

**Electronic Cigarette Exposure:
Inflammatory and Immunological
Implications in Structural Lung Cells
and the Potential Public Health
Consequences**

by Jack Edward Bozier

Thesis submitted in fulfilment of the requirements for
the degree of

Doctor of Philosophy

under the supervision of Professor Brian G.G. Oliver and
Hui Chen

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This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Awards, Presentations and Posters Arising from This Work

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E-cigarette Vapor Induces Cellular Senescence in Lung Fibroblasts and may Contribute to Lung Pathology

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Combined E-cigarette and cigarette use reduces efficacy of dexamethasone to attenuate neutrophilic inflammatory markers

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Table of Contents

<i>Acknowledgements</i>	<i>II</i>
<i>Awards, Presentations and Posters Arising from This Work</i>	<i>IV</i>
Awards	IV
Posters and Presentations	IV
<i>Publications Included in this Thesis</i>	<i>VI</i>
<i>Publications Included as an Adjunct to this Thesis</i>	<i>VI</i>
<i>Statements of Contributions of Authors</i>	<i>VII</i>
How Harmless are E-cigarettes? Effects in the Pulmonary System.....	VII
The Evolving Landscape of e-Cigarettes: A Systematic Review of Recent Evidence.....	VIII
Heightened Response to E-cigarettes in COPD.....	XI
E-cigarette Vapor Induces Cellular Senescence in Lung Fibroblasts and may Contribute to Lung Pathology	XII
Combined E-cigarette and cigarette use reduces efficacy of dexamethasone to attenuate neutrophilic inflammatory markers	XIV
<i>Table of Figures</i>	<i>XIX</i>
<i>List of Tables</i>	<i>XX</i>
<i>Abstract</i>	<i>XXI</i>
<i>List of Abbreviations</i>	<i>XXIII</i>
<i>Chapter 1 Introduction</i>	<i>1</i>
1.1 History of Tobacco use.....	1
1.2 Harmful effects of cigarette smoke exposure.....	2
1.3 Chronic Obstructive Pulmonary Disease	5
1.4 Symptoms of COPD.....	7
1.5 Diagnosis.....	7
1.6 COPD Pathophysiology	8

1.7 COPD Treatment.....	10
1.8 How harmless are E-cigarettes: effects in the pulmonary system	12
1.9 The Evolving Landscape of E-cigarettes: A Systematic Review of Recent Evidence	19
1.10 Outline of Chapters.....	49
1.11 Aims and Hypothesis	51
Chapter 2.....	55
Methods.....	55
2.1 Checklist for Reporting Results of Internet E-Surveys (CHERRIES)	55
2.2 Primary Cell Isolation.....	61
2.3 Cell Culture.....	62
2.4 E-vapour Extract Generation	63
2.5 Cigarette Smoke Extract Generation.....	63
2.6 MTT Assay.....	64
2.7 Enzyme Linked Immunosorbent Assay – ELISA.....	64
2.8 Western Blot	65
2.9 RNA isolation and purification	70
2.10 cDNA Synthesis.....	71
2.11 Real Time Quantitative Polymerase Chain Reaction - RT – qPCR.....	72
2.12 Senescence Induction by Paraquat	73
2.13 Wound Healing Assay	73
2.14 Senescence Associated β -Galactosidase Staining - SA- β Gal staining	74
2.15 Mouse model of E-cigarette and cigarette smoke exposure.....	75
2.16 Dexamethasone Pre-treatment	75
Chapter 3.....	76
E-cigarette use in Young Australians: perceptions of harms or benefits of emerging tobacco products	76

Chapter 4.....	104
Heightened Response to E-cigarettes in COPD.....	104
Chapter 5.....	109
E-cigarette vapor induces cellular senescence in lung fibroblasts and may contribute to lung pathology	109
Chapter 6.....	121
Dual E-cigarette and cigarette use reduces dexamethasone sensitivity in-vitro	121
Chapter 7.....	146
7.1 Discussion, Conclusions and Future Directions.....	146
7.2 Perceptions of harm and usage patterns of E-cigarettes in Australia	147
7.3 Harms Associated with E-cigarette use in COPD Patients	151
7.4 Limitations and Future Directions of this Research	157
7.5 Final Conclusion.....	159
Chapter 8 References	161

Table of Figures

<i>Figure 1.1 – Decline of FEV1 in susceptible smokers with COPD, non-susceptible smokers, ex-smokers and non-smokers.....</i>	<i>6</i>
<i>Figure 3.1 – Respondents first introduction to E-cigarettes</i>	<i>81</i>
<i>Figure 3.2 – Respondents reasons for E-cigarette use.....</i>	<i>82</i>
<i>Figure 3.3 – Respondents opinion of nicotine and non-nicotine containing E-cigarettes as a cessation tool.....</i>	<i>83</i>
<i>Figure 3.4 – Respondents opinion of whether nicotine and non-nicotine containing E-cigarettes are bad for your health</i>	<i>84</i>
<i>Figure 3.5 – Respondents opinion of whether nicotine and non-nicotine containing E-cigarettes can cause similar damage to the lungs as cigarettes</i>	<i>85</i>
<i>Figure 3.6 – Respondents opinion of whether use of nicotine and non-nicotine containing E-cigarettes had an increased risk for lung cancer.....</i>	<i>86</i>
<i>Figure 3.7 – Respondents rating of health risks related to E-cigarette use compared to common habits.....</i>	<i>87</i>
<i>Figure 3.8 – Respondents opinions on whether cigarettes, alcohol, nicotine E-cigarettes or non-nicotine E-cigarettes should be regulated</i>	<i>88</i>
<i>Figure 7.2 – EVE stimulates IL-8 release from primary ASMCs and Fibroblasts</i>	<i>152</i>
<i>Figure 7.3 – E-vapour stimulates induction of cellular senescence.....</i>	<i>154</i>
<i>Figure 7.4 – Combined EVE and CSE stimulation results in steroid insensitive inflammation</i>	<i>156</i>

List of Tables

Table 2.1 – CHERRIES Checklist	55
Table 2.2 – Reagent volumes for polyacrylamide gels used in western blotting	66
Table 2.3 – Buffers and reagents for Western blots	67
Table 2.4 – Primary and secondary Antibodies used for Western blots	68
Table 2.5 - TaqMan® Gene expression Assays	72
Table 2.6 – Hi-ROX qPCR Protocol	73
Table 2.7 - SA-β-Gal Staining solution	74
Table 3.1 - Characteristics of complete survey respondents	80
Table 3.2 – E-cigarette use dependant on smoking status	80

Abstract

Electronic cigarettes have rapidly become the consumer preferred alternative to tobacco cigarettes, but very little is known about the harms associated with their use. Electronic cigarettes are often proposed as a cessation device from a harm reduction standpoint, but this overlooks the lack of evidence for reduced harms and the numerous new vapers who have never smoked that are exposed to harms they otherwise would have avoided. Studies within this thesis provide essential evidence in the harm reduction debate.

In Chapter 3 we surveyed perceptions of young Australians towards E-cigarettes. We hypothesised that they would believe E-cigarettes to be less harmful than tobacco cigarettes, and that they would be misinformed about E-cigarette regulations in Australia due to a lack of education from regulatory bodies. In Chapters 4, 5 and 6 of this thesis we used *in vitro* models of exposure to determine potential health risks associated with E-cigarette use. Chronic obstructive pulmonary disease (COPD) patients have been identified as a high-risk population of E-cigarette users, hence our studies focused on the potential effect E-cigarette exposure may have on mechanisms related to the underlying pathophysiology of COPD.

In Chapter 4, we developed an *in vitro* E-cigarette exposure model to determine the cytotoxic and inflammatory effects of E-cigarette exposure in COPD and non-COPD primary human airway smooth muscle cells. In this study we confirmed earlier suspicions on cytotoxicity and provided the first evidence that COPD cells are hyper-responsive to E-cigarettes. In Chapter 5 we provided the first evidence that E-cigarettes have the potential to induce cellular senescence. This finding gives further support to avoiding use of E-cigarettes in COPD patients, given the role cellular senescence plays in COPD pathophysiology. In Chapter 6 we provided evidence that combined cigarette and E-cigarette use is significantly more harmful than using either product alone. Furthermore, we found that the inflammatory response induced by dual exposure was glucocorticoid resistant. Glucocorticoid

resistance is one of the hallmarks of COPD, and thought to contribute to uncontrolled inflammation in pre-COPD (symptomatic smokers) so dual use should be avoided.

Importantly, this thesis elucidates pathological harms associated with E-vapour exposure. The evidence provided in the studies within this thesis should be used to inform clinicians, researchers and patients on the harms associated with E-cigarette use to improve clinical outcomes in terms of morbidity and mortality in COPD.

List of Abbreviations

AKT - Protein kinase B
ALI – Air liquid interface
APS – Ammonium persulfate
ASM – Airway smooth muscle
ASMCs – Airway smooth muscle cells
BALF – Bronchoalveolar lavage fluid
BME - β -mercaptoethanol
cDNA – Complementary DNA
COPD – Chronic obstructive pulmonary disease
CFTR – Cystic fibrosis transmembrane conductance regulator
CS – Cigarette smoke
CSE – Cigarette smoke extract
CXCL1 – chemokine (C-X-C motif) ligand-1
CXCL2 – chemokine (C-X-C motif) ligand-2
CXCL8 – chemokine (C-X-C motif) ligand-8 or Interleukin-8
DNA – Deoxynucleic acid
DMEM – Dulbecco's modified eagle medium
ELISA – Enzyme linked immunosorbent assay
EVALI – E-cigarette or vaping product use associate lung injury
EVE – E-vapour extract
FBS – Fetal bovine serum
FEV1 – Forced expiration volume in 1 second
FEF – Forced expiratory flow
FOXO1 – Forkhead box protein O1
FVC – Forced vital capacity
GOLD – Global Initiative for Chronic Obstructive Lung Disease
GM-CSF – Granulocyte-macrophage colony-stimulating factor
HBECs – Human bronchial epithelial cells
HR – Heart rate
HRP – Horseradish peroxidase

HREC – Human research ethics committee
IL-1 – Interleukin-1
IL-1 α - Interleukin-1 alpha
IL-1 β – Interleukin-1 beta
IL-6 – Interleukin-6
IL-8 – Interleukin-8
LABA – Long acting beta agonist
LAMA – Long acting antimuscarinic
MCP-1 – Monocyte chemoattractant protein-1
MMP – Matrix metalloproteinase
mTOR – Molecular target of rapamycin
MTT - 3,4,5- dimethylthiazol-2-(yl)-2,5-disphenyltetrazolium
NAC – N-acetyl cystine
NASEM – National Academies of Science, Engineering and Medicine
NDSHS – National drug strategy household survey
NE – Neutrophil elastase
NF- κ B – Nucleation factor-kappa B
NIH – National Institute of Health
NLR – Neutrophil to lymphocyte ratio
NRT – Nicotine replacement therapy
NSCLC – Non-small cell lung carcinoma
p38 MAPK - p38 mitogen-activated protein kinases
PAHs – Polycyclic aromatic hydrocarbons
PBS – Phosphate buffered saline
PI3K – Phosphoinositide 3-kinase
PG – Propylene glycol
PM – Particulate matter
PMNs – Polymorphonuclear cells
PQ - Paraquat
RNA – Ribonucleic acid
ROS – Reactive oxygen species

RT – Room temperature
RT – qPCR – Real time quantitative polymerase chain reaction
SASP – Senescence associated secretory phenotype
SA- β Gal – Senescence associated beta-galactosidase staining
T-PBS – Phosphate buffered saline supplemented with 0.05% Tween (v/v)
T-TBS – Tris Buffered Saline supplemented with 0.05% Tween
TAFE - Technical and Further Education
TEMED - N,N,N',N'-Tetramethyl ethylenediamine
TGF- β – Transforming growth factor beta
TLR – Toll like receptor
TMV – Terminal mucous velocity
TNF α - Tumor necrosis factor alpha
TSANZ – Thoracic Society of Australia and New Zealand
TSNAs – Tobacco specific nitrosamines
VAPI – Vaping associated pulmonary injury
VG – Vegetable glycerin
VOCs – Volatile organic compounds