

The validation of human decomposition  
fluid as a cadaver-detection dog training  
aid

by

REBECCA C. BUIS

A thesis submitted for the degree of  
Doctor of Philosophy (Science)

Centre for Forensic Science  
University of Technology, Sydney

2016

# **Certificate of Original Authorship**

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

Date: 23 May, 2016

**In Loving Memory of**

**Sharon G. Buis**

*Missing May 23, 2014*

# Acknowledgements

I would like to begin by thanking my supervisors, Dr Shari Forbes, Dr Barbara Stuart, and Dr Tapan Rai. Without your guidance and support, I could not have reached this point in my degree. Your expertise, your critiques and your encouragement have been integral to the completion of my degree.

I must also extend my sincere gratitude to the NSW Police Dog Unit's Cadaver-detection dogs and their handlers, because without your eagerness to participate in our research, this research project would never have gotten off the ground. It has been a sincere pleasure to be involved in your training, and I can only hope that this research gives back in turn.

Thanks go to Mohammed Shareef and Nafisa Shaikh from the UTS Facility for Anatomical and Surgical Sciences. You have been incredibly patient with my requests for tissue samples, and have bent over backwards to make sure this research could happen. I especially appreciate your good sense of humour and strong stomachs when the pieces of tissue that you handed out came back in less than ideal condition!

I would also like to acknowledge the technical staff from the Chemistry department: Dr Ronald Shimmon, Dr David Bishop, and R. Verena Taudte. You have been wonderful sources of support during this research process. I am deeply appreciative of your abilities and your patience, especially during those times when things haven't been going as smoothly as we would have liked them to with our instruments. Thank-you, also, to the team at LECO Australia for the work you put in to ensure that our instrument was repaired promptly and our research could continue with minimal disruption.

The saying that "it takes a village to raise a child" can also be applied to the production of a doctoral thesis. In that regard, I must extend my most sincere thanks to members of Professor Forbes' research group: Dr Katie Nizio, Dr Katelynn Perrault, Maiken Ueland,

Latara Rust, Laura McGrath, Amanda Troobnikoff, Kate Trebilcock, Prue Armstrong and Miroslava Ross. Thank-you all for the time and effort you have expended helping me with the myriad aspects of my research, from helping out with the dog trials to guiding me through the use of the software involved in my data analysis. It has been a pleasure getting to know you all, and I am deeply grateful for the support you have provided throughout this project.

On a more personal note, I must express deep gratitude to Rose, Andy, and Matthew Boyd for becoming my surrogate family while I have been here in Australia. It has been a true blessing to have you as my support system, and I shall miss you terribly when I return home. Thank-you, also, to Karlijn Stouten, for all the editing you have done, as well as for encouraging me, and for the humour you inserted into the situation every time things got rough. I think John McIlwaine is having a right laugh at us from heaven over some of the things we've come up with! To Shannon Hill, you have my undying gratitude for the support and encouragement you've given as I struggled through the last few months of writing this thesis. Having someone in the same boat, who could encourage me and work with me was amazing. Best of luck with your thesis and defence! To Janelle Vogel: thank-you for fitting me into your crazy schedule and doing some last minute editing. You are amazing!

Finally, I have to thank Mom, Dad, Court and Josh, all my friends and extended family members back home. A PhD is an intellectual and emotional rollercoaster, and you have never failed to support me or celebrate with me as needed.

As I reflect on the end of this stage in my journey, I have no doubt that you have all made a lasting contribution to my research and my life, and I extend my heartfelt gratitude for your support. I wish you all the best as we travel onwards.

# Table of Contents

<b>ABSTRACT</b> .....	<b>x</b>
<b>LIST OF TABLES</b> .....	<b>xi</b>
<b>LIST OF FIGURES</b> .....	<b>xii</b>
<b>RELATED PUBLICATIONS</b> .....	<b>xvii</b>
<b>CHAPTER 1: INTRODUCTION</b> .....	<b>1</b>
1.1 Cadaver-detection Dogs.....	2
1.1.1 <i>Detection of Decomposition Odour</i> .....	3
1.1.2 <i>Effectiveness and Limitations</i> .....	6
1.1.3 <i>Training and Training Aids</i> .....	7
1.2 Cadaver Decomposition.....	9
1.3 The Chemistry of Decomposition .....	13
1.3.1 <i>Protein Degradation</i> .....	13
1.3.2 <i>Lipid Degradation</i> .....	15
1.3.3 <i>Carbohydrate Degradation</i> .....	17
1.3.4 <i>Volatile Organic Compounds (VOCs) Produced by Decomposition</i> .....	19
1.4 Collection and Analysis of Decomposition VOCs .....	20
1.4.1 <i>Sorbent Tubes</i> .....	21
1.4.2 <i>Solid-Phase Micro-Extraction (SPME)</i> .....	22
1.4.3 <i>Gas Chromatography — Mass Spectrometry (GC-MS)</i> .....	24

1.5 Comprehensive Two-Dimensional Gas Chromatography — Time-of-Flight Mass Spectrometry (GC×GC-TOFMS).....	26
1.6 Research Aims and Objectives.....	30
<b>CHAPTER 2: METHOD OPTIMISATION .....</b>	<b>32</b>
2.1 Introduction.....	33
2.2 Materials and Methods.....	35
2.2.1 Decomposition Fluid Samples.....	35
2.2.2 Sorbent Tubes and Thermal Desorption.....	35
2.2.3 Solid-Phase Micro-Extraction (SPME).....	37
2.2.4 One-dimensional and Two-dimensional Gas Chromatography — Mass Spectrometry.....	38
2.3 Results.....	40
2.3.1 Sorbent Tubes and Thermal Desorption.....	40
2.3.2 Solid-Phase Micro-Extraction (SPME).....	42
2.3.3 Comprehensive Two-dimensional Gas Chromatography — Time-of-Flight Mass Spectrometry.....	43
2.3.4 A Comparison of Sorbent Tubes and Solid-Phase Micro-Extraction.....	44
2.4 Discussion.....	47
2.5 Conclusions and Future Works.....	50
<b>CHAPTER 3: Odour Profiling of Human Decomposition Fluid.....</b>	<b>51</b>
3.1 Introduction.....	52
3.2 Materials and Methods.....	54

3.2.1 Decomposition Samples.....	54
3.2.2 Comparison of Decomposition Fluid and Human Cadaveric Profiles.....	54
3.2.3 Storage and Ageing Trials.....	55
3.2.4 Dilution and Ageing Trials.....	56
3.2.5 Data Analysis.....	57
3.3 Results.....	60
3.3.1 Comparison of Decomposition Fluid and Human Cadaveric Odour Profiles.....	61
3.3.2 Storage and Ageing Trials.....	63
<u>3.3.2.1 Decomposition Fluid Stored and Aged at Room Temperature.....</u>	68
<u>3.3.2.2 Decomposition Fluid Stored and Aged in the Refrigerator.....</u>	72
<u>3.3.2.3 Decomposition Fluid Stored and Aged in the Freezer.....</u>	75
3.3.3 Dilution and Ageing Trials.....	80
<u>3.3.3.1 Dilutions Aged for 3, 6, 9, and 12 months.....</u>	83
<u>3.3.3.2 Dilutions Aged for 15, 18, 21 and 24 months.....</u>	88
3.4 Discussion.....	94
3.4.1 Comparison of Decomposition Fluid and Human Cadaveric Odour Profiles.....	94
3.4.2 Storage and Ageing Trials.....	97
3.4.3 Dilution and Ageing Trials.....	101
3.5 Conclusions.....	105

<b>CHAPTER 4: CADAVER-DETECTION DOG TRIALS.....</b>	<b>108</b>
4.1 Introduction.....	109
4.2 Materials and Methods.....	111
4.2.1 <i>Decomposition Fluid Samples.....</i>	111
4.2.2 <i>Training Sessions.....</i>	112
4.2.3 <i>Data Analysis.....</i>	115
4.3 Results.....	115
4.3.1 <i>Fresh Diluted Decomposition Fluid.....</i>	116
4.3.2 <i>Aged Diluted Decomposition Fluid.....</i>	117
4.3.3 <i>Correct vs. Incorrect Responses.....</i>	120
4.3.4 <i>Comparison of Odour Profiles to Dog Responses.....</i>	122
4.4 Discussion.....	129
4.4.1 <i>Fresh and Aged Dilutions of Decomposition Fluid.....</i>	129
4.4.2 <i>Correct vs. Incorrect Responses.....</i>	131
4.4.3 <i>Comparison of Odour Profiles to Dog Responses.....</i>	132
4.5 Conclusions.....	133
<b>CHAPTER 5: CONCLUSIONS AND FUTURE WORKS.....</b>	<b>134</b>
5.1 Summary of Thesis.....	135
5.2 Future Recommendations.....	136
<b>REFERENCES.....</b>	<b>143</b>
<b>APPENDICES.....</b>	<b>157</b>

Appendix A: List of Significant VOCs Obtained from Human Decomposition Fluid.....	158
Appendix B: List of Significant VOCs Obtained from Human Cadaver Decomposition Literature.....	177
Appendix C: PCA Plots for Samples Stored and Aged at Room Temperature.....	181
Appendix D: Predominant VOCs Found in Samples Stored and Aged at Room Temperature.....	184
Appendix E: PCA Plots for Samples Stored and Aged in the Refrigerator.....	187
Appendix F: Predominant VOCs Found in Samples Stored and Aged in the Refrigerator.....	190
Appendix G: PCA Plots for Samples Stored and Aged in the Freezer.....	194
Appendix H: Predominant VOCs Found in Samples Stored and Aged in the Freezer.....	196
Appendix I: Predominant VOCs Found in Diluted Samples of Decomposition Fluid.....	199
Appendix J: Predominant Compounds Found in Human Decomposition Fluid.....	202

# Abstract

Cadaver-detection dogs are used by police services to locate human remains. Due to ethical restrictions, the dogs are not trained using human cadavers, but rather, using pseudo-scents or human tissues, such as blood and decomposition fluid. However, the chemical profile of these training aids has not been comprehensively investigated and their accuracy as substitutes for decomposed remains has not been determined. The aim of this study was to validate human decomposition fluid as a training aid for cadaver-detection dogs. The study examined the odour profile of decomposition fluid, including the changes in the profile over time (aged for one year) and under different storage conditions (room temperature, refrigerator and freezer) in order to determine the optimal conditions for its use as a training aid. The study also examined the dogs' sensitivity to decomposition fluid and compared their responses with the chemical odour profiles.

The odour profile of the decomposition fluid was collected using Solid-Phase Micro-Extraction (SPME) and analysed using Comprehensive Two Dimensional Gas Chromatography—Time of Flight Mass Spectrometry (GC×GC-TOFMS). The volatile organic compounds (VOCs) identified in decomposition fluid were compared to the VOCs reported in the literature for human cadaver decomposition odour. A wide range of characteristic decomposition VOCs were identified in the decomposition fluid. While individual VOCs were not comparable to human remains, the compound class proportions of the odour profiles were deemed similar. Variable odour profiles were observed under different storage conditions; room temperature and refrigeration were suitable, but freezing was not recommended for sample storage.

The decomposition fluid was also serially diluted to 1 part-per-trillion to determine the sensitivity of cadaver-detection dogs to this training aid. The samples were presented to three cadaver-detection dog teams under standard indoor training conditions over 14 training sessions. The dogs were capable of detecting the 1 part-per-trillion dilutions after several exposures to the fluid. The samples were subsequently analysed using SPME-GC×GC-TOFMS to determine the odour profile for all dilution levels. A range of VOCs were detected, although their abundances decreased in the lowest dilutions. The results of this study suggest that decomposition fluid closely mimics the odour profile of a decomposing cadaver and is a suitable training aid for cadaver-detection dogs when stored appropriately.

# List of Tables

<b>Table 2-1:</b> Parameters optimised for Sorbent Tube VOC collection. Those highlighted in red are the optimised methodology.....	41
<b>Table 2-2:</b> The parameters tested for SPME VOC collection. Those highlighted in red indicate the optimised procedure.....	43
<b>Table 2-3:</b> Summary of the optimised GC×GC-TOFMS method developed for the odour profiling of decomposition fluid.....	44
<b>Table 3-1:</b> The 35 compounds found in 95% or more of the stored, aged, and diluted decomposition fluid samples.....	104
<b>Table 4-1:</b> Summary of the classifications for dog responses to target odours.....	115

# List of Figures

<b>Figure 1-1:</b> Primary scent cone.....	4
<b>Figure 1-2:</b> Vertical scent cone created by heat.....	5
<b>Figure 1-3:</b> Secondary scent pool caused by a natural barrier 1) primary scent pool, 2) secondary scent pool.....	5
<b>Figure 1-4:</b> Stages of decomposition, as demonstrated by porcine remains: a) fresh, b) bloat, c) active decay, d) advanced decay, e) dry/remains.....	12
<b>Figure 1-5:</b> Protein degradation pathways during the decomposition of soft tissue.....	14
<b>Figure 1-6:</b> Lipid degradation pathways during the decomposition of soft tissue.....	16
<b>Figure 1-7:</b> Carbohydrate degradation pathways during the decomposition of soft tissue.....	18
<b>Figure 1-8:</b> A schematic of the Thermal Desorption (TD) Unit for desorbing and concentrating sorbent tube samples.....	22
<b>Figure 1-9:</b> A schematic of a GC-MS instrument.....	25
<b>Figure 1-10:</b> A schematic of a GC×GC-TOFMS system.....	28
<b>Figure 1-11:</b> A chromatogram of decomposition fluid headspace VOCs. Compounds (indicated by black dots) are separated in two dimensions, according to the two column properties. Intensity of the colour (blue to green to yellow to red) indicates the abundance of the compound. The second dimension separation of numerous co-eluting compounds in the first dimension can be observed in the red boxes.....	29
<b>Figure 2-1:</b> The purge and trap system for trapping VOCs on sorbent tubes.....	36
<b>Figure 2-2:</b> SPME fibre sampling from 20 mL glass headspace vial with decomposition fluid in block heater.....	38

<b>Figure 2-3:</b> Proportion of compound classes collected by sorbent tubes. The proportionately larger classes collected by sorbent tubes compared to SPME are indicated with an asterisk (*).	45
<b>Figure 2-4:</b> Proportion of compound classes collected by SPME. The proportionately larger classes collected by SPME compared to sorbent tubes are indicated with an asterisk (*).	46
<b>Figure 2-5:</b> Proportion of compounds collected by each sample collection method.	47
<b>Figure 3-1:</b> The decomposition fluid samples (left) and controls (right) in 20 mL glass headspace vials for the room temperature storage condition study.	56
<b>Figure 3-2:</b> The proportion of compound classes found in a) the odour profile of human cadaver decomposition, as reported in the literature, and b) the odour profile of human decomposition fluid, as determined in this study.	62
<b>Figure 3-3:</b> PCA plots for the storage conditions trial: a) Scores plot showing the differentiation between the samples stored and aged: at room temperature (RT, blue), in the refrigerator (F, red) and in the freezer (Z, green), b) Loadings plot indicating the VOCs influencing the differentiation between storage conditions as the samples aged.	64
<b>Figure 3-4:</b> A comparison of the number of compounds present within the overall odour profile of decomposition fluid, categorised by compound class between each storage condition.	65
<b>Figure 3-5:</b> Variations in the proportions of compounds by compound class as decomposition fluid samples aged (Day 0 to Day 350): a) at room temperature, b) in the refrigerator, and c) in the freezer.	67
<b>Figure 3-6:</b> Overall proportions of compound classes present in the odour profile of decomposition fluid when samples have been stored at room temperature for a year.	69
<b>Figure 3-7:</b> PCA plots for the odour of decomposition fluid stored and aged at room temperature, sorted by compound class: a) Scores plot with 95% CI, b) loadings plot with correlation loadings ellipse, c) bi-plot overlapping A and B to show the influence of the loadings on the scores.	70
<b>Figure 3-8:</b> Day-to-day change in the proportion of compound classes present in the odour profile of decomposition fluid as it aged at room temperature.	71

**Figure 3-9:** Overall proportions of compound classes present in the odour profile of decomposition fluid when samples have been stored in the refrigerator for a year.....72

**Figure 3-10:** PCA plots for the odour of decomposition fluid stored in the refrigerator, sorted by compound class: a) Scores plot with 95% CI, b) loadings plot with correlation loadings ellipse, c) bi-plot overlapping A and B to show the influence of the loadings on the scores.....74

**Figure 3-11:** Day-to-day change in the proportion of compound classes present in the odour profile of decomposition fluid as it aged in the refrigerator.....75

**Figure 3-12:** PCA plots for the odour of decomposition fluid stored and aged in the freezer, with outliers removed: a) Scores plot sorted by days and all compounds, original outliers removed, 95% CI, b) Loadings plot demonstrating the VOCs influencing the variations seen among the samples in plot A.....76

**Figure 3-13:** Overall proportions of compound classes present in the odour profile of decomposition fluid when samples have been stored in the freezer for a year.....77

**Figure 3-14:** PCA plots for the odour of decomposition fluid stored and aged in the freezer, sorted by compound class: a) Scores plot with 95% CI, b) loadings plot with correlation loadings ellipse, c) bi-plot overlapping A and B to show the influence of the loadings on the scores.....78

**Figure 3-15:** Day-to-day change of the proportion of compound classes present in the odour profile of decomposition fluid as it aged in the freezer.....79

**Figure 3-16:** PCA plots for the odour profile of diluted and aged decomposition fluid, with all significant compounds present: a) Scores plot displaying all dilution levels and ages of samples, b) loadings plot demonstrating significant VOCs influencing differentiation of samples seen in plot A.....81

**Figure 3-17:** PCA plots for the odour profile of diluted and aged decomposition fluid, sorted by compound class: a) scores plot displaying separation of aged samples by dilution level, b) loadings plot demonstrating the compound classes influencing the differentiation seen in plot A.....82

**Figure 3-18:** Proportion of compound classes present in the odour profile of 3 month aged and diluted decomposition fluid.....84

<b>Figure 3-19:</b> Proportion of compound classes present in the odour of 6 month aged and diluted decomposition fluid.....	85
<b>Figure 3-20:</b> Proportion of compound classes present in the odour profile of 9 month aged and diluted decomposition fluid.....	86
<b>Figure 3-21:</b> Proportion of compound classes present in the odour profile of 12 month aged and diluted decomposition fluid.....	87
<b>Figure 3-22:</b> Proportion of compound classes present in the odour profile of 15 month aged and diluted decomposition fluid.....	89
<b>Figure 3-23:</b> Proportion of compound classes present in the odour profile of 18 month aged and diluted decomposition fluid.....	90
<b>Figure 3-24:</b> Proportion of compound classes present in the odour profile of 21 month aged and diluted decomposition fluid.....	91
<b>Figure 3-25:</b> Proportion of compound classes present in the odour profile of 24 month aged and diluted decomposition fluid.....	92
<b>Figure 4-1:</b> Serially diluted decomposition fluid samples for dog trials.....	112
<b>Figure 4-2:</b> Concrete bricks in a standard scent training line-up.....	113
<b>Figure 4-3:</b> Sample cans inside the scent line-up bricks.....	113
<b>Figure 4-4:</b> The percentage of dogs responding correctly to dilute samples of decomposition fluid in the fresh fluid trials (Trials 1-6). Green indicates that all dogs correctly located the sample; red indicates that no dogs correctly located the sample, and shades of orange indicate the degree to which the dogs were able to locate the sample.....	117
<b>Figure 4-5:</b> Percentage detection rates of diluted samples for Trials 1-6 (fresh fluid).....	118
<b>Figure 4-6:</b> The percentage of dogs responding correctly to dilute samples of decomposition fluid in the aged fluid trials (Trials 7-14). Green indicates that all dogs correctly located the sample; red indicates that no dogs correctly located the sample, and shades of orange indicate the degree to which the dogs were able to locate the sample.....	119

**Figure 4-7:** Percentage detection rates of diluted samples for Trials 7-14 (aged fluid).....120

**Figure 4-8:** A comparison of correct and incorrect responses in all 14 trials. Note: any missing bars indicate that the team was not available for that particular trial.....121

**Figure 4-9:** A comparison of chemical profiles to dog detection rates for the 3 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates.....124

**Figure 4-10:** A comparison of chemical profiles to dog detection rates for the 6 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates.....125

**Figure 4-11:** A comparison of chemical profiles to dog detection rates for the 9 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates.....125

**Figure 4-12:** A comparison of chemical profiles to dog detection rates for the 12 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates.....126

**Figure 4-13:** A comparison of chemical profiles to dog detection rates for the 15 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates.....126

**Figure 4-14:** A comparison of chemical profiles to dog detection rates for the 18 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates.....127

**Figure 4-15:** A comparison of chemical profiles to dog detection rates for the 21 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates.....127

**Figure 4-16:** A comparison of chemical profiles to dog detection rates for the 24 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates.....128

## Related Publications

**Buis R**, Rust L, Nizio K, Rai T, Stuart B, Forbes S. (2015). Investigating the sensitivity of cadaver-detection dogs to decomposition fluid. *Journal of Forensic Identification*, 65(6): 985-997.

Rust L, **Buis R**. (2015). The Scent of a Crime. *Australasian Science*, 36(7), 16-18.