain electrical activity also varies in the temporal, spatial and frequency domains. Analysing all of these is extremely tedious with no guarantee of success, so we choose to look at the frequency domain and see if there are TCA-related changes to be seen. This is valid, as it is well known that arousal, and mental activity, and sleep, all alter the frequency domain. The brain exhibits electrical activity, or electrical power, over a range of nearly 0 Hz to above 40 Hz. 'Power' in these situations is really a measure of the number of neurons (nerve cells) which are actively firing. Their tiny electrical signals are recorded and added together if the cells fire in synchrony. Because the total number of neurons firing at any point in time is variable the total 'power' also varies; similarly, the level of signal recorded from different people may also vary.

Rather than assume a constant level of brain activity during our experiments, we compute the 'relative power' where we add up all the signal levels we have measured in each of the circumstances and equate these to 100% and then report the different frequencies as the relative proportion of this. In this way we can make reasonable comparisons and also combine the number obtained from different people for the purposes of statistical analysis. In reality all frequencies are represented to some extent at all times, but in sleep there is an increase in the lower frequencies (less then 8 Hz) over rested awake state. At rest with little stimulation, the frequency band in which most of the brain's activity occurs is in the "alpha" range which is between 8 Hz and 12 Hz. The overall trend is for arousal to move the information processing to higher frequencies and for reductions in brain activity frequencies to be more representative of calming, sedated states. While these are generalisations, they are sufficient for the current purposes.

Another factor in the analysis of these data is that there is decreasing 'power' or activity levels in the higher frequencies, so there is little point in examining those above 20 Hz. We directed our interest at the frequencies below 20 Hz and looked for differences caused by the inspiration of the TCA in ethanol at three different 'concentrations'. As mentioned before there are 'spatial' issues with the brain. It is known that some electrical activity found in certain regions of the brain are related to certain types of processing: vision, hearing, sensory, speech are all located predominately – but not exclusively – in certain anatomical regions. That is there is a spatial separation of processing.

While the brain is a very extensive anatomical structure, traditionally it is divided up in to two hemispheres which are in turn divided in to mirror image regions. From previous work on the EEG and olfaction we know that the most likely regions in which odour-induced changes are going to occur are those towards the front (the frontal regions in which it is known that a lot of association of ideas and processing of higher concepts can occur); the temporoparietal region which lie close to the ears and are known to involve sensory functions. These regions – four in all – are those we analyse data from. People were asked to report, at the end of the delivery runs, their experiences on *Likert* scales for '*Liking'*, '*Strength'* and '*Complexity'*. In our results we have reported that there were trends rather than significant results in the regions and the frequency bands to TCA. The reason the original number of participants were required was to ensure – from our extensive experience in recording EEG to odours – that we were in a strong position to get significant results. Having achieved fewer than half of the number we expected, we are forced to report analysis on less than desirable numbers. Yet, we had both trends and probabilities which were approaching significance! We are confident that had we been able to obtain the requisite numbers then these results would have been more definitive. It is fully expected that with more than a doubling of the participants who should have been involved in the EEG trial that these statistics would have been significant.

5.6 Relative % Power Analysis to determine odour effects across concentrations

The results of a comparison of responses across TCA concentrations showed that TCA produced a change in the 4 Hz for TCA in the left frontal region associated with increasing concentration of the TCA; that is, a change in 4 Hz responses was associated with the high level of TCA. A more detailed analysis of this shows that the trend is in fact made up of a two-part response. With the delivery of the small amount of TCA there was a reduction in the left frontal activity compared with the straight ethanol, which could be taken to be a 'turning off' of brain activity in this region in this frequency. For the intermediate amount of TCA the response was similar, but when the greatest level of TCA was administered the response was a large increase in the level of activity in the left frontal region in the 4 Hz region. Contrasting this is the brain activity which occurred in the 11 Hz region where only the middle amount of TCA caused an increase in the activity with the TCA, while the small and large amounts resulted in a decline in activity at this frequency compared with ethanol alone. A similar response was noted for the right temporoparietal region at 11 Hz. Had these trends been significant then it would suggest that we have a discriminating pattern of EEG responses related to concentration. The lack of significance, however, does mean that such a claim is unwise without extra data from more recordings. Post-hoc analysis and application of rigorous statistical criteria ($p<0.004$) showed that the left temporoparietal region at both 6 Hz and 14 Hz had a significant difference in power for the low and middle TCA amounts between *Like* and *Dislike/Neutral*. No other location or frequency investigated showed this clear significance. They, did however, approach this rigorous level of significance, so the presumptions as to where in the brain the differences may reside were not inappropriate. Differences between the left and right sides of the brain in relation to preference have been shown previously in our laboratory with other odorous stimuli.

The examination of the subjective *Strength*/EEG relationship revealed that the left temporoparietal regions had a rigorously determined significant difference ($p<0.004$) between the large amount of TCA and the small and medium amounts in the 6 Hz and 11 Hz frequencies. The difference between the 11 Hz and 14 Hz of the liking relationship (see above) may not be critical or of concern, so the two subjective reports of *Liking* and *Strength* of the TCA may, in fact, be related to similar frequency changes. They definitely relate to the same region of the brain. A similarly rigorous analysis of the *Complexity* rating also involved the left temporoparietal region and the 6 Hz frequency where *Complex* was different from *Simple* and *Mid* ratings. It is not appropriate to infer some deeper meaning to these results other than to say that the EEG changes were able to decode relationships which existed between *Liking*, *Strength* and *Complexity* which were not apparent in the subjective ratings when measured alone. As a reminder, there were no significant differences in either of the three rating scales (when considered alone) across the concentration of TCA. The uncertainty in this comparison is of course the level of exposure to TCA of the two different delivery techniques.

5.7 Comparison of Relative % Power responses for Liking sub-groups at different TCA levels

By selecting the extreme response people from the original 62 and analysing their power spectra, the extreme responses could be compared with the mean results from the remaining more moderate responding people. The issue with the data from the majority is that it represents a group of people who were neutral in their responses rather than extreme. Whether this is due to their lack of expertise, or not, is debatable, but this must have be an influence in their judgement. The logic behind the 'extreme' response argument is that one can check to see if there were major differences between those who reported a strong dislike and those who reported a strong liking. The statistical power of this approach is very limited because of the small numbers found in these sub-groups, however, the trend is the important

issue. It can never be guaranteed how many people in a sample of a population will fall into these arbitrary groupings: in fact there is no guarantee that if the questions were asked at a different time if the responses would be consistent. Such are the vagaries of asking opinions, particularly on an unfamiliar smell. A very worthwhile future study would be to examine this point: how consistent are people in their subjective rating of a taint such as TCA and how does the situation affect their view, in other words, if the testing was carried out in a group panel situation, instead of individually, would the responses alter?

The graphs of the analyses of the sub-groups (*Low Dislike*, *Low Like*, *High Dislike*, *High Like*) revealed some interesting patterns; some of the differences ($p=0.04$ for *Low Dislike* versus *Low Like* at 9 Hz) were approaching significance. One group of interest are those who expressed a view that the TCA was disliked and their opinion was maximum value reported. Similarly, at the other extreme were those who strongly liked the TCA.

With the 'alpha' range (9 Hz to 12 Hz) the very dramatic difference – but not significant because of the small numbers in this group – was at 10 Hz (right in the middle of the alpha band). At this frequency, *Low Dislike* represented a situation where the participants had a lower percentage of this frequency when they were exposed to the TCA than without. In contrast to this, the *High Dislike*, in the same alpha band, showed a lower percentage when exposed to the TCA in the 11 Hz frequency; and, there was an approaching significant difference with the High Like group ($p=0.08$). Those who reported their opinion to be in the High Like sub-group had an increase in alpha frequencies when they were exposed to the TCA, particularly at 9 Hz and 12 Hz. Although the data probably don't support general statements, there is one possible conclusion from these results: increasing alpha is usually taken to indicate a more "relaxed" state. Do the trends observed at 9 Hz and 12 Hz for the High Like sub-group tell us that they were more 'relaxed' about the TCA because it wasn't unpleasant for them? Superficially these results may support this proposal.

This conclusion has a pitfall which is behind all of these general views of the function of the brain and its reduction to a few frequency bands. If the 9 Hz, 10 Hz, 11 Hz and 12 Hz results were added together the alpha band result would alter to be a slight overall increase. The inner detail of the changes within the alpha band are lost in the generalisation, however, our understanding of the working of the brain is not yet sophisticated enough for us to be able to know if we are 'reading' too much into the patterns.

This issue is supported by the observation that the largest relative change in the theta band (5 Hz to 8 Hz) was at 7 Hz where the *High Like* sub-group showed a very large increase in the relative power to the presence of TCA. Again the *High Dislike* sub-group had a decrease in the 6 Hz frequency to the presence of TCA. Realistically, the relevance of these differences in the frequency is quite indeterminate at the current state of knowledge. Do 7 Hz and 9 Hz represent some essential, fundamental characteristic of neural processing? Probably they do not. They, most likely, are the best hints at underlying processing that our technology can reveal.

There are three observations from the analysis of the multi-factor graphs (shown in Appendix 5). In an attempt to include as many of the variables simultaneously as possible we created bar graphs which allowed a direct comparison of all the results from the EEG study. Twelve graphs were created. Six were from data from each of the cortical/scalp regions (three left and three right frontal; three left and three right temporoparietal). Within each set of three there was one for the low concentration, one for the middle concentration and one for the high concentration. The bars plotted the changes to the electrical activity levels in response to the presentation of TCA in ethanol as a percentage difference relative to the total power obtained. There were 13 sets of bars; one for each of the frequencies of interest (4 Hz to 16 Hz). Lastly, in each set of the bars there was the level for that frequency of the different groups of people based on their responses to the experience (*Dislike*, *Neutral* and *Like*).

These sorts of data do not lend themselves to reasonable statistical analysis as the number of comparisons are great, and the data cannot be assumed to be from normal

populations and are not really 'paired' as they cannot be assumed to be obtained under identical conditions. From these, though, there are six obvious patterns. Firstly, the results for the cortical/scalp regions for the middle concentrations are an amalgam of the low and high patterns across frequencies. This is what would be expected as we have a discontinuous transition for individuals as the concentration is increased in the three steps. The reason why the middle concentration pattern is not simply the sum of the other two lies in the nature of the variable TCA thresholds of our participants and the mixture of the preferences (dislike, neutral and like). Our participants were predominately of the view that they 'Liked' the TCA more than they disliked the TCA, but at what concentration they 'switched', became conclusive, or had the underlying brain changes in response to the TCA alter, is likely to have been highly variable. The most likely situation is that the sub-group of our participants who had large changes in brain activity would swamp those with smaller changes. In addition there is no guarantee that we all have identical brain changes in response to stimulation intended to elicit an hedonic response. There is nothing to say that person A might dislike the TCA and the predominant change in their frequency is at 5 Hz while person B says the dislike it, but in reality is not so adamant and their change is at 6 Hz; or, even at 6 Hz when they also definitely dislike the odour. So, the superficial similarity between the middle concentration results and the high and low concentration results is pleasing, but not one that would be able to be interpreted further.

Two very prominent differences may be more revealing. The first was a large increase in the 4 Hz power in the left frontal in those who expressed *Dislike* at the *High* concentration. If this was simply an experimental situation aberration then it could be assumed to be present in all the left frontal conditions, but it is not present in the *Low* and *Mid* concentration graphs. It is logical to assume that at the highest concentration we used, we would expect that largest number of people in our study would be detecting the TCA. Logically, the TCA would be expected to be at its most 'offensive' for those who disliked it. This conclusion may gain support from the fact that there was a trend in the same direction for the right frontal recordings as well as in the left temporoparietal, although neither of these looked as dramatic as the left frontal.

The second of the prominent differences was the increase in activation with the delivery of *High* concentration of TCA for those who reported they liked the stimulus. In this situation, the participants who so reported showed increases in the percentage of power for most of the alpha band frequencies in the right temporoparietal region, less dramatically there were increases in the left temporoparietal for the same people. Large increases in relative power were observed for those who reported liking the TCA at the *Low* concentration in the left temporoparietal region at the 6 Hz frequency with a smaller increase at 7 Hz. To emphasise the complexity inherent in this interpretation of consequences associated with this unknown mechanism and relationship, in the same region, there were reductions in activation for the TCA for those who *Like* the experience in the 4 Hz and 5 Hz frequencies. Our state of knowledge of the meaning of changes in electrical activity of the brain is so primitive that we cannot be sure of the significance of these observations. We can be sure that it is only observed in those who reported they *Liked* the TCA and only in these frequencies recorded from this region. It is tempting to suggest that these sorts of patterns are our first exposure to this sort of information where preference, regions, frequencies and TCA are all linked. A further feature of the data is the similarity between these changes and ones seen in the records for those who *Liked* the TCA at the *Mid* concentration. A prominent decrease was seen at 4 Hz here as well. The situation at 6 Hz was the inverse for the *Mid* frequency, however, but at 12 Hz there was an increase where with the *High* concentration results an 11 Hz increase occurred for liking and left temporoparietal. In both the left and right frontal regions there was a similar increase, but at 6 Hz, for liking the TCA.

These observations are both intriguing and frustrating. There are hints of underlying consistencies in various cortical/scalp regions at certain frequencies for *Like* and *Dislike* which vary with concentration of the TCA. The data are frustrating because of the residual variation which precludes specific definitive conclusions. Unfortunately, this is a

consequence of dealing with enormous detail which is linked to a high degree of variability in the preferences/opinions and experiences of the participants. One possible solution is to harness the experience of the experts to see if there are consistencies between their responses and those of the naïve [non-expert] consumer. It would be interesting to determine if, by virtue of their training, experience and expectation, the experts were more alike – less variable - in their responses; that is more of a mono-culture. Aspects from their results – most likely based on reports of dislike – which may parallel results from the naïve participants, could then help distinguish the common thread linking naïve and expert in the nature of brain activity changes. Until there is a way of reducing the variability of subjective hedonic responses, the results from the study of the EEG will remain a tantalising, but frustrating, window into the mechanisms of what happens when we like, or dislike something.

6. CONCLUSIONS

The presence of the over-riding individual variability is a constant confound. The relationship between brain electrical activity and preference is one which is being examined with no conclusive information as yet, probably because of the previous confound and the variations which may occur in each of us when we process the same opinion. Further, the degree of variation in what is represented by a 'strong' or 'weak' descriptor further muddies the waters. The accepted, but very limited and crude division of brain activity into frequency bins may be counter-productive if the actual changes are occurring in different ways inside these frequency groups, such as an increase in one frequency at the same time as a fall in an adjacent one. If the premise that increasing power in the alpha frequency band is related to a change to more quiet, relaxed activity, is combined with the proposal that unpleasant things are likely to be 'warnings' and negative reactions are going to arouse us and so enable us to avoid them, then our results are very informative. The trend for the extreme dislike responders is to have evidence of reductions in activity in the alpha band, as well as nearby frequencies in response to the presence of the TCA. Conversely, the trend in those who reported strong liking for the TCA had overall increases in the alpha band. While it may be seen by some to be over-interpreting these results, at some time we must have data which can assist in decoding the relationship between electrical activity and perception, preference and detection. This study is one in which some of these relationships were expected to be available for analysis and consideration.

The original design of the project was predicated on the expectation that with a big enough sample of the population, the numbers of people in these sub-groups would have meant a realistic statistical analysis. What occurred was that most of our participants were not extreme in their views of TCA: they neither disliked it strongly, nor liked it strongly. They were, it appears, neither turned off nor turned on by the TCA at the concentrations we delivered to them. As these were a somewhat random population, it is assumed that their responses are not likely to be dissimilar to the wider population of people who are not expert in wine issues. In which case, the results indicate that TCA is not a major issue for these sorts of potential consumers. That they were predominately young, university students, it may be argued that they may not be totally representative of the bulk of the population, however, it could also be argued that they – as future professionals – may be very representative of those who may be significant consumers of quality wines in the future.

The results from the threshold testing of the three wine taints – TCA, 4-EP and 4-EG – revealed interesting relationships which have to be considered in light of the situation where these participants would have minimal familiarity with these compounds. We have a series of comparisons where the taints were offered in four situations of 'increasingly complex' background conditions: simplest, artificial delivery in the form of odour pens; slightly more complex and relevant delivery in ethanol; next most complicated and relevant delivery in white wine; most complicated and relevant delivery in red wine. As expected in every case the threshold for the taint was lowest when delivered in the odour pens. This supports the previous conjecture that the ability to detect these taints at low levels in inexperienced people

is going to be confounded by the complexity of the background. The more distraction present, the less they will be able to distinguish the taint; a condition we would argue is overcome in experts by their experience. Surprisingly, the taints were next best detected in the two wines with approximately similar detection thresholds in both white and red wine. The most concentrated levels for detection were in the ethanol. As a pure compound, the ethanol is missing all the subtleties of the wines, and yet the participants needed more of each taint to be sure of its detection. Generally, the levels of 4-EP and 4-EG are within the ranges suggested in the literature; however, the level we found for TCA is probably much higher than those suggested for experts.

This project tackled the somewhat risky task of evaluation of responses to taints in a group of non-experts who were young and with no obvious issues regarding their ability to smell. While the participants were screened for a normal ability to smell according to an internationally-accepted olfactory ability test, there is always some underlying doubt as to the actual ability to smell the specific odour which is not part of the screening test. This aspect was overcome by developing a similarly delivered screening test for TCA, using the same pen-based technology. While the pens were of a different type to those in the commercial test kit, the development of the TCA pens followed a rigorous analysis which leaves little doubt as to their efficacy. The link is then from the screening test through the TCA pen-test of threshold, to the rest of the experiments. This rigorous approach reduced the likelihood that participants would be included who couldn't smell TCA, that is, they had a specific anosmia to the taint, or, they were so high in their threshold for the material that they would not be able to detect it at the levels we were using. What transpired was that all of the participants were able to show they had a threshold to detect TCA in the range of the pens; no anosmia to TCA.

By including young people predominately, the project avoided the issue of declining olfactory ability in the more mature. It also minimised the impact of experience – specifically of wine taints – on the expressed views about the 'quality' of the olfactory experience. That the results revealed a less than dramatic attitude to the TCA, even though the participants were able to detect it, suggests that the issue of TCA – in this group – was not a strongly held negative response. While this is concentration limited, we didn't use a wide range of concentrations, the levels we aimed for fall within the lower limits of those recognised in the literature. The question that is then posed is why did we not use stronger concentrations where dislike might have been more consistent? The answer lies in the desire to be able to avoid contamination of the location of the study, to avoid the risk of saturation (particularly when we had to deliver multiple exposures in the EEG trials), and to keep the experience such that we would not risk participants withdrawing from the project because of unnecessary unpleasantness. The object of the project was determine the responses of naïve people, not to create a totally negative response, which might be permanent, to the exposure of high levels of TCA.

Concluding Summary

Overall, the project has revealed important industry issues.

- Taints are not universally disliked by the inexperienced.
- Taints are, in fact, sometimes described positively by the inexperienced.
- Detection of taints at low concentrations has been confirmed by changes in brain electrical activity to the taint, which can be the only cause of the changes in our paradigm.
- The use of EEG as a method for probing the development and characterisation of hedonic responses has been further supported by the nature of the results.
- It has not been possible to definitively state what sort of brain electrical change is always aligned with 'liking' or 'dislike', but there are strong suggestions of a pattern.
- Inexpert consumers are very diverse and the need to eliminate TCA is not recommended, but that 10% of inexperienced consumers may find it unpleasant.
- Inexpert consumers may include 10% of people unable to detect TCA even at 1 ng.l^{-1}.

- Of the population in our study, the majority could detect the TCA at 1 ng.l^{-1}, and, they were more likely to state a liking for it, than a dislike based on our 100 point scale with 'neutral' set at 50.

7. OUTCOMES

This project was an investigation into high-risk, innovative and strategic research to culminate in a new approach to determining non-expert consumer responses to wine taints.

7.1 Outputs and Performance Targets

This study succeeded in meeting the proposed performance targets and outputs in successfully correlating differences in subjective and objective measures of wine taint responses with untrained consumers.

- TCA sensory panel thresholds were determined incorporating doses of TCA in difference wine varieties and in ethanol, for comparison with TCA delivered in pen-like devices.
- Sensory odour panel sessions were conducted using 4-ethyl guaiacol, 4-ethyl phenol in a replication of the TCA sensory panel tests, for comparison with threshold and subject responses to TCA.
- The use of different concentration levels of different concentration levels TCA attained the performance targets, with successful evaluation of the brain activity responses associated with concentration and preference (liking) responses.
- Preliminary data for the 4-ethyl guaiacol, 4-ethyl phenol sensory testing were reported to the industry at the AWITC 2004. Journal publications will follow to report all aspects of the now completed project.

The only aspects of the current project which did not succeed were related to:

- Lack of success in recruiting the anticipated numbers of participants for the proposed recordings. Recordings continued throughout the project, but due to the need for repeated sessions with each participant, there was an increased "failure rate" in participant completion that far exceeded all previous projects and therefore was not anticipated. This was further compounded by the periods of reduced availability of student participants throughout the academic year.
- The lack of completion of recordings using eucalyptus as a wine taint. This had been proposed later in the development of the project, in addition to the main focus of investigating the other taint responses, but the eucalyptus testing was not completed, as the research became focussed on completing the key aspects of the study with TCA, 4-ethyl guaiacol and 4-ethyl phenol.

7.2 Project Implications and Conclusions

- The sensory and physiological techniques used in this project provided evidence of reliable differences in brain activity processing between like and dislike and between the presence of alcohol and an odour plus alcohol.
  - Detection of taints at low concentrations has been confirmed by changes in brain electrical activity to the taint, which can be the only cause of the changes in our paradigm.
  - The use of EEG as a method for probing the development and characterisation of hedonic responses has been further supported by the nature of the results.
  - Inexpert consumers are very diverse and the need to totally eliminate TCA is not recommended, because only 10% of inexperienced consumers may find it unpleasant.

- The degree of variability in responses to TCA was clearly evident in this study in both subjective and EEG responses.

Although this research still contains substantial unknowns in terms of the fine detail of the nature of the relationship between brain activity and preference, the potential for the application of objective techniques to contribute valuable information about flavour responses has been demonstrated.

7.3 Research and Communication Strategies

The results of this project will be communicated to the scientific and wine industry communities through scientific publications, conference presentations and industry seminars.

Further research will assist in determining the consistency of these responses across a larger subject group, while correlating with measured sensitivity and subjective reports of responses to wine odours.

This research could:

- Be related to the interaction of taints in wine with different flavour components
- Focus on the interaction of wine flavours in different styles of wines
- Variations in sensitivity and subjective responses to TCA and other wine flavours and taints should be further investigated in the consumer population in comparison with wine experts, to establish a greater understanding of the varying needs and demands of the wine consumer market.

7.4 Practical Implications for the Australian Grape and Wine Industry

This study has taken important steps in demonstrating the complexity of human preferences and variety of experiences which contribute to the complexity of flavour responses. The results reported here provide unassailable evidence of a link between EEG structure and preference for odours, supporting pilot studies conducted for the wine and dairy industries.

The combined objective and sensory techniques used in this research provide access to subjective reports from untrained consumer groups which can be correlated with the objective and language independent odour-induced brain responses, independent of the participant's conscious awareness of the odour.

This consumer-based work can be extended to investigate differences in age groups, different cultural groups (nationally and internationally) with practical implications for the development of new wines and markets.

Greater understanding of consumer flavour responses will assist the industry in developing new wine markets, with the potential to tailor different wines to different markets, both nationally and internationally. This then has further implications and potential benefits for the marketing of Australian wines both nationally and internationally.

7.5 Project Benefits

While advances have been made in chemical analysis, the extent and variety of responses to wine flavours is still largely reliant on subjective reports and awareness of the odour. Individuals vary greatly in sensitivity to odours, reporting both positive (enhancers) or negative (taint) responses. This is in part due to physiological differences and to differences in experiences. In addition, subjective responses to odours and taints are strongly influenced by the degree and quality of experience with the odour, and its associated emotional and memory responses.

This project used subjective and objective techniques to monitor perceived and physiological responses to wine-related odours in a preliminary investigation of the variations in human sensitivities and the perceived effects of concentration of TCA on consumer responses to wine odours.

This study demonstrated the variation in consumer responses associated with preferences for the wine taints odours (TCA, 4-EP and 4-EG), and with the type of wine or alcohol the taint was presented in, providing the wine industry with evidence of consumer preference responses to these wine taints

Overall, the project has revealed important industry issues.

- Taints are not universally disliked by the inexperienced.
- Taints are, in fact, sometimes described positively by the inexperienced.
- Detection of taints at low concentrations has been confirmed by changes in brain electrical activity to the taint, which can be the only cause of the changes in our paradigm.
- The use of EEG as a method for probing the development and characterisation of hedonic responses has been further supported by the nature of the results.
- Inexpert consumers are very diverse and the need to totally eliminate TCA is not recommended, because only 10% of inexperienced consumers may find it unpleasant.
- Inexpert consumers may include 10% of people who cannot detect the TCA even at 1 $ng.l^{-1}$.
- Of the population in our study, the majority could detect the TCA at 1 $ng.l^{-1}$, and, they were more likely to state a liking for it than a dislike, based on our 100-point scale with 'neutral' set at 50.
- The level of inconsistency in our sub-population was marked: TCA responses ranged from like to dislike, but the trend was for the rating of the dislike to be less extreme than the like rating.
- Even at low concentrations there was evidence of EEG responses to the stimulus.
- Even at the lowest concentration used, the non-expert participants all expressed a subjective preference for the TCA when compared with the baseline control carrier.

This study has demonstrated that, together with traditional sensory techniques, the use of objective EEG measures has provided evidence of differences in responses to wine flavours independent of conscious awareness or recognition of the flavour.

This would suggest this approach has potential for application in further research to provide the Australian wine industry, and the food and beverage industries in general, with a greater understanding of variability in sensitivity and perceptual responses to wine flavours.

8. RECOMMENDATIONS

This project was designed to use sensory, psychophysiological and EEG recordings to establish if detailed EEG signal changes correlate with subliminal or conscious detection of wine odours (enhancers and taints) together with either, or both, hedonic response to, or preference for, an odour. As indicated, the sensory and physiological techniques used in this project provided evidence of reliable differences in brain activity processing between like and dislike and between the presence of alcohol and an odour plus alcohol.

The study has been successful in every regard other than obtaining the preferred numbers of participants and while this has impacted on the ability to be definite about some of our observations, many are quite definitive. They have also pointed the way to the need for some future studies in the broad area of the influence of taints, but also wine flavours, on perception of a product in the inexpert consumer. The issues raised by this study are likely

to apply to all the various positive and negative wine components. Simply because the participants were inexpert, they had few, if any, pre-conceptions and pre-conditioning. Surely, those features of wine, so much described by vitners and experts, are just as unfamiliar to the inexpert consumer? If so do we need to determine the features of wine that is significant to the non-expert? Using the current paradigm, but substituting wine flavour compounds for the taints, may reveal some interesting relationships between preferences and thresholds.

8.1 Future Research Directions

A more definitive examination of the changes over time in preferences and thresholds for taints is needed to track the development of improved detection. At present there is a gap in our knowledge as to how our expertise in odour detection may develop. By using non-expert consumers, their alteration in sensitivity, preference for, and discrimination of, TCA could be traced over time. Similarly the question of the non-expert ability to distinguish taints in the presence of other taints needs elucidation. Do they remain distinguishable from each other in mixtures, or do they become masked by one? How does the ability to individually identify them develop over time? Does our preference for these taints drift towards negative responses as we become more capable of identifying them at lower concentrations?

There seems to be a need, based on our results, to see how the performance of experts and non-experts relate in the various tests. While a great deal has been done with experts in many trails and experiments, most of these are looking at a complex situation: taints in real wines. How, though, do experts and non-experts compare in basic tests such as the odour pen threshold test? How do experts compare with non-experts when they are given taints in mixtures and combinations? Do experts and non-experts have a similar capacity to distinguish taints when presented in a substrate like ethanol? Would we see a similar performance from experts if they are presented with a taint 'out of context'?

A potentially valuable study would be to engage wine experts in an EEG study looking at their responses to TCA, and other taints, when their subjective preference is one of strong dislike. The difficulty of obtaining participants in this study who were from non-expert pool who had extremes of preference was one which might be overcome by tapping into a group who have already formulated a strong 'view' about the taints. If the data obtained supported the information we have obtained suggesting that the TCA is able to engage changes in brain electrical activity towards those associated with a 'calming' influence (liking), or away from a 'calming' influence (dislike), then a strong case for defining these changes as meaning 'like' and ''dislike' would be made. There are few cases where such links between activity patterns and higher cortical function of like and dislike preferences have been made. Confirmation of this observation would be a very substantial step forward in our understanding of the brain. More relevant for the wine industry we may then have ways in which we could objectively determine the preference for a complex mixture such as a wine, if the research was able to show that the same opposite changes occur in the brain electrical activity when like, or dislike is expressed for a stimulus.

8.2 Broader Industry Practices

This study has taken important steps in demonstrating the variety of human preferences and experiences which contribute to the complexity of flavour responses. The reported results provide unassailable evidence of a link between EEG structure and preference for odours.

This research has important implications for broader industry practices associated with the assessment of quality and flavour management. As stated in the *GWRDC Five Year Research & Development Plan (2002-2007)*, the industry needs quality and flavour measurement technologies which enable greater control of quality and more potential for differentiating wine to meet market preference. This study has demonstrated the great variability in consumer responses, and the differences in their responses in comparison with

expert assessment of TCA, as reported in the literature. Further development of the research techniques reported here will provide the industry with access to information which will contribute to a greater understanding of flavours and qualities associated with different market preferences.

8.3 Priorities for Future R&D

As stated in the *GWRDC Five Year R&D Plan (2007-2012): the beginnings of thee new Research and Development Plan 2007-2012*, consumers are the key drivers of the wine market, and it is therefore important for the industry to keep up with changes in the consumer demographics and in consumption patterns.

The research reported in this document has the potential to provide a valuable contribution to understanding consumer responses, particularly in comparison with the information available about wine expert responses.

Future research priorities to contribute to the industry's understanding consumers is needed:

- To investigate variations in sensitivity to TCA and wine taints in the consumer population versus wine experts.
- To investigate variations in sensitivity to individual wine flavours in the consumer population to establish the relative importance of these in wine appreciation by non-experts.
- To establish a greater understanding of the subjective assessment of wine flavours and the interaction of different flavours and concentrations of flavours in wines.
- To investigate the appreciation/preference of the various key wine flavour compounds in non-experts.
- To investigate whether changes in sensitivity and appreciation occur with peer pressure and ongoing exposure to taints over time (familiarity).

9. APPENDIX 1: COMMUNICATION

Conference Presentations and Industry Seminars

Preliminary results were reported in a poster presentation at the AWITC scientific meeting:

- *AWITC 2004*: Owen C.M., Patterson, J and Frank, D. (2004) What the consumer knows – an analysis of sensory threshold and preference responses to wine taints.. *Proceedings: Australian Wine Industry Technical Conference, Melbourne*.

Publications

Manuscripts will be submitted to industry-related journals to assist in communicating the results of this study to the industry.

Further Communication Activities

Industry seminars and presentations will be undertaken by the researchers to provide industry with access to the developments of this project and the potential to become involved in further investigations of consumer flavour and preference responses.

Such interactions will also provide the researchers with valuable information about the future and developing research interests of the industry.

10. APPENDIX 2: INTELLECTUAL PROPERTY

This research has extended olfactory and flavour research in general by investigating the interaction of sensory and electrophysiological responses to wine odours and further demonstrating the consistency of the left frontal response associated with liking an odour, together with changes in concentration responses associated with a liking response.

Previous studies have demonstrated the effect of odours on responses (and subsequently behaviour) even when the individual is not aware of the odour. This has implications in wine quality assessment, with the complexity of the wine flavours and quality incorporating the subtle and subconscious interaction of the complex flavours, associated with the psychophysiological aspects of the flavour responses (variations in thresholds, adaptation and masking effects).

These objective measurement techniques have now been explored in the Australian Wine Industry and [independently funded] in the Australian Dairy Industry. The breadth of information available from this study has now provided the Wine Industry with an indication of the valuable information which is potentially accessible in consumer responses.

- The valuable information arising from this research is based on the measurement of flavour responses using techniques which are language and experience independent.
- The variations in responses to different levels of wine odours and the great sensitivity of brain recording techniques to provide additional evidence of flavour responses at the basic sensory level (prior to the formation of conscious responses) provide significant information to the industry associated with the emotional and physiological responses involved in the complexity of the flavour response.
- The use of non-expert participants has provided the industry with a broader perspective on the variations in wine consumer responses, which can only contribute to the understanding of responses to wine taints and flavours.

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12. APPENDIX 4: STAFF

Dr John Patterson BSc, MSc, PhD.

Director, Sensory Neuroscience Laboratory, Swinburne University of Technology.

Dr Patterson pioneered the development of a novel technology allowing subjects to breathe normally and be exposed to very small quantities of odour with over 200 successful recordings. With this, innovative work investigating EEG changes to normally inspired odours is being undertaken. This methodology offers a significant opportunity to advance knowledge in this field to Australia's benefit.

Dr Caroline Owen. BA (Hons), PhD.

Deputy Director, Sensory Neuroscience Laboratory, Swinburne University of Technology.

Dr. Owen is project leader in the olfactory research conducted at the laboratory, and is involved in coordinating the psychophysiological and electrophysiological recordings, initiating new research and industry contacts, preparation of publication and presentations.

Research Associates:

Dr Damian Frank. BSc (Hons), PhD.

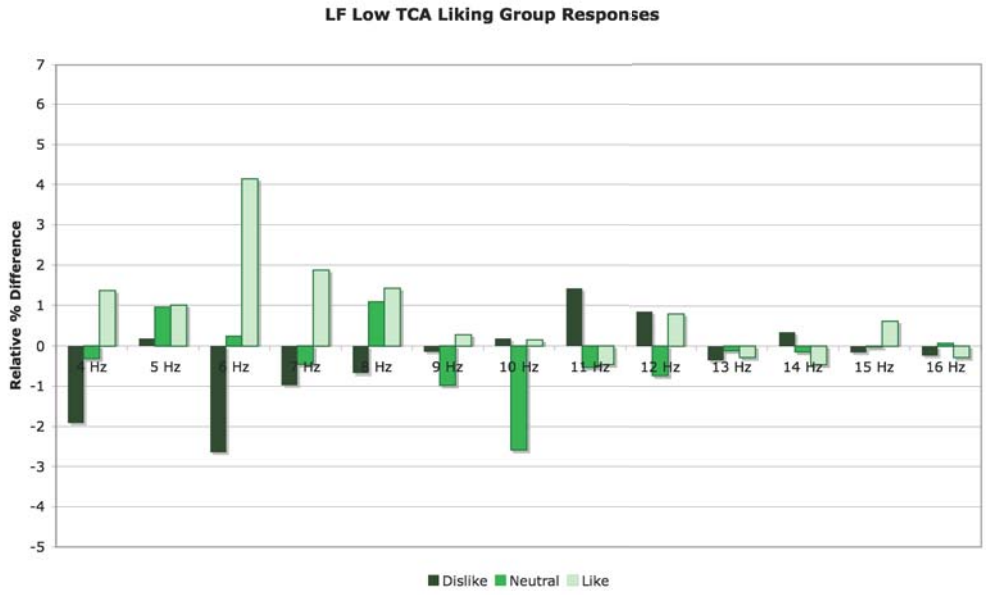
Research Fellow, Sensory Neuroscience Laboratory, Swinburne University of Technology.

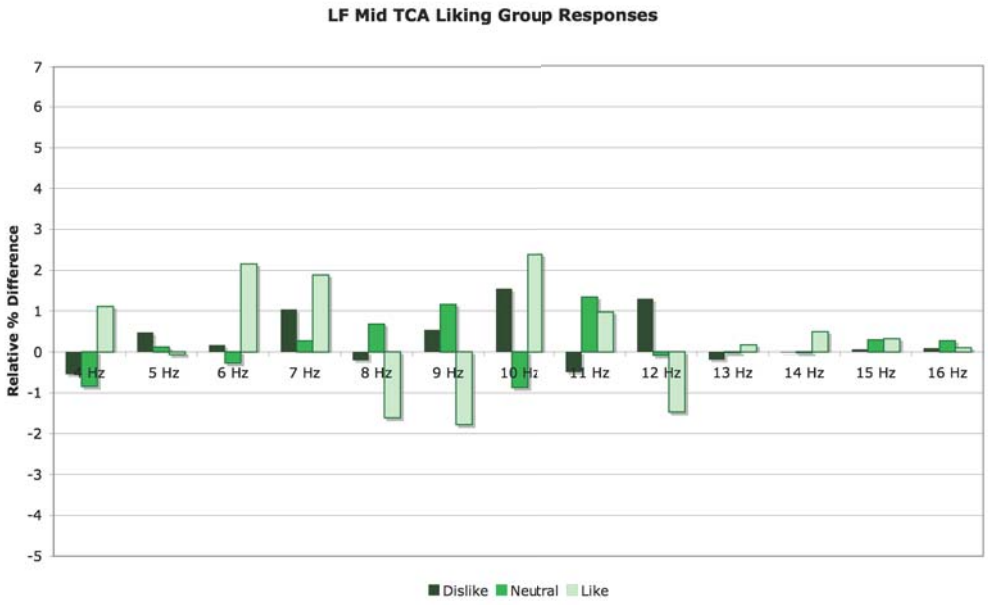
Dr. Frank was involved in the analysis of the wine flavour components, and preparation of the TCA odour stimuli for the EEG recording and sensory rating sessions.

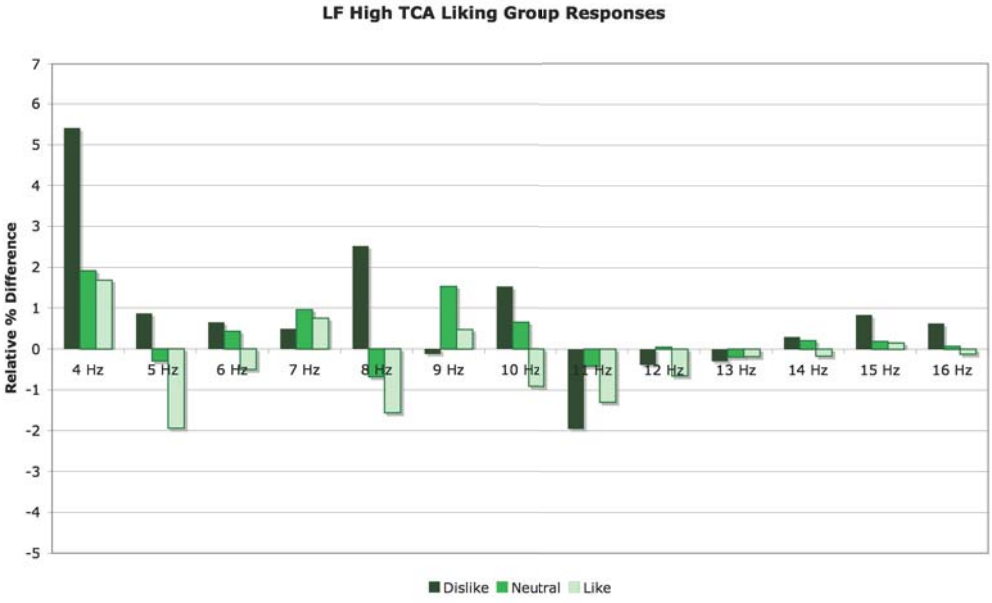
Research Assistants, involved in the electrophysiological and olfactory performance sessions and data analysis:

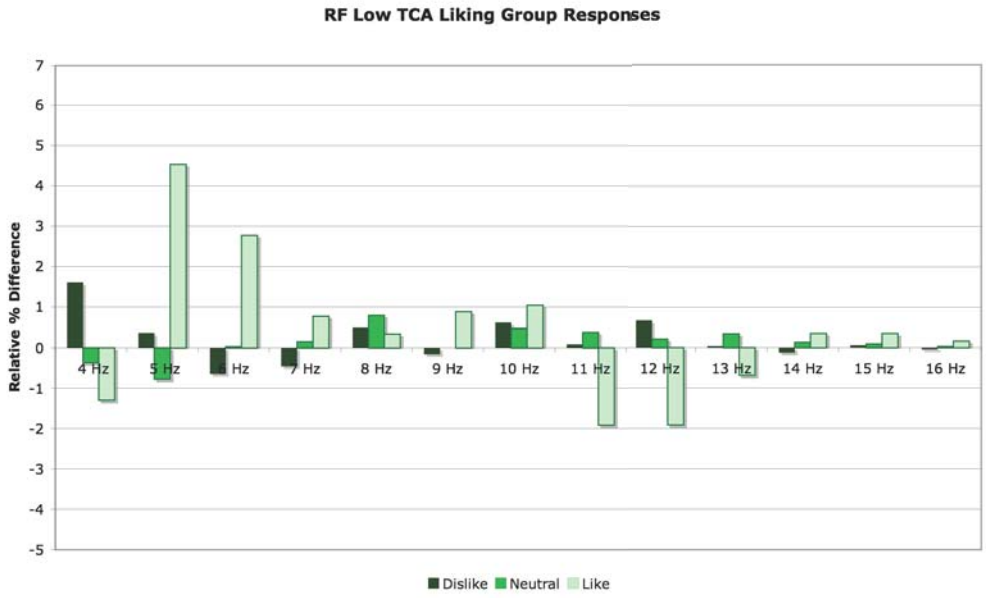
- Natalie Michael BSc (Hons)
- Simon Danckert. BSc (Hons)
- Rachid Annab BSc (Hons), BA (Hons)
- Sarah Nelder BSc (Hons)
- Cameron Czerczyk BSc

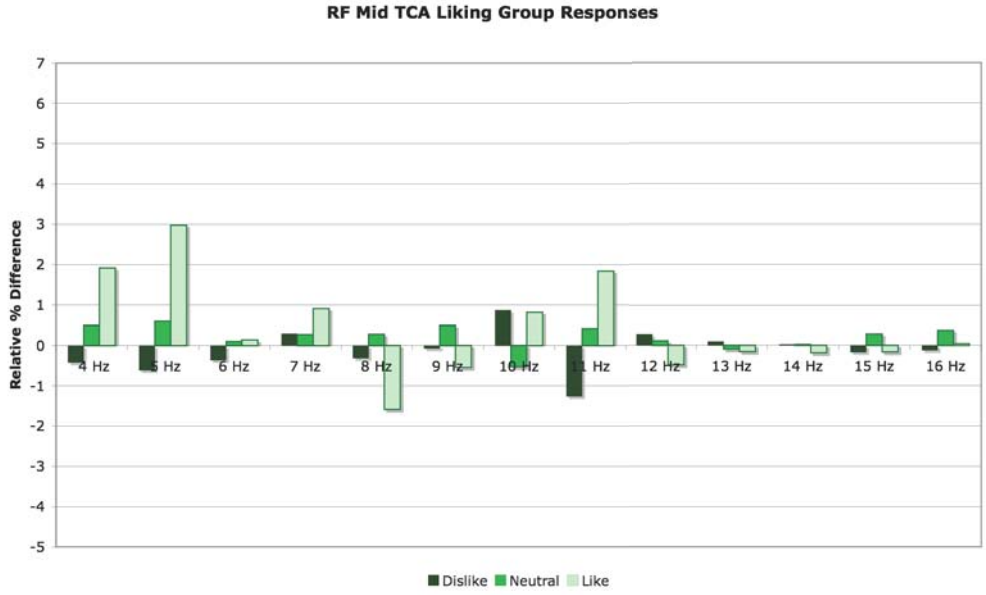
13. APPENDIX 5: LIKING RELATIVE % DIFFERENCE RESPONSES FOR ALL FREQUENCIES AND CONCENTRATIONS

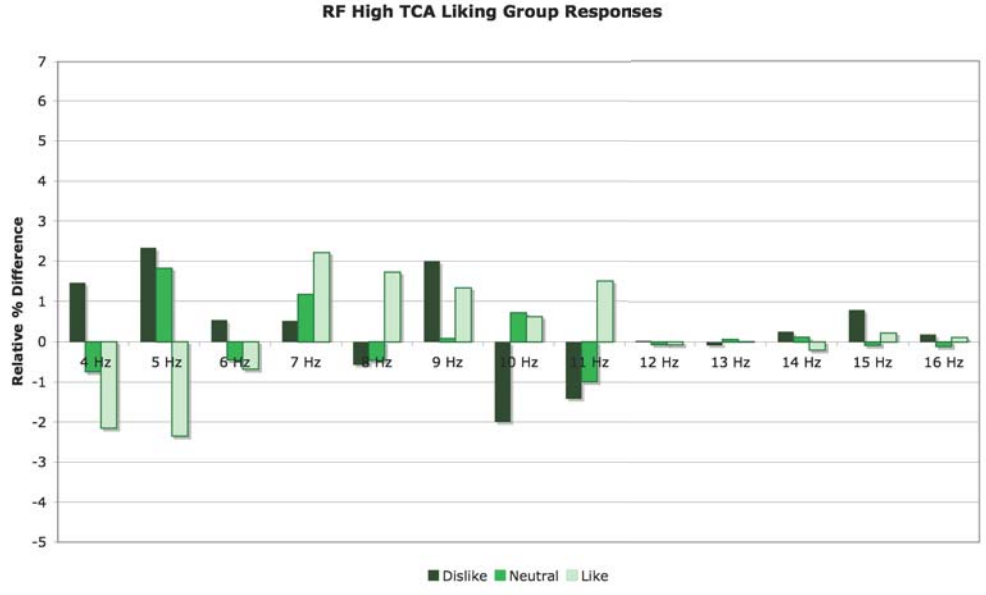

LF Low TCA Liking Group Responses
Relative % Difference
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
5 Hz
9 Hz
10 Hz
11 Hz
12 Hz
13 Hz
14 Hz
15 Hz
16 Hz
Dislike
Neutral
Like


LF Mid TCA Liking Group Responses
Relative % Difference
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
5 Hz
6 Hz
7 Hz
8 Hz
9 Hz
10 Hz
11 Hz
12 Hz
13 Hz
14 Hz
15 Hz
16 Hz
Dislike
Neutral
Like

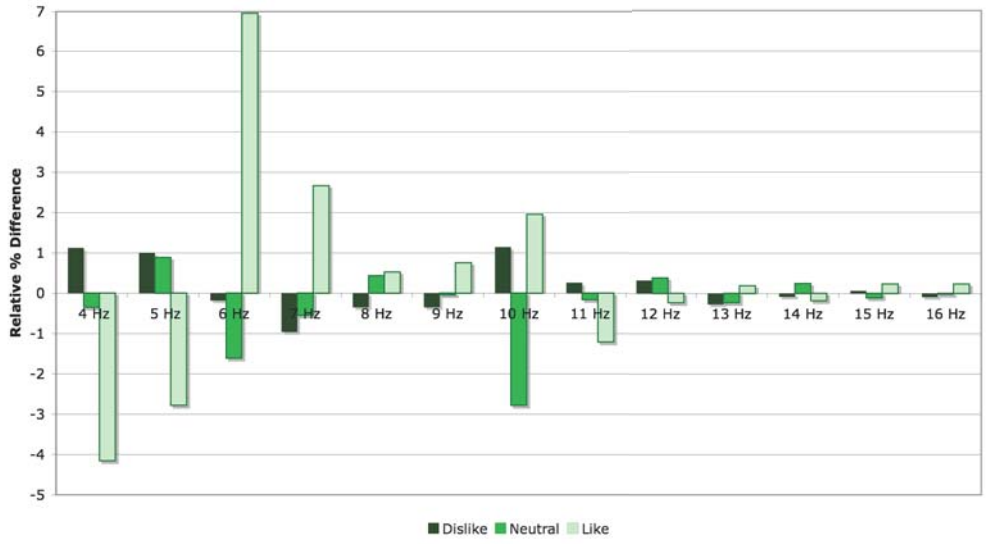

LF High TCA Liking Group Responses
Relative % Difference
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
4 Hz
5 Hz
6 Hz
7 Hz
8 Hz
9 Hz
10 Hz
12 Hz
13 Hz
14 Hz
15 Hz
16 Hz
Dislike
Neutral
Like


RF Low TCA Liking Group Responses
Relative % Difference
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
4 Hz
5 Hz
6 Hz
7 Hz
8 Hz
9 Hz
10 Hz
11 Hz
12 Hz
13 Hz
14 Hz
15 Hz
16 Hz
Dislike
Neutral
Like

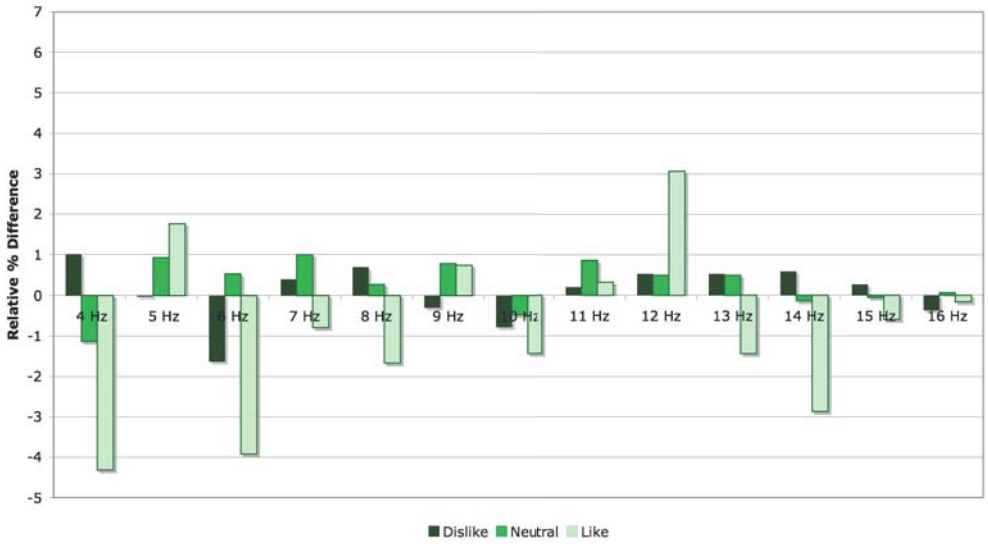

RF Mid TCA Liking Group Responses
Relative % Difference
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
4 Hz
6 Hz
7 Hz
12 Hz
13 Hz
14 Hz
15 Hz
16 Hz
Dislike
Neutral
Like


RF High TCA Liking Group Responses
Relative % Difference
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
5 Hz
6 Hz
7 Hz
9 Hz
12 Hz
13 Hz
14 Hz
15 Hz
16 Hz
Dislike
Neutral
Like

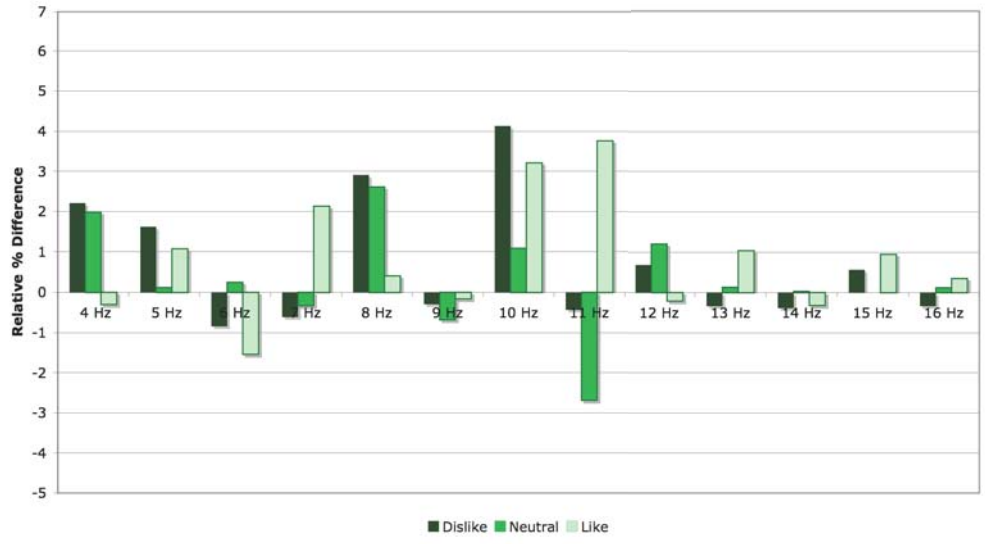
LTP Low TCA Liking Group Responses



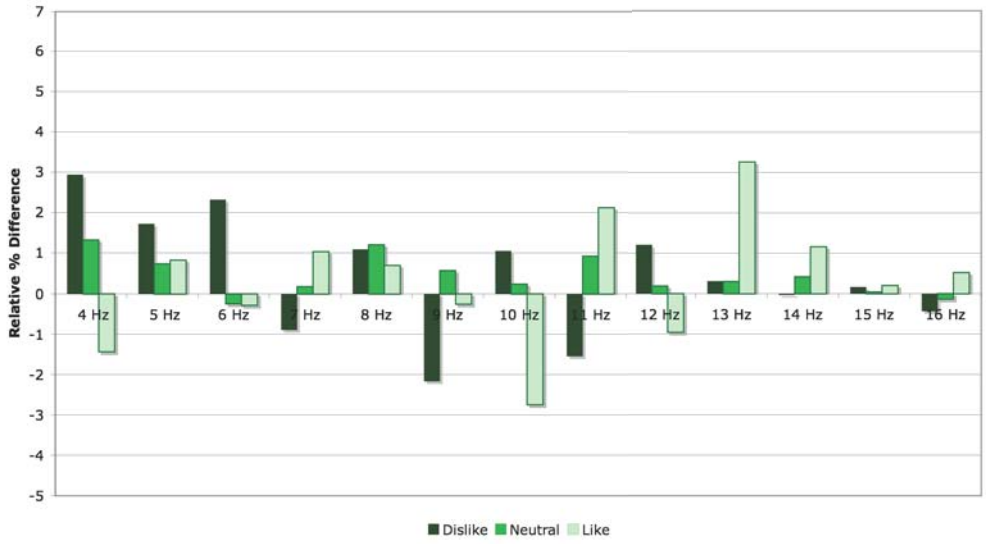
LTP Mid TCA Liking Group Responses



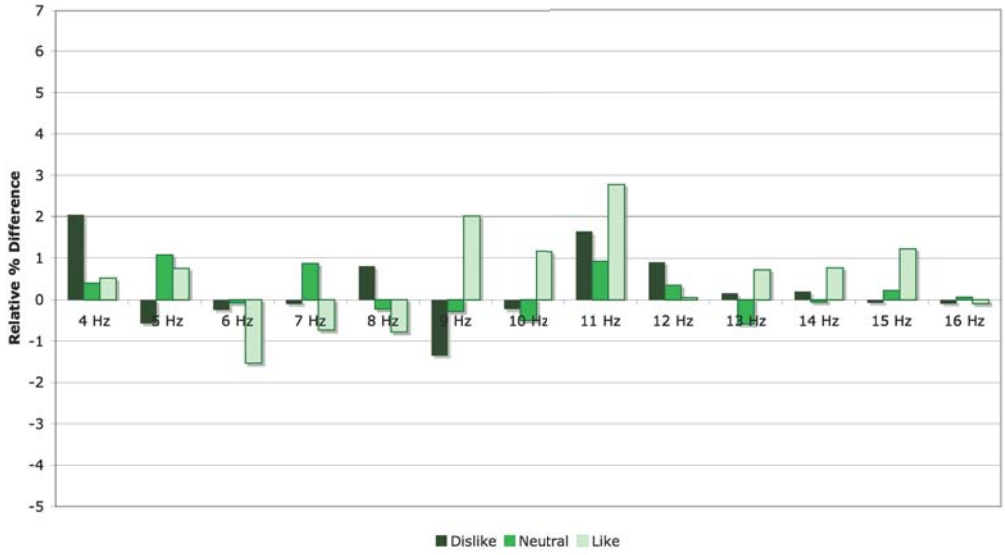
LTP High TCA Liking Group Responses



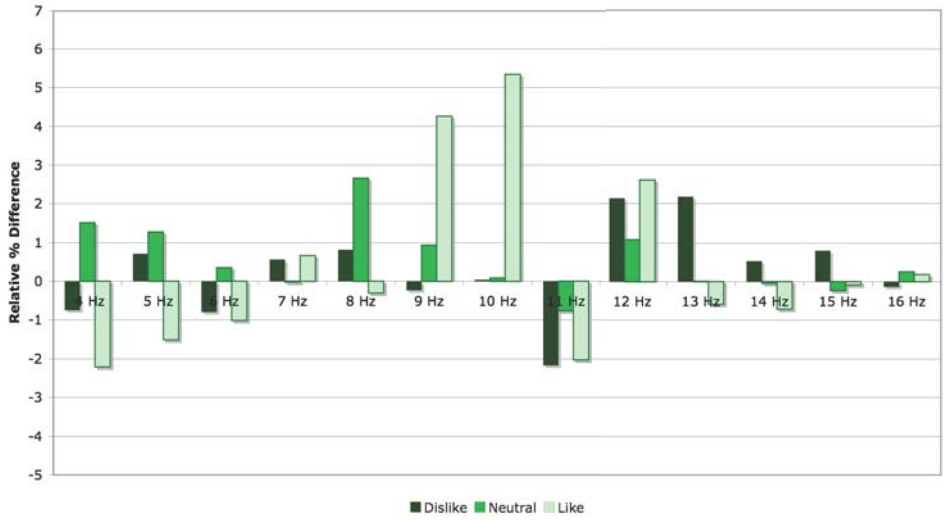
RTP Low TCA Liking Group Responses



RTP Mid TCA Liking Group Responses



RTP High TCA Liking Group Response



14. APPENDIX 6: BUDGET RECONCILIATION

GRAPE & WINE RESEARCH & DEVELOPMENT CORPORATION
Statement of Receipts and Expenditure - **FORM B**
For the year ending 30 June 2006
Final Project Budget Reconciliation

Trust Fund :	RESEARCH TRUST FUND	FUNDING	$
Project No :	SUT02_01	Salaries	30,000
Grantee :	Swinburne University of Technology	Travel	3,000
Title of Project :	A multi-disciplinary analysis of subjective & objective responses to TCA in wine, using sensory, chemical & electrophysiological techniques	Operating	71,760
		Capital	24,600
		Total Funding	129,360

EXPENDITURE										
	Salaries		Travel		Operating		Capital		Total	
	$	¢	$	¢	$	¢	$	¢	$	¢
A Uncommitted (c/f 1 July)	NIL		NIL		NIL		NIL		NIL	
B Outstanding Commitments (c/f 1 July)	NIL		NIL		NIL		NIL		NIL	
C Refunds of funding	NIL		NIL		NIL		NIL		NIL	
D Cash Received From Trust Fund	30,000		3,000		71,760		24,600		129,360	
E Approved transfers (from Form C)	NIL		NIL		NIL		NIL		NIL	
F Cash available (A+B-C+D±E)	30,000		3,000		71,760		23,600		129,360	
G Expenditure	30,000		3,000		71,600		23,600		129,360	
H Outstanding Commitments (30 June)	NIL		NIL		NIL		NIL		NIL	
I Total funds Committed (G-H)	30,000		3,000		71,600		23,600		129,360	
J Uncommitted (30 June) (F-I)	NIL		NIL		NIL		NIL		NIL	
K Other income (Paid to Trust Funds)	N/A		N/A		N/A		N/A		N/A	

Note : *Row B should be the same as Row H from the previous year and Row A the same as Row J from the previous year.*

I hereby certify that this statement of expenditure is correct.

…… …… ……
Signature Printed Name Date