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GENETIC DAMAGE IN NEW ZEALAND VIETNAM WAR VETERANS

A thesis presented in partial fulfilment of the requirements for
the degree of Master of Science in Genetics at Massey
University, Palmerston North,
New Zealand

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2006

ABSTRACT

From July 1965 until May 1971, New Zealand Defence Force Personnel fought in the Vietnam War. During this time the United States military forces sprayed more than 76,500,000 litres of phenoxylic herbicides over parts of Southern Vietnam and Laos. The most common herbicide sprayed was known as 'Agent Orange'. All of the Agent Orange sprayed during the Vietnam War was contaminated with 2,3,7,8-tetrachlorobenzo-*para*-dioxin (known simply as TCDD), a known human carcinogen. Since returning to New Zealand more than 30 years ago, New Zealand Vietnam War veterans have expressed concern about the numerous health problems experienced by both themselves and their children. New Zealand Vietnam War veterans attribute these health problems to exposure to Agent Orange while serving in Vietnam.

This study aimed to ascertain whether or not New Zealand Vietnam War veterans have incurred genetic damage as a result of service in Vietnam. The Sister Chromatid Exchange assay (SCE) is a very sensitive and widely applied assay used to detect genetic damage induced by an environmental agent or clastogen. In the current study a group of New Zealand Vietnam War veterans and a control group were compared using an SCE analysis in order to determine if genetic damage had been sustained by the Vietnam War veterans. All participants were screened to reduce the possible influence of factors that could severely impact on findings and to eliminate any bias in the SCE results.

The results from the SCE study show a highly significant difference between the mean of the experimental group and the mean of the control group ($p < 0.001$). This result indicates that New Zealand Vietnam War veterans have sustained genetic damage; this damage can be attributed to service in Vietnam (possibly as a result of exposure to Agent Orange). This result is strong and indicates that further scientific research on New Zealand Vietnam War veterans is required.

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge and thank my supervisor, Dr Al Rowland. Your assistance, support and belief in me have been invaluable. Thank you.

I am truly blessed with a family who support and encourage me through everything I do, without them I would not have made it through. I would like to especially thank my parents. Mum, thank you for your amazing kindness and thank you for giving me the strength to carry on, even during the hardest times. Dad, thank you for instilling in me the drive to always achieve my goals and to never give up. Thank you for your participation in my project, without your assistance it would not have been possible. Thank you to my sisters, Rebecca and Elizabeth and to my brother Matthew, you are truly amazing people. A special thank you must be sent out to Michael, thank you for always being interested in how my research is going and for encouraging me so much, thank you also for frequently taking me out for meals! Thank you to Grandma, Aunty and Catherine for your prayers and encouragement, you are very special to me. A special thanks to Jane, Paul, Tereska and all my young cousins – I could not have done it without you, I love you all.

To my amazing partner Daniel, thank you for always being there for me – even when we were living in different cities. Thank you for understanding my excitement when I gained promising results, thank you for always being willing to give me advice, and thank you for letting me explain every aspect of the Vietnam War to you (more than once). Most of all thank you for supporting me through tears and triumph, through the hard times as well as the good times. Thank you for understanding me; you are truly amazing and I love you so much.

A huge thank you to Pam Sisterson for always being there and listening to me ramble about Vietnam, even when I'm sure you had heard it many times before. Thank you for being there for me for the past three years, I am absolutely sure that without you I would never have succeeded. Thank you so much to Corey Laverty, for all your professional advice, and for reading my thesis so many times, thank you for always having a smile on your face, even through the tough times. You are a great friend.

Mohammed Abdul Wahab, Chad Johnson and Ruth Major for your technical advice, your never-ending help and your smiling faces in the lab – thank you. Thank you to Chris Kendrick for your assistance in blood collections. Thank you to John Podd for your very willing assistance with the statistical analysis. To Roger Mortlock, Piers Reid, Ken Avenell and John Govern for giving me so much help in finding participants for the project – thank you.

Lastly, I would like to extend my sincere gratitude and respect to all of my volunteers, especially my veterans, thank you for all you have given, you have served your country well, and you will always be remembered for the price you have paid. Thank you.

ABBREVIATIONS

In addition to the chemical symbols from the Periodic Table of Elements and the *Système International d'Unités* (SI), the following abbreviations are used:

2,4-D	2,4-dichloropheoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxacetic acid
AhR	Aryl Hydrocarbon Receptor
ANCOVA	Analysis of Covariance Assay
Arnt	Aryl Hydrocarbon Receptor Nuclear Translocator
BrdU	5-bromo-2-deoxyuridine
c-metaphse	Colchicine-treated cell in metaphase
CINCPAC	Scientific Advisory Group of the Commander in Chief Pacific
CF	Clastogenic Factor
CYP	Cytochrome P-450
dH₂O	Distilled Water
DNA	Deoxyribose Nucleic Acid
DRE	Dioxin Response Element
<i>et al.</i>	<i>Latin, and others</i>
ES	Effect Size
FISH	Fluorescence <i>in situ</i> Hybridisation
G	Gauge
h	Hour
HIV	Human Immunodeficiency Virus
IARC	International Agency for Research on Cancer
ICMESA	Industrie Chimiche Meda Società
IPCS	International Programme on Chemical Safety
MB	Megabytes
MqH₂O	Milli-Q Water
n	Sample Size
NHL	non-Hodgkin's Lymphoma
p	Probability

<i>pers. comm.</i>	Personal Communication
PHA	Phytohaemagglutinin
PCC	Premature Chromosome Condensation
ppm	Parts Per Million
ppt	Parts Per Trillion
RAM	Random Access Memory
rpm	Revolutions Per Minute
SCE	Sister Chromatid Exchange
Std.	Standard
Std. Dev.	Standard Deviation
Std. Error	Standard Error
SOD	Superoxide Dismutase
TCDD	2,3,7,8-tetrachlorobenzo-<i>para</i>-dioxin
USA	United States of America
UV	Ultra Violet Light
WBC	White Blood Count
w/v	Weight per volume

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1 CHAPTER ONE: INTRODUCTION

In the 1950s South East Asia was an area of the globe in severe political turmoil. Emerging from the post-colonial era, nations were attempting to establish their own identity. For reasons beyond the scope of this thesis, New Zealand became embroiled in one of the most bitter wars of the last century outside of the two World Wars: the Vietnam War.

In 1958, several religious and political groups, most notably the North Vietnamese Communists (Viet Cong), revolted against the South Vietnamese government. New Zealand Defence Force Personnel were based in Vietnam from June 1964. In July 1965, New Zealand troops moved into a combatant role supporting the USA in an attempt to stop invasion of South Vietnam by its North Vietnamese neighbours. New Zealand's troops continued to fight in Vietnam for almost 6 years; the last leaving in May 1971. The Australian Task Force base at Nui Dat, in Phuoc Tuy Province, was established in June 1966, and although most New Zealand troops spent some time here, New Zealand soldiers generally served in Long Khanh, Bien Hoa, Binh Duong, Gia Dinh and Hua Nghia provinces as well as Phuoc Tuy province (Irvine, 2003) (Figure 1.1)

During the Vietnam War the United States military forces sprayed an estimated 76,540,964 litres of phenoxylic herbicides (Duchnowicz *et al.*, 2005) over approximately 3.6 million hectares of Vietnamese and Laotian land in order to remove forest cover, destroy crops and clear vegetation from the perimeters of the US bases as part of their military strategy. A consequence of this decision was a legacy of ill health, not only amongst the Vietnamese population themselves, but also in thousands of American, Australian and New Zealand Vietnam War veterans, and their children.

In 1961, the USA government commenced an aerial spraying programme (codenamed Operation "Ranch Hand") of a group of defoliants, the most common of which was known as 'Agent Orange'. The concentration at which herbicides were sprayed by USA forces was more than an order of magnitude greater than that for similar domestic weed control.



Figure 1.1 South Vietnam 1965-1972

Most New Zealand troops spent some of their time in Vietnam at the Australian Task Force base in Nui Dat, (in Phuoc Tuy Province). The dark colour around Nui Dat indicates the area where New Zealand troops served (Chadwick, 2004)

Between 1961 and 1972 various herbicide mixtures, nicknamed by their coloured identification barrels, were used by the USA and the Republic of Vietnam forces to defoliate forests and mangroves in order to clear perimeters of military installations and to destroy “unfriendly” crops as a tactic for decreasing enemy shelter and food supplies (Stellman *et al.*, 2003).

Operation Ranch Hand dispersed around 95 % of all the herbicides used in Operation Trail Dust, the overall herbicide programme. Other branches of the USA armed services and the Republic of Vietnam forces used hand sprayers, spray trucks, helicopters and boats to disperse the remainder.

Current literature substantiates the view that exposure to Agent Orange and other herbicides can lead to adverse health effects and cause genetic damage in humans (Akhtar *et al.*, 2004; Bukowska, 2004; Duchnowicz *et al.*, 2005; Eriksson *et al.*, 1981; Hardell, 1979; Palmer, 2005; Schechter *et al.*, 1995). With the amount of information that is now available, it is accepted that New Zealand Vietnam War veterans were exposed to Agent Orange and other herbicides during their service in Vietnam. The current study has therefore been established to investigate genetic damage (if any) that has been sustained by New Zealand Vietnam veterans. The Sister Chromatid Exchange Assay (SCE) has been chosen to analyse Vietnam veterans in the current study. The SCE Assay is a reliable and widely applied assay used for detecting genetic damage. This assay has been used successfully in previous studies involving chemical exposure and possible genetic damage (Akin *et al.*, 2005; Arias, 2002; Bhattacharya *et al.*, 2005; Garaj-Vrhorac & Zeljezic, 2001; Iannuzzi *et al.*, 2004; Zober *et al.*, 1993).

The detection of SCE in dividing blood lymphocytes is used to evaluate genetic damage from exposure to environmental genotoxic agents (Sarto *et al.*, 1985; Tucker *et al.*, 1993). Exchanges occur when DNA is replicating after an initial change in the form of DNA base damage (Uggla & Natarajan, 1983). In 2000, the IPCS (International Programme on Chemical Safety) published guidelines for the monitoring of genotoxic effects in humans (Albertini *et al.*, 2000). In defining the significance of the endpoint and application of the sister chromatid exchange assay, the report states "The readily quantifiable nature of SCEs with high sensitivity for revealing toxicant-DNA interaction and the demonstrated ability of genotoxic chemicals to induce a significant increase in SCEs in cultured cells...has resulted in this endpoint being used as an indicator of DNA damage in blood lymphocytes of individuals exposed to genotoxic (agents)." The SCE assay is thus acceptable as an indicator of *in vivo* damage. Furthermore, it is an accepted tenet in the current study that any damage to DNA may lead to ill health and possibly result in intergenerational effects. Follow-up studies on individuals exposed to genotoxic agents have clearly demonstrated the predictive value

of high chromosomal damage for subsequent health risk (Hagmar *et al.*, 1994, 1998, 2001).

1.1 Aim

- **To determine whether or not New Zealand Vietnam veterans have incurred any genetic damage as a result of their service in Vietnam.**

In order to achieve this aim, the following objective is stated: An SCE analysis will be conducted to establish whether or not a sample group of Vietnam veterans have a statistically higher frequency of sister chromatid exchange than a control group of men who did not serve in Vietnam.

1.2 Hypothesis

- **That New Zealand Vietnam veterans have incurred genetic damage as a result of their service in Vietnam.**

The null hypothesis is that New Zealand Vietnam War veterans did not sustain genetic damage. If the null hypothesis is true then we would predict, according to the current objective, that no statistically significant difference in mean SCE frequency between the Vietnam veterans group and the control group would be detected.