Genetic Damage In New Zealand Vietnam War Veterans

Participants Report

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A B S T R A C T

From July 1965 until November 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 a small sample of 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 as a bioindicator of genetic damage induced by an environmental agent or clastogen. In the current study a group of 24 New Zealand Vietnam War veterans and 23 control volunteers were compared using an SCE analysis. 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 suggests, within the strictures of interpreting the SCE assay, that this particular group of New Zealand Vietnam War veterans has been exposed to a harmful substance(s) which can cause genetic damage. Comparison with a matched control group would suggest that this can be attributed to their service in Vietnam. The result is strong and indicates that further scientific research on New Zealand Vietnam War veterans is required.

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T A B L E O F C O N T E N T S

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. 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 revolted against the South Vietnamese government, most notably the Vietcong, coupled with invasion from the north. In response, some western nations became involved in the defence of South Vietnam, including New Zealand. 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 over 6 years; the last leaving in November 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; Schecter *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).

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. Futhermore, 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, within the strictures of the assay applied, 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 incur 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.

2 CHAPTERTWO: LITERATURE REVIEW

2.1 Agent Orange and Health Effects

Over the duration of Operation Ranch Hand, 6 major herbicides were aerially spayed: Agent Pink (approximately 51,000 L); Agent Green (approximately 31,000 L); Agent Purple (approximately 1.8 million L); Agent Orange (unknown volume, but in excess of 50 million L); Agent White (approximately 20.5 million L) and Agent Blue (approximately 4.7 million L).

Approximately 65 % of the herbicides used contained 2,4,5-trichlorophenoxacetic acid (2,4,5-T), and all of the 2,4,5-T used contained 0.5 to 100 ppm of 2,3,7,8-tetrachlorobenzo-*para*-dioxin (known simply as TCDD or Dioxin) as a manufacturing contaminant (Gough, 1991). It is the TCDD that is considered to be the prime cause of detrimental health and genetic effects from these herbicides (Neuberger *et al*., 1999; Palmer, 2005; Pavuk *et al*., 2005; Pearce & Mclean, 2005).

Military herbicide operations in Vietnam became a matter of scientific controversy right from their inception. In April 1970 2,4,5-T was banned from most USA domestic uses and from many other countries on the basis of evidence of its teratogenicity[1] (Stellman *et al*., 2003). Even given this knowledge, the military strategy of using herbicide spray in Vietnam was considered a greater priority at the time.

Estimates of exactly how much TCDD was deposited in Vietnam are based on the volume of 2,4,5-T-containing herbicide sprayed, and on TCDD contamination levels, but are hard to predict. In 1970 when Operation Ranch Hand finally ended, over 3.6 million hectares of forest and villages in Central and Southern Vietnam had been covered with millions of litres of toxic herbicide (Tuyet & Johansson, 2001). Any humans or other living organisms situated in these 3.6 million hectares of forests would have almost certainly come into direct contact with these toxic substances.

[1] A '**teratogen**' is a term used to describe any agent with the potential to cause genetic deformities.

Agent Orange was the most common of the herbicides used by the USA, and comprised a 1:1 mixture of 2,4-dichloropheoxyacetic acid (2,4-D) and 2,4,5-T (Figure 2.1). Agent Orange was used for the longest duration, and was one of the most toxic herbicides used. It was commonly made available in hand-sprayers to be used by soldiers around the perimeters of their camps. No precautions while using Agent Orange were generally enforced, thus giving soldiers the impression that this substance was harmless. Aerial spraying and hand spraying missions not only brought soldiers and the Vietnamese living in the area into direct contact with the toxic herbicide, but also caused contamination of drinking water and many food sources such as fish and crops. Exposure to Agent Orange and the toxic contaminant TCDD therefore occurred very easily during the Vietnam War, and was usually unavoidable. Dai (2000) estimates that during the War about 17 million people living in South Vietnam, and about one million from the North, were directly exposed to TCDD-contaminated herbicides.

In studies conducted by Schecter *et al.* (1995) comparing Vietnam veterans with contemporary veterans who had served elsewhere, TCDD levels were found to be significantly elevated among those who had served in Vietnam.

2.1.1 2,3,7,8-tetrachlorobenzo-*para*-dioxin (TCDD)

Known simply as TCDD or Dioxin, this chemical is produced as a by-product of many industrial processes. It is a contaminant of particular phenoxylic herbicides that contain 2,4,5-T and have been manufactured and used in many countries through out the world. TCDD is formed during the incomplete combustion of organic material where chlorine is available in the feedstock or in the air supply. It is also produced at trace levels in various industries. Focus on dioxins as contaminants began in the 1940s and 1950s in industrial settings, where manufacture of chlorinated phenoxy herbicides occurred, as a result of exposed workers exhibiting particular health problems (Aylward & Hays, 2003). The ability of TCDD to affect the endocrine system, and toxic effects on experimental animals, prompted several studies into the possible effects of dioxin on humans, especially regarding their reproductive ability. TCDD behaves as a multi-site human carcinogen, and is thought to induce tumours in humans indirectly (Albertini *et al.*, 2000). The biological mechanism of TCDD-induced carcinogenesis is not

completely clear. Thus exposure to TCDD results in a broad spectrum of biological responses, including altered metabolism, disruption of the normal hormone signalling pathways, and reproductive and developmental effects.

2.1.2 TCDD Half Life in Humans

The half life of a particular chemical is of great importance for hazard assessment because it allows an estimation to be made of the persistence of a chemical in living aquatic and terrestrial organisms. The half life is the time required to reduce the concentration of a chemical by one-half in tissue, organ or in the whole organism (Geyer *et al*., 2000).

It is known that TCDD is very persistent and has a long half-life in organisms (Geyer *et al*., 2000; Li *et al*., 1999; Van den Berg *et al*., 1994). Geyer *et al*. (2002) states that the average half life of TCDD in humans is approximately 2,840 days (7.78 years). A half life as large as this means that New Zealand Vietnam veterans who were exposed to TCDD more than 30 years ago are still very likely to have elevated TCDD levels when compared to non-veterans. As recently as 1995, TCDD blood levels were found to be between 25 and 170 times higher in people living in sprayed areas of Vietnam, compared to people living in unsprayed villages in Northern Vietnam (Palmer, 2005).

2.1.3 Health Effects Caused by Exposure to TCDD

Spraying of Agent Orange during the Vietnam War represents the world's largest ever TCDD contamination to date. Health effects associated with exposure to TCDD have not been fully characterised. In 1997 the International Agency for Research on Cancer (IARC) classified TCDD as a Group 1 human carcinogen, based largely on four highly-exposed industrial studieds that showed an excess of all cancers (Steenland *et al*., 1999). The largest of the four groups considered by the IARC is the USA study group of 5,172 workers at 12 plants that produced chemicals contaminated with TCDD. These workers were exposed to high levels of TCDD. The workers were found to have on average 286 times more TCDD in their blood than the general population. This population was also found to have a 46 % greater mortality rate caused by cancers.

A recent study contrasting cancer incidence rates in white male USA Air Force veterans involved in Operation Ranch Hand with the USA white male population reported increases in prostate cancer and melanoma in Ranch Hand Veterans (Akhtar *et al*., 2004). Pavuk (2005) reported statistically significant associations between TCDD and all types of cancer in USA Air Force veterans selected as comparisons in the Air Force health study.

Farm and agricultural workers who are exposed to a range of chemicals as part of their job have been the subject of many scientific studies. Illing (1997) found an increased rate of cancer in farmers and agricultural workers that was directly related to their occupational background exposure to organochlorines (including TCDD) and other pesticides. Dich & Wiklund (1998) reported a statistically significant increased risk of prostate cancer among pesticide applicators. In Britain a study was conducted around a pesticide factory, revealing an excess of skin melanoma, lung, stomach, pancreas and prostate cancers (Wilkinson *et al*., 1997). A series of case-control studies in Sweden have found increased risks of soft-tissue sarcoma and malignant lymphoma among agricultural workers who had been exposed to phenoxy herbicides (Eriksson *et al*., 1981; Hardell & Sandstrom, 1979).

Research on ex-servicemen from the Vietnam War has shown significant associations between TCDD exposure and certain kinds of cancer, including soft tissue sarcoma (Eriksson & Hardell, 1990; Lynge, 1993), non-Hodgkin's lymphoma (Pearce & Mclean, 2005) and multiple myeloma (Bertazzi *et al.*, 2001). One major investigation that warrants special attention is the on-going study of the residents of Seveso, a small town in Italy. In 1976 an explosion at the ICMESA (Industrie Chimiche Meda Società) chemical plant near Seveso resulted in the highest exposure to TCDD in a residential population to date. The earliest related health effect was chloracne in children who were outdoors and in the path of the toxic cloud (Caramaschi *et al*., 1981). In the following years other adverse health effects were observed and found to be linked to TCDD exposure. These include spontaneous abortions (Revich *et al*., 2001), cytogenetic abnormalities (Bertazzi *et al*., 2001), congenital malformations (Mastroiacovo *et al*., 1988; Revich *et al*., 2001), impaired liver function and lipid metabolism (Ideo *et al*., 1985; Mocarelli *et al*., 1986).

In one particular study of the Seveso population, Bertazzi *et al.* (2001) discovered a two-fold increase in rectal cancer-induced deaths and an excess of "other" digestive cancer-induced deaths. Lung cancer was also in moderate excess; nearly twice as many lymphohemopoietic neoplasms were observed than expected. Bertazzi *et al*. (2001) also reported particular increases in Hodgkin's disease, multiple myeloma, and acute myeloid leukaemia. All of these cancers are well established as cancers arising from specific genetic malfunction. Moderate to significant increases were also observed in chronic obstructive pulmonary disease and diabetes.

In addition to known carcinogenic properties, Steenland *et al.* (1999) reported that TCDD exposure is a possible cause of heart disease. Elevated ratios for mortality from heart disease were found in a large multi-country study. Steenland *et al.* (1999) also reported a positive relationship with total cholesterol and TCDD exposure.

Exposure to Agent Orange also has major effects on the reproductive system of humans; TCDD is an endocrine-disrupting chemical with a highly toxic effect on the human reproductive system (Rogan & Ragan, 2003). Even at low doses TCDD can seriously disrupt normal reproduction in humans; it can lower fertility, increasing antenatal mortality and the risk of endometriosis, and can also cause many birth defects (Lawson *et al*., 2004). Egeland *et al*. (1994) conducted a study on male reproductive endocrine function of subjects occupationally exposed to TCDD and found that exposed individuals had lower testosterone and higher gonadotrophin levels in a dose-dependent relationship with increasing serum dioxin concentrations. In addition to these illnesses, Agent Orange exposure was also found to be associated with the onset of porphyria cutanea tarda[2] (Frumkin, 2003).

Henriksen *et al*. (1997) performed a cross-sectional medical study of the Operation Ranch Hand participants and found a 50 % higher prevalence of diabetes among those individuals with the highest levels of TCDD in serum, compared with non-exposed control individuals. Steenland *et al*. (2001) found an increased risk of diabetes with TCDD exposure. Kern *et al*., (2002a) biochemically linked TCDD exposure and

[2] **Porphyria cutanea tarda** is a disorder of heme biosynthesis due to a defective liver enzyme. Symptoms of this disorder include photosensitivity; hepatic dysfunction; discolored teeth, gums and skin; excessive hair; and psychiatric symptoms.

diabetes in humans, complementing the many epidemiological studies that had been conducted previously.

Although the majority of current literature supports the claim that exposure to TCDD causes detrimental health effects, there are some studies that disagree. Most of these studies are related to the reproductive outcome of those exposed to TCDD. Wolfe *et al*. (1995) conducted a study on reproductive outcomes in Vietnam War veterans and found no elevation in the risk of spontaneous abortion or still birth. Some elevations were found in birth defects but these were reported to be non-significant and there was no increase in birth defect severity. Schnorr *et al*. (2001) found no association between paternal TCDD levels at the time of conception and spontaneous abortion or sex ratio among pregnancies fathered by men exposed to TCDD.

Goetz *et al*. (1994) investigated neurological disorders and brain tumours in Vietnam veterans exposed to TCDD; it was concluded that there was insufficient evidence to determine an association between neurological disorders and exposure to TCDD in Vietnam. However, it was found that there was limited evidence to suggest no association between exposure and brain tumours.

2.2 Exposure of New Zealand soldiers to herbicide sprays

Past claims of herbicide exposure have largely relied on anecdotal evidence. More recently however, a major Select Committee report conducted by the New Zealand Government and published in October 2004 has resulted in more convincing information being presented on exactly where New Zealand soldiers were at certain times, which corresponds exactly to times and places of Agent Orange release. The case is no longer circumstantial and the evidence strongly substantiates the claim that New Zealand troops were exposed to Agent Orange. The claim that Vietnam veterans were exposed to herbicide spraying as part of Operation Trail Dust was recognised by the Government in December 2003, 32 years after the New Zealand Vietnam War veterans left Vietnam.

On 3 December 2003 a new report was submitted to Parliament's Health Select Committee (Chadwick, 2004). The Chief of the Defence Force had requested an investigation be conducted into the spraying of herbicides in Phuoc Tuy province in South Vietnam between 1965 and 1971 (Figure 1.1). The report concluded that while serving in Vietnam between 1965 and 1971, New Zealand Vietnam War veterans were exposed to large quantities of the defoliants Agent Orange, Agent Blue[3] and Agent White[4]. It was estimated that at least 1.8 million litres of the defoliants Agent Orange, White and Blue was sprayed in the Phuoc Tuy Province between November 1965 and June 1968 (Taylor, 2003).

Phuoc Tuy Province was the location of the first Ranch Hand missions conducted in January 1962. The head of the Scientific Advisory Group of the Commander in Chief, Pacific (CINCPAC) reported that 227,125 litres of defoliant was sprayed on the Phuoc Tuy Province between December 1965 and January 1966. New Zealand Vietnam veterans were serving in this province during this time (Irvine, 2003).

Yet more evidence that New Zealand War Veterans became exposed to harmful defoliants during their service in Vietnam was presented upon examination of the military command zones which the American commanders divided South Vietnam into: I Corps, II Corps, III Corps and IV Corps (Figure 2.3). New Zealand and Australian units operated exclusively in III Corps. Operation Ranch Hand records show that III Corps received 21,521,614 litres of Agent Orange, 563,852 litres more than the other three zones combined.

The evidence is substantial to support the claim that New Zealand troops were both directly and indirectly exposed to the TCDD-containing herbicide Agent Orange while serving in Vietnam.

[3] **Agent Blue** was a mixture of herbicides containing Cacodylic acid, commonly known as Arsenic. It was used specifically to kill rice in Vietnam.
[4] **Agent White** was a 4:1 mixture of 2,4-D and Picloram. Picloram is a herbicide used on woody plants.

2.3 Consequences of Herbicide Exposure in Vietnam

During the 1970s, returned Vietnam Veterans began to report skin rashes, cancers, psychological symptoms, extreme fatigue, congenital abnormalities as well as handicaps in their children, and many other health problems. In the USA some 300,000 veterans have undergone medical tests and an estimated 2,000 children of veterans are suffering from the birth defect spina bifida (Palmer, 2005). A Columbia University study also estimates that up to 4 million people may be directly affected by Agent Orange (Stellman *et al*., 2003). Most veterans are concerned that Agent Orange exposure might have contributed to these health problems. These concerns helped to initiate a series of scientific studies on Agent Orange and its carcinogenic contaminant TCDD. In addition to polluting the environment, exposure to the toxic TCDD-containing herbicides has been found to cause many diseases, including several types of cancers (Hardell & Eriksson, 1999; Hardell & Sandstrom, 1979; Khuder *et al*., 1998; Lynge, 1998; Pavuk *et al*., 2005; Safi, 2002; Saracci *et al*., 1991), as well as causing increased rates of endometriosis (Igarashi *et al*., 2005; Rier & Foster, 2003), congenital birth defects (Barrow *et al*., 2002; Lawson *et al*., 2004) and other health problems (Aoki, 2001; Lawson *et al*., 2005; ten Tusscher *et al*., 2003) in the children of those exposed.

Tuyet and Johansson (2001) conducted a study on Vietnamese women and their husbands who were exposed to Agent Orange during the Vietnam War. The authors found that 66 % of all children had some type of major health problem. Thirty-seven percent of these children were born with some visible malformation or disability while 27 % had developed a disability during the first year of life. Of the 60 children suffering from health problems, 40 were unable to attend school but were able to help with agricultural work and domestic chores. Twenty children were very severely physically and mentally disabled, and required 24-hour care; needing to be attended to by their parents for every daily need. There were no cases of congenital malformation nor other disabilities among unexposed siblings of the husbands and wives, nor among the children of their siblings.

Giri *et al*. (2004) concluded that exposure to Agent Orange is associated with an increased risk of prostate cancer. Men who had been previously exposed to Agent

Orange were at least two times more likely to be diagnosed with prostate cancer as unexposed men. Pavuk *et al*. (2005) also reported that prostate cancer was significantly associated with service in South East Asia (including, or exclusively in, Vietnam).

2.4 USA & Australian Reactions to Agent Orange Exposure

The United States Department of Veteran Affairs now accepts a link between Agent Orange exposure and Hodgkin's Disease, multiple myeloma, non-Hodgkins lymphoma, respiratory cancers (lung, bronchus, larynx and trachea), soft-tissue sarcoma, prostate cancer, chronic lymphocytic leukemia, porphyria cutanea tarda, acute and subacute peripheral neuropathy, and adult-onset diabetes.

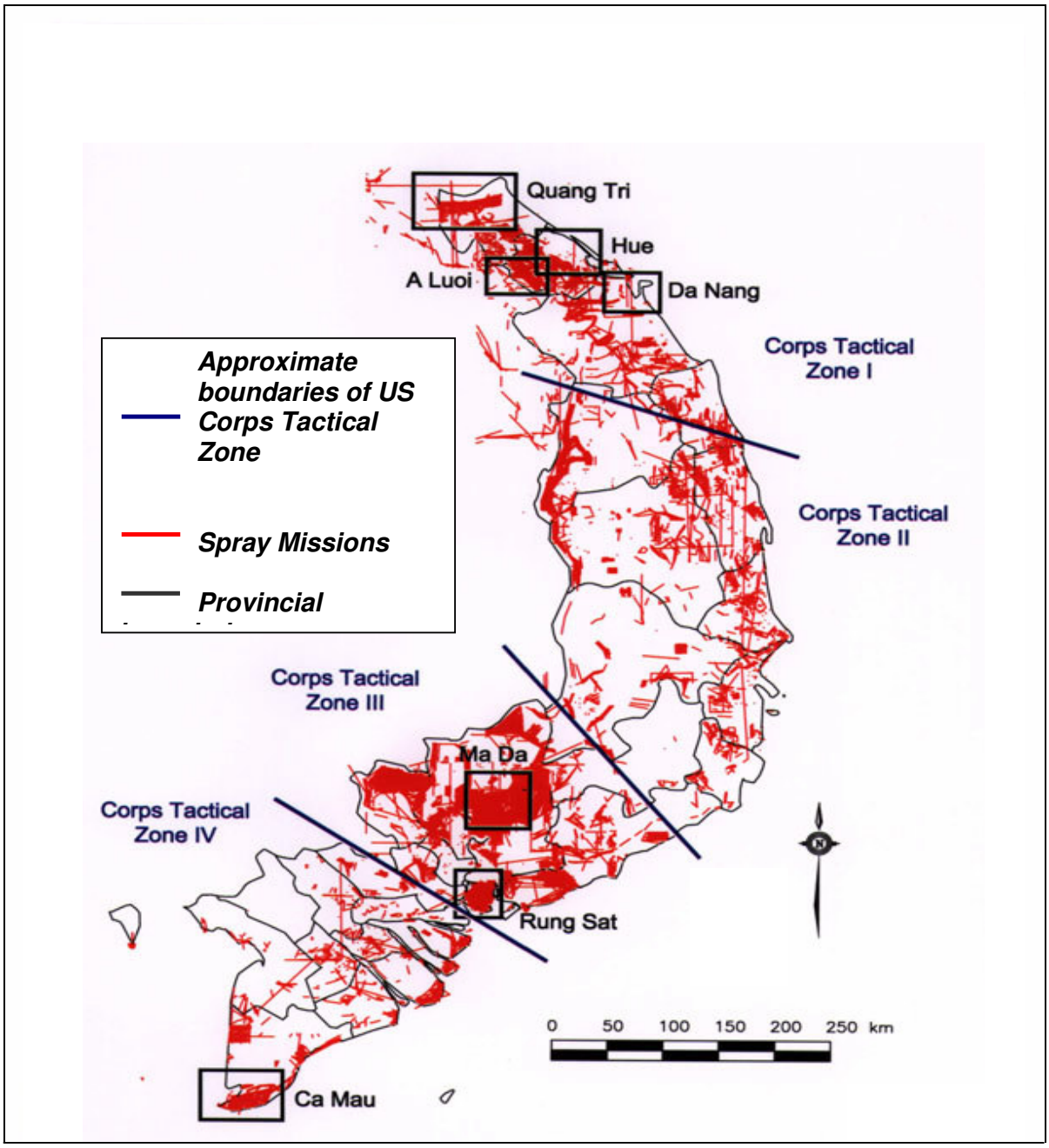

Figure 2.3 Map of Agent Orange Spraying

Map of South Vietnam, showing the aerial herbicide spray missions of Agent Orange from 1965 to 1971 as part of Operation Ranch Hand. New Zealand troops served exclusively in III Corps. III Corps received the greatest volume of Agent Orange.

US Department of the Army (Irvine, 2003)

The USA Government also accepts a link between Agent Orange and spina bifida in children of male veterans and a link between all birth defects that are not caused by familial disorder, birth-related injury, or foetal or neonatal infirmity in children of female veterans. Compensation and health care are provided to veterans and children of veterans suffering from these illnesses (Chadwick, 2004; Irvine, 2003).

According to Irvine (2003), the Australian Department of Veteran Affairs does not accept a link between Agent Orange and these health problems; yet they give the benefit of the doubt to veterans on a case-by-case basis. They also provide treatment to any Vietnam veteran with cancer. The Australian Department of Veteran Affairs (while still not accepting a link) in partnership with the Department of Health and Ageing, provides treatment to children of Vietnam veterans born with cleft lip or palate, spina bifida, acute myeloid leukemia, and adrenal gland cancer.

2.5 Detection of Genetic Damage

Although concerned about the direct health effects of Agent Orange, Vietnam War Veterans are most fearful of long term genetic damage, with the possibility of this damage being passed on to their children and further generations. Several molecular assays are now available to researchers to determine if genetic damage has occurred in humans. One of the most sensitive and widely applied tests for clastogenicity[5] is the Sister Chromatid Exchange (SCE) assay. Due to the time constraints on the current study it was possible to conduct only one of the cytogenetic tests mentioned here. SCE was chosen due to its high sensitivity and success rate in studies on clastogens.

2.5.1 Determination of Genetic Damage using SCE Assay

As noted earlier (Section 1), a statistically significant increase in the average SCE frequency in an experimental group compared to a matched control group, is, according to Albertini *et al.* (2000), indicative of genetic damage. To reiterate, the SCE

[5] A **'clastogen'** is the term used to describe any environmental agent which results in damage to DNA. A clastongen may or may not be a 'mutagen' (resulting in mutations directly), a 'teratogen' (resulting in genetic deformities) or a 'carcinogen' (resulting in cancer).

cytogenetic test is used to visualise the number of SCEs in cells, and is considered a bioindicator of any genetic damage that has been sustained by the subject. It is therefore a test that indicates the harmfulness of particular chemicals.

Changes in the genetic fingerprint induced by environmental mutagens may produce harmful genetic effects on human health, causing mutations in sex cells and somatic cells. Sex cell mutations create a genetic risk for hereditary diseases and congenital defects while somatic cell mutations result in various diseases including cancer (Kaioumova & Khabutdinova, 1998). The SCE test has been previously applied successfully to animals and humans to determine whether or not genetic damage has been incurred as a result of exposure to dioxins and other chemicals (Akin *et al*., 2005; Arias, 2002; Bhattacharya *et al*., 2005; Garaj-Vrhovac & Zeljezic, 2001; Iannuzzi *et al*., 2004; Zober *et al*., 1993). Zober *et al.* (1993) show that the SCE technique is suitable to evaluate possible genotoxic effects of particular chemicals even if exposure occurred many years ago. It is therefore appropriate that the SCE test be performed as a test for ascertaining evidence of genetic damage, if any, sustained by New Zealand Vietnam Veterans as a result of exposure to herbicides while serving in Vietnam. The results of the current study will add to the body of knowledge so far gathered on the genetic health status of Vietnam War veterans.

2.6 The Sister Chromatid Exchange Assay

The incidence of sister chromatid exchanges in a cell increases due to many variables including age, smoking, some medications, and exposure to any substances that cause damage to DNA. A wide variety of agents that are known to cause chromosome breaks have also been found to induce SCE. Numerous studies have utilised SCE analysis to explore the extent of genetic damage caused by environmental agents. Rowland & Harding (1999) found that SCE frequency increased in female smokers between the ages of 16 and 25 years, compared to a non-smoking control group. A study conducted on methamphetamine abusers also found significant increases in SCE frequencies (Li *et al*., 2003). Iannuzzi *et al*. (2004) reported increases in the frequencies of SCEs in sheep flocks that had been exposed to specific dioxins in herbicides. Exposure to pesticides, iodine-131, wood dust, and many other environmental factors have also shown

increases in SCE frequency when compared with matched controls (Elavarasi *et al*., 2002; Sonmez *et al*., 1997; Zeljezic & Gavaj-Vrhovac, 2002). These studies highlight the usefulness of SCE analysis in the biomonitoring of human populations exposed to a variety of agents.

Evidence of genetic damage is accepted if the number of SCEs in an experimental group is more statistically significant than a selected control group (Albertini *et al*., 2000). A significant increase in SCE frequency is accepted as an indication that the DNA of a target group has been damaged in some way. Any damage to DNA is universally accepted as being detrimental to a person's well-being.

3 CHAPTER THREE: RESULTS/ DISCUSSION

3.1 SCE Analysis

The Sister Chromatid Exchange Assay (SCE) is a very sensitive and widely applied assay to study genetic damage induced by an environmental agent or clastogen. In the current study a group of New Zealand Vietnam veterans and a control group were compared using an SCE analysis, with the aim of exploring the possibility of genetic damage in the experimental group.

The SCE analysis initially included 25 men in each of the veterans and control group, a total of 50 participants. The personal questionnaire (Appendix IV) was completed and returned to the researcher by 47 of these participants. Of the remaining three men, two failed to return a questionnaire by the conclusion of the study, and one returned an incomplete questionnaire that was unsuitable for use in statistical analysis for the current project. Hence 24 veterans and 23 control individuals made up the study group. All results and statistical analyses for the current study were therefore calculated using this group of 47 individuals only.

The results from the sister chromatid exchange assay show a highly significant difference between the mean of the experimental group and the control group. A difference between these two groups was expected if genetic damage, according to the limitations of the SCE assay as a bioindicator, has been sustained by the veterans group while serving in Vietnam. However, a statistical analysis was required to ascertain whether the difference seen between the two groups was statistically significant or possibly just due to coincidence. It was essential to determine what confounding factors may be acting on individuals in the current study and perhaps altering the results, leading to inaccurate conclusions. Therefore, a close examination of the confounding factors known to have

an effect on SCE rates was conducted. The following is a summary and explanation of all results obtained leading to the conclusions drawn from the study overall.

	Mean SCE/Cell	Standard Deviation	Number of Participants
Veteran Group	**10.99**	**3.05**	**24**
Control Group	**8.24**	**1.10**	**23**

Table 3.1 SCE Analysis Results
Descriptive statistics for the mean SCE rates of the veteran and control group. Each set of statistics was calculated from the 50 cells analysed for each participant.

Table 3.1 shows the overall results for the SCE analysis – Mean SCE/Cell shows the average SCE frequency per cell for each of the veterans and controls. This average value was taken from the mean of 50 consecutive cells counted for each participant. The difference seen here with raw data (approximately 2.75 SCE/cell) appears to be large.

The graph below shows exactly the same information as in Table 3.1 but in a graphical form. The large difference between the two groups is much more easily seen in this representation. The 95% confidence is also indicated on the graph. The 95% confidence interval is an important parameter because it says that we can be 95% confident that the actual average SCE/cell for each group falls within these confidence intervals. Because there is no overlap between the two 95% confidence intervals shown on the graph below, we can be very confident that the differences between the two groups is meaningful.

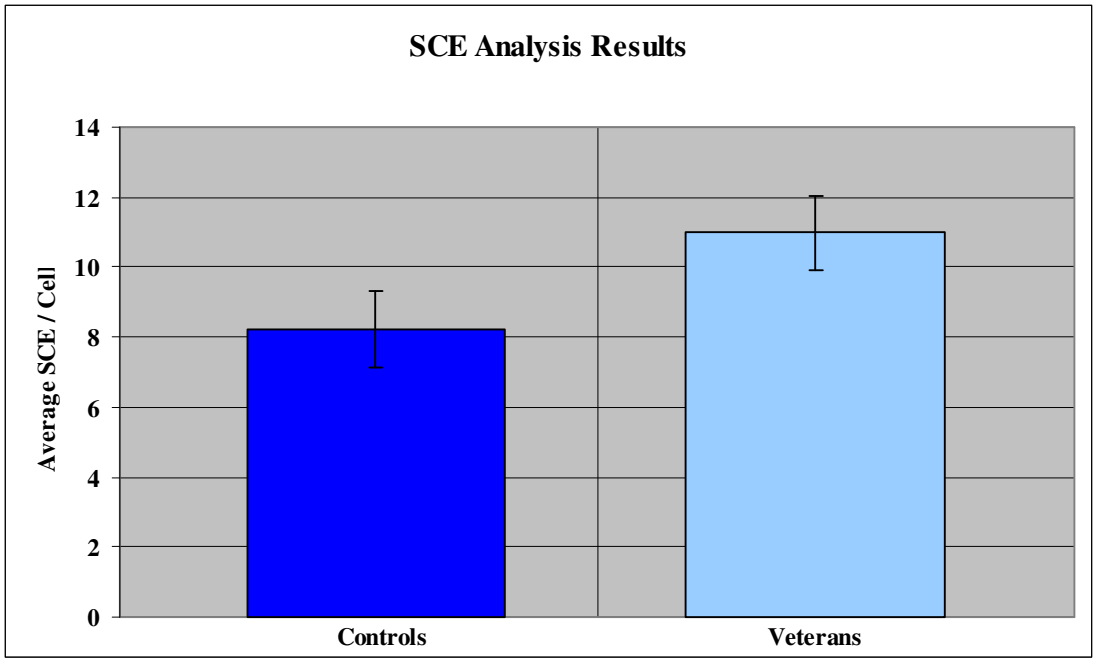

Figure 3.1 SCE Analysis Results
Graphical representation illustrating the descriptive statistics for the SCE rates (Table 4.1). The increase in average SCE per cell between the two groups can be clearly seen. The 95% confidence intervals are indicated on the error bars; the confidence intervals do not overlap.

3.2 Confounding Factors

It is possible that there are factors other than service in Vietnam that may affect the SCE results obtained in the current study. Any factor which affects SCE results other than the factor being tested (in this case service in Vietnam) is known as a confounding factor. These confounding factors could be causing the difference seen between the groups. Careful consultation of current literature revealed that the biggest confounding factor with regard to SCE studies is cigarette smoking, with smokers consistently having significantly higher SCE rates compared to non-smokers (Barale *et al.*, 1998; Biro *et al.*, 2002; Burkvic *et al.*, 1998; Burgaz *et al.*, 1998; Karaoguz *et al.*, 2005; Lambert *et al.*, 1982; Lazutka *et al.*, 1992; Sardas *et al.*, 1991; Testa *et al.*, 2005). Karaoguz *et al.* (2005) and Popp *et al.* (1994) also found alcohol consumption to be a confounding factor in SCE studies. Age has also been consistently reported as having a significant association with SCE rates in humans (Burgaz *et al.*, 1998; Gulten *et al.*, 2002; Kaul *et*

al., 2001; Kelsey *et al*., 1992). The review of current literature clearly indicated three confounding factors that needed to be statistically corrected for: age, cigarette smoking and alcohol consumption; all have the potential to affect SCE results. Information on age, smoking rates and average alcohol consumption of participants was available in the personal questionnaires.

When investigating confounding factors it is important to note that the SCE assay only detects exchanges that are occurring in the bloodstream at the time the blood is drawn. Therefore, it is only necessary to correct for those confounding factors which are having an effect currently. Approximately six months after cessation of cigarette smoking, SCE frequencies return to normal (Lazutka, *pers. comm.*). Therefore although some participants in the current study may have smoked earlier in life, the SCE assay will not indicate any damage from this past smoking, providing it was at least 6 months prior to the blood used in the current study being drawn. Examination of all personal questionnaires revealed that all non-smokers in both the veteran and control group had not smoked for a minimum of 12 months.

Although every effort has been made to correct for all confounding factors, which could be affecting SCE rates, it is possible that one or more confounding factors have been overlooked due to the fact that they are unknown to the researcher. It appears likely, however, for the data obtained, that there is some factor(s) involving the veterans group that has resulted in them showing higher average SCE rates compared to New Zealand army personnel who did not serve in Vietnam. By controlling for all known confounding factors, we can assume this difference is caused by service in Vietnam.

After correction for confounding factors the results were not changed, indicating that these factors were having no significant effect on the SCE results.

3.3 Statistical Analysis

The statistical analysis that was conducted gave a *p*-value of less than 0.001, which indicates that the difference in means between the experimental and control groups was highly statistically significant. Accepting the SCE assay as an indirect bioindicator, this result would suggest that New Zealand Vietnam veterans may have sustained genetic damage as a result of service in Vietnam. Further investigation into the results was conducted providing more statistical data to support this finding.

3.3.1 Within-Group Variability

When analyzing the descriptive statistics for the raw SCE data, it became apparent that although there was a significant difference between the veteran and the control groups, the spread of the data within these groups was also very different. The standard deviation for the control group was 1.10, the veterans group had a standard deviation of 3.05, almost three times higher than that of the control group. It was therefore necessary to investigate the spread of data in each of these groups to try and ascertain the reason for this large difference. It was possible that the higher standard deviation and perhaps the higher mean seen in the veterans group was due to a small number of very high SCE frequencies skewing the distribution. The average SCE frequency per cell for each of the veterans and controls was plotted (Figure 3.2 below) to investigate the spread of these groups and compare them with one another. Figure 3.2 illustrates the difference in spread between the two groups. It can be seen that the difference in standard deviation between the groups is due to the overall spread of data being greater in the veterans group compared to the control group; the large difference in spread between the two groups is not due to a small number of outliers that are skewing the veterans group distribution, although the distribution is slightly skewed.

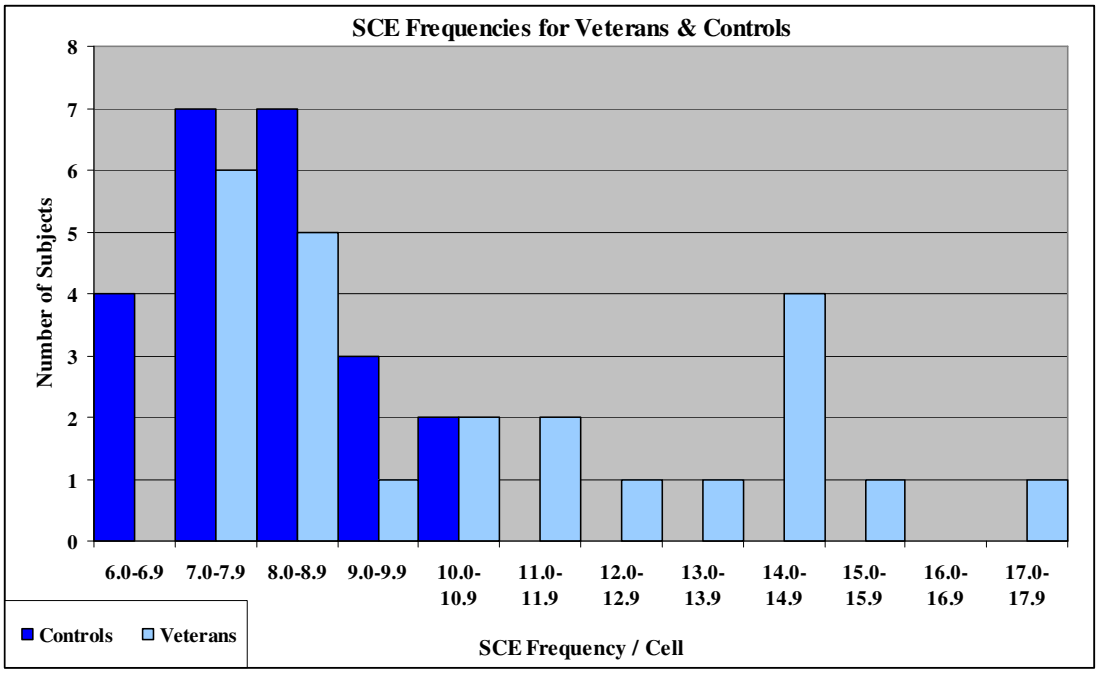

Figure 3.2 Plot of SCE Raw Data
The raw data (SCE averages) for each of the 24 veterans and 23 controls being statistically analysed. This plot clearly displays the difference in spread between the two groups. It is evident from this plot that the difference in means between the two groups is not due to a few high value outliers.

Note that out of the 24 veterans included in this analysis, 12 have higher SCE frequencies than the highest SCE frequency recorded for the entire control group (10.54 SCEs/cell). Therefore, half of all veterans studied have a higher SCE frequency than the highest obtained SCE frequency among the controls. This illustrates the fact that the experimental group have much higher SCE rates as a group when compared to the control group and the statistical analysis reflects this. The 95 % confidence intervals for the estimated means show no overlap between the veterans and the control group (Figure 3.1), giving further evidence that the difference in the means is significant, despite the difference in spread. It is also important to note that the estimated statistics show very similar values for the standard error of each distribution respectively. This again reiterates the fact that the difference between the two groups is highly significant.

3.3.2 Statistical Effect Size

The Effect Size (ES) is a measure of the magnitude of a treatment effect; in the case of the current study a measure of the magnitude of exposure effect. The ES is independent of sample size. Cohen (1988) defined any ES of 0.8 or larger as being a large effect. The ES obtained for the current study, 1.226, is therefore a large ES, and indicates that the effect of service in Vietnam (perhaps caused by exposure to Agent Orange), is very large.

3.3.3 Conclusions

- The SCE results obtained show a highly statistically significant difference between a group of New Zealand Vietnam veterans and a group of matched controls ($p < 0.001$)

- Following statistical correction for influence of confounding factors, the difference in mean between the two groups remains highly significant ($p < 0.001$)

3.4 Overall Summary

It is important to consider that statistical significance of genetic damage should be interpreted cautiously with regard to the biological significance. The SCE assay cannot be applied as a diagnostic tool. Although it is interpreted as a sensitive and powerful bioindicator of genetic damage, it cannot predict specific health outcomes. However, genetic damage to any degree has the potential to result in adverse health effects.

In the current study, a significantly higher frequency of SCE was observed in a sample group of New Zealand Vietnam War veterans compared to a matched control group of New Zealand army and ex-army personnel. Elevated frequency of SCE in a target group is an accepted indicator of clastogenicity/genotoxicity.

3.4.1 Conclusion

According to the guidelines published by the ICPS (Section 1 of this report) defining the significance of the endpoint of the SCE assay as a bioindicator of genetic damage, it is possible to draw the following conclusion from the results obtained from the current study:

- **The SCE assay conducted on a small sample of New Zealand Vietnam veterans in this study would suggest that these men have been exposed to a harmful clastogenic agent as a result of service in Vietnam. Within the strictures in interpreting the biological significance of this particular assay, there is an indication that these men may have incurred genetic damage.**

4 CHAPTER FOUR: RECOMMENDATIONS

It is acknowledged by the author that time constraints of a two year project have resulted in the small sample size of the current study. However, the strong results that have been obtained are interpreted, with limitations, as being indicative of genetic damage in New Zealand Vietnam War veterans and should be seen as an alert signal.

4.1 New Zealand Vietnam War Veterans

The current study has obtained highly significant results and strong evidence that New Zealand Vietnam War veterans have been exposed to a clastogen which can cause genetic damage as a result of service in Vietnam. The results therefore warrant a larger study of New Zealand Vietnam War veterans. A larger study would consist of a significantly larger sample size (minimum of 50 veterans and 50 controls) than the current study. In addition to a larger sample size, a number of cytogenetic assays, such as those mentioned in Section 2.5 should be used for analysis.

4.2 Children of New Zealand Vietnam War Veterans

The current study has detected high SCE frequencies in New Zealand Vietnam War veterans, but in a small sample. Nonetheless, the convincing results suggest that a similar scientific investigation of the children of these veterans be conducted using other assays. Whilst not wanting to appear alarmist, inherited genetic damage can be passed on to the next generation, possibly causing detrimental health effects through many generations to come.

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