New Zealand Nuclear Test Veterans' Study – a Cytogenetic Analysis

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Responsibility for all information presented in this report lies with the authors.

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❖ OVERVIEW

This report presents the findings of three assays performed to assess the genetic status of those New Zealand military personnel who participated in Operation Grapple in 1957-58. Two of the assays: the G2 assay and the micronucleus (MN) assay show no difference between the veterans and the matched controls, which suggests that DNA repair mechanisms in the veterans are not deficient.

The results reported here using the mFISH assay, however, show elevated translocation frequencies in peripheral blood lymphocytes of New Zealand nuclear test veterans 50 years after the Operation Grapple series of nuclear tests. The difference between the veterans and the matched controls with this particular assay is highly significant. The total translocation frequency is 3 times higher in the veterans than the controls who showed normal background frequencies for men of this age group. This result is indicative of the veterans having incurred long term genetic damage as a consequence of performing their duties relating to Operation Grapple.

A careful comparison of the veterans and the controls for possible confounding factors, together with a close analysis of the scientific literature in related studies, leads us to a probable defining cause for the chromosome anomalies observed. Ionizing radiation is known to be a potent inducer of chromosome translocations. We submit the view that the cause of the elevated translocation frequencies observed in the veterans is most likely attributable to radiation exposure.

❖ INTRODUCTION

In 1957/58 the British Government conducted a series of nuclear tests at Christmas Island and Malden Island in the mid-Pacific Ocean. This series of detonations was given the codename “Operation Grapple”. These islands were previously part of the Line Islands group but are now part of the country known as Kiribati. Operation Grapple consisted of 9 nuclear detonations between May 1957 and September 1958. A series of 3 atomic (fission) detonations occurred over the ocean near Malden Island. A further 4 detonations of atomic (fission) devices occurred over the ocean at Christmas Island in addition to 2 smaller thermonuclear (fusion) devices over land.

The Grapple series involved several naval vessels from Britain and New Zealand. Two New Zealand frigates attended the series of detonations: the HMNZS Pukaki and the HMNZS Rotoiti. Over the course of these tests a total of 551 New Zealand naval personnel manned these ships. Their duties consisted of witnessing the detonation of the nuclear devices and collecting weather data.

During the Operation Grapple tests, the New Zealand vessels were stationed at various distances of between 20 and 150 nautical miles upwind from ground zero, the point on the ocean surface above which the devices were detonated (Crawford, 1989). The Pukaki was present in all of the 9 tests, while the Rotoiti was present only at the first 4 tests. Table 1 (page 2) shows the detonation and distance information for each of these ships.

The unavailability of data from film badges worn by the participants during these tests makes it difficult to establish with certainty whether or not these individuals received any radiation dosage, or if they did, to what degree. Nevertheless, since the tests, veterans have claimed, rightly or wrongly, that their quality of life has been affected as a direct result of their participation in Operation Grapple.

Table 1. The location and yields of each Operation Grapple test, and the position of each ship at the time of each detonation (Crawford, 1989).

					Distance From Ground Zero (Nautical Miles)	
Round	**Date**	**Island**	**Height (m)**	**Yield**	**Pukaki**	**Rotoiti**
Grapple 1	15/05/1957	Malden	2400 m	Megaton	50	150
2	31/05/1957	Malden	2300 m	Megaton	50	150
3	19/06/1957	Malden	2300 m	Megaton	150	50
X	08/11/1957	Christmas	2250 m	Megaton	132	60
Y	28/04/1958	Christmas	2350 m	Megaton	80	-
Z1	22/08/1958	Christmas	450 m	Kiloton	28	-
Z2	02/09/1958	Christmas	2850 m	Megaton	35	-
Z3	11/09/1958	Christmas	2650 m	Megaton	35	-
Z4	23/09/1958	Christmas	450 m	Kiloton	20	-

The veterans have also claimed that there is an increased prevalence of genetic disorders among them and their offspring. There have been reports of an increased frequency of multiple myelomas present in British veterans of such tests, based on the analysis of medical records for several thousand of the participants (Rabbitt Roff, 1999). Many veterans have had a history of afflictions such as cataracts (Phelps-Brown et al., 1997) and arthritis, or have died due to diseases that could be attributed to radiation exposure, such as gastrointestinal or respiratory disorders and some types of cancers (Rabbitt Roff, 1997). Although several epidemiological studies have been conducted regarding the health of nuclear veterans from Britain, USA, Australia and New Zealand, all have yielded results that are inconclusive or non-significant (Pearce et al., 1990a,b; Rabbitt Roff, 1999; Dalager et al., 2000; Muirhead et al., 2003), as have studies involving the health of their offspring (Reeves et al., 1999; McLeod et al., 2001a,b).

The small number of participants in the New Zealand group (551) was always going to make epidemiological studies difficult, as any radiation-induced cancers that might result would not easily be detectable against background and expected range of different cancers that may arise spontaneously (McEwan, 1988). Nevertheless, some studies have found moderately significant increases in the incidences of haematological cancers in the New Zealand veterans, such as leukemia, which may have arisen due to radiation exposure from the Operation Grapple tests (Pearce, 1990a). However, a comparison of the morbidity of the control group to the national cancer statistics showed that the group had abnormally low incidences of cancer, which may have skewed the results (McEwan, 1988). All of the claims made by the New Zealand nuclear test veterans thus far have been based on epidemiological evidence or anecdotal evidence and have yet to be supported experimentally.

For this reason a controlled genetic study was conducted to determine whether or not the New Zealand naval personnel who witnessed the Operation Grapple series of tests have incurred any genetic damage. The report written here follows that of a parallel study conducted by this laboratory of a sister chromatid exchange (SCE) assay on 50 veterans and 50 controls (Rowland et al., 2005), together with an MSc thesis performed by Chad Johnson (2004) at Massey University on the same sample group using the COMET assay. The SCE study detected a small but nonetheless significantly higher frequency of sister chromatid exchange in the nuclear test veterans compared to a matched control group of New Zealand ex-servicemen. In the current study reported here, a further three assays were performed: G2 assay, Micronucleus (MN) assay, and mFISH (multicolour fluorescent *in situ* hybridisation).

G2 assay

Chromosome instability and loss or gain of chromosomes are changes characteristic of many tumour cells and human disorders (Griffin, 2002). Several studies have established a link between cancer predisposition and

radiosensitivity and that impaired DNA repair capacity appears to play an important role in genetic instability and cancer development (Sanford et al., 1989; Terzoudi et al., 2000; Smart et al., 2003). Deficient DNA repair capacity can be measured by increased G2 chromosomal sensitivity (Sanford and Parshad 1999; Bryant et al., 2002). This is achieved by irradiating cells about 1.5 hours prior to harvest then examining c-metaphase chromosomes for chromosome and chromatid breaks. Such cells would have been in the G2 phase at the time of irradiation. The G2 assay was performed in the current study to ascertain levels of radiosensitivity in New Zealand nuclear test veterans.

Micronucleus assay

The cytokinesis-block micronucleus assay is internationally recognized as a sensitive biomarker for measuring the sensitivity of chromosomes to ionizing radiation. In human studies it has become a routine and reliable method for assessing *in vivo* and/or *in vitro*, radiation-induced chromosome damage in peripheral blood lymphocytes (Fenech and Morley, 1985; Prosser et al., 1988; Mueller et al., 1996; Thierens et al., 2000; Fenech 1993, 2000; Gutierrrez and Hall, 2003; Lorge et al., 2006; Clare et al., 2006). Increased sensitivity as a consequence of exposure to ionizing radiation is thus well documented and has been correlated to a large number of heritable cancer-prone conditions (Scott et al., 1998). The explanation for increased sensitivity is attributed to defects in the processing of radiation induced DNA damage which could contribute to cancer predisposition in these genetic disorders.

The micronucleus assay applied in the current study has thus been widely used in the past to evaluate the extent of chromosomal damage in human populations exposed to clastogenic or genotoxic agents. The technique is straightforward in its design. Cells are irradiated in G1 of the cell cycle and subsequently form broken or detached chromosomes that are separated from the spindle apparatus. After these irradiated cells undergo mitosis, the fragments which are not reinserted into the genome become trapped in the cell cytoplasm and form

micronuclei. In order to retain all the fragments along with the 2 daughter nuclei in the one parental cell, a cytokinesis-block called cytochalasin B is applied. This allows the researcher to count the number of fragments not “healed” back into the genome and thus provides the investigator with a measure of DNA repair efficiency.

mFISH

Ionizing radiation is a well known inducer of chromosome aberrations (Lazutka, 1996; Slozina et al., 1997; Bauchinger et al., 1993, 1997; Cologne et al., 1998; Edwards, 2002; Hsieh et al., 2002; Maffei et al., 2004; Hei et al., 2005; Soyfer, 2002). With the relatively recent development of a cytomolecular technique called multicolour fluorescent *in situ* hybridisation (mFISH) or “chromosome painting”, a rich variety of aberration types can be seen, including stable translocations and unstable dicentric chromosomes. With this technique, each homologous pair of chromosomes in the human genome is stained (painted) a different colour, thereby allowing investigators to detect exchanges between chromosomes along with many other aberrant events. Translocation studies often involve painting only 3 chromosomes and counterstaining the rest of the genome. This method has an efficiency of approximately 30% (Whitehouse et al., 2005).

In our study, we employed the multicolour fluorescent *in situ* hybridisation (mFISH) procedure where every chromosome in the genome is painted a separate colour. This gives a greater degree of efficiency in recording the total number and type of translocation events, rather than a projected estimate. Furthermore, we deemed it wise to select our own control population, as New Zealanders may have been placed at risk in the past from several atmospheric atomic bomb tests in French Polynesia. Indeed New Zealand took France to the International Court of Justice, The Hague, over this issue in 1978.

It is an accepted tenet 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, 1998a,b, 2001). We wish to emphasise, however, that the current study makes no claims on the health status of the veterans. This study concentrates on the genetic status of the Experimental (veterans) group.

One of the reservations the researchers had in embarking upon this study was whether one could detect any evidence of genetic damage that could be attributed to an event which took place so long ago. Fifty years or more is a long time and few like studies have ever been attempted. Nevertheless, research conducted by several authors supports our view that the study was a worthy endeavour. Hande et al. (2003) working in David Brenner's laboratory at Columbia University, New York, showed convincingly that past exposure to densely ionizing radiation can leave a unique permanent signature in the genome. Their research confirmed that radiation products can remain in the body for many decades and result in long term genetic effects. They conducted a study of healthy former nuclear-weapons workers who were occupationally exposed from 1949 onwards in the former Soviet Union. The radiation workers were employed either in plutonium manufacturing/processing facilities or in a nuclear reactor facility. High yields of chromosome aberrations were seen in both the highly exposed workers and in the reactors. Significantly, they demonstrated long term retention of a fraction of the plutonium intake. Autopsy data were used to calculate lung clearance of plutonium. For the plutonium workers studied by Brenner's group, an average of 50% of the bone marrow plutonium dose was deposited in this tissue after 1983, 25% was deposited after 1993 and 8% was deposited after 1998. This means that for some workers who were exposed in 1949, it has taken nearly 50 years for the plutonium to be deposited in a different extrapulmonary organ.

Furthermore, it has been known for some time that some lymphocytes are very long-lived, in excess of 20 years, which means that radiation-induced aberrations can still be observed in cells that were present as peripheral lymphocytes at the time of exposure (Awa et al., 1978; Buckton et al., 1983).

A search of the literature relevant to the current study showed that people who had been exposed to radiation several years previously still showed evidence of genetic damage. These studies include single cases, with accidentally incorporated tritiated water 11 years previously (Lloyd et al., 1998) and an Estonian accident in 1994 (Lindholm and Edwards, 2004), as well as group studies of radiation workers with 11 – 22 years of employment (Bauchinger et al., 1997), Chernobyl workers examined up to 8 years after their exposure (Lazutka and Dedonyte, 1995; Salissidis et al., 1994, 1995; Snigiryova et al., 1997) and from A-bomb survivors measured about 50 years after exposure (Lucas et al., 1992, 1996; Nakamura et al., 1998).

All of the above research encouraged us to embark upon an investigation into whether or not New Zealand military personnel who took part in Operation Grapple incurred long term genetic damage. Notwithstanding the above research, however, our views were tempered by other studies such as that of highly exposed victims of the Goiania accident in Brazil in September 1987 (Straume et al., 1991; Natarajan et al., 1998). A decline of damage over time is noted when observing some parameters (dicentric frequencies in lymphocytes decrease with time) whereas other parameters of damage remain high (translocation, deletion, aneuploidy and frequency of hypoxanthine guanine phosphoribosyltransferase-deficient ($HPRT^-$ mutants)).

The researchers were also conscious of the fact that an investigation such as the one conducted here has the potential to be highly contentious. Thus it was crucial that considerable attention be devoted to the design of the study in order to isolate the variable of interest as far as was feasible, i.e. participation in

Operation Grapple. For this reason, psychology researchers who are experienced in conducting human studies were pivotal in this investigation. Their expertise was valuable in constructing the selection process for both the veterans and the control group.

The procedure by which these two groups were selected is detailed in the Materials and Methods section. Strict criteria were applied for inclusion of participants in the study, together with the gathering of extensive personal information on lifestyle history, occupational history and medical history in an attempt to account for as many confounding factors as possible which may have a bearing on the results. Selection was stratified across the North Island of New Zealand to ensure similar geographic location of veterans and controls, in case for some unknown reason locality was a factor influencing the results.

❖ MATERIALS AND METHODS

(1) Population and sampling procedure

Fifty male New Zealand naval nuclear test veterans (Experimental group) and 50 male age-matched Controls who had also undergone military or police training when they were younger participated in the study. Participant age (at the date of their interview) ranged from 58 to 76, with the mean age for the Experimental and Control groups being 65.9 years (SD = 3.1) and 66.5 years (SD = 3.8), respectively. All were North Island residents, selected by the following procedure.

Names of volunteer veterans were communicated to the researchers through the Office of Veterans' Affairs. A letter of invitation from the Veterans' Affairs office was mailed out to all nuclear test veterans in the North Island listed on the Board's database. Also included with the letter was a Preliminary Inclusion Criteria Questionnaire (see Appendix I) along with an addressed FREEPOST envelope which was returned to the research team at Massey University with the completed questionnaire. Information furnished enabled the researchers to decide whether a veteran was included/excluded from the potential participant pool.

A respondent database was compiled from all those who posted their completed Preliminary Inclusion Criteria Questionnaire to Massey University. A *potential participant pool* was formed by excluding any respondents who failed to meet specific inclusion criteria. A *final participant pool* was formed by randomly selecting the specified number of participants from the potential participant pool database.

Matched control subjects were selected from a pool of volunteers according to criteria identical to the veterans, but with the essential difference that they did not participate in Operation Grapple. Ex-servicemen were selected as controls where possible, most from the army. Some ex-policemen were also chosen. Ex-naval servicemen were excluded as control subjects on the grounds of controversy as to whether the frigates involved were completely "clean" upon returning to New Zealand and subsequently manned by other crew who may have been theoretically exposed to contamination. Ex-airforce personnel, except for ground crew, were also excluded for reasons of possible increased past exposure to cosmic radiation. Vietnam veterans were also not included in either the Control or Experimental group because there is a risk that these people have been adversely affected by possible exposure to defoliants. Neither was any man selected, Control or Experimental, who had previously worked in the timber industry, received prolonged exposure to solvents, or was currently receiving chemotherapy or radiotherapy. Selection of both

Experimentals and Controls was stratified across the North Island to achieve a random geographical distribution of participants.

Selected final participants were sent an Information Sheet (E = Experimental; C = Control), Consent Form and Detailed Questionnaire (Appendix II) that gathered information relating to their life events and general health. This was necessary in order for the researchers to take into account any other factors that may be causing chromosomal damage, if it appeared, other than participation in Operation Grapple. The participants were asked to sign the Consent Form, fill in the Detailed Questionnaire and return these to the researchers at Massey University.

On receipt of the Detailed Questionnaire, a face-to-face interview was arranged and conducted by a psychologist skilled in eliciting memory recall. This was in order to clarify if necessary any incomplete details in their responses, and secure more information related to any substances that might potentially affect the blood sample that would be used for analysis. It was important in this study that we obtained the best recall data possible to validate our results, which is why a face-to face interview with a trained interviewer was essential. A blood sample was collected at the same time as the interview, or else arrangements were made to collect a sample from the participant at a later convenient date. The whole study was conducted following strict ethical guidelines as specified by the World Medical Association Declaration of Helsinki. Ethics approval to conduct the study was given by the Massey University Human Ethics Committee (PN Protocol 01/61) and the following regional hospital ethics committees: the Manawatu/Whanganui Ethics Committee, the Taranaki Ethics Committee, the Hawke's Bay Ethics Committee, the Bay of Plenty Ethics Committee, the Wellington Ethics Committee and the Auckland Ethics Committee.

Each blood sample was collected by an independent phlebotomist and coded with a number so that the researchers could eventually link a name with that code. This code, no name, was written on the side of each blood tube and delivered to the Massey University Student Health Clinic in Palmerston North. Medical assistants at the Clinic recoded each tube with a new number and kept a record linking the codes which were eventually revealed at the conclusion of the study. This ensured that no member of the research team could identify an Experimental from a Control. The blood samples were then collected from the Clinic for genetic analysis. The study was conducted blind in order to remove bias from the analysis. The codes were broken and Experimentals/Controls identified only after all genetic analyses were completed. The blood collected was used only for chromosome analysis and for no other purpose. All genetic information obtained about an individual remained strictly confidential.

Inclusion/Exclusion Criteria

There were 8 exclusion criteria, the first 3 involving potential genetic damage. Participants were excluded if they met the following criteria:

1) Service in a theatre of war or nuclear-related area
2) Exposure to toxic substances[1] for a year or more
3) Having received radiation treatment or chemotherapy
4) Aged over 75 (to avoid confounding effects of age)
5) Air Force aircrew (exposed to cosmic radiation, confounding nuclear radiation exposure)
6) Too ill to participate
7) Death subsequent to survey completion, and
8) Resident in the South Island. (There were insufficient funds to cover trips to interview and collect blood from the 15 eligible South Islander veterans.)

Controls

Controls were obtained through regional Returned Services Associations, and the assistance of Exposed participants through personal contacts. Controls were age-matched individually where possible. The inclusion criterion was service in the NZ Army or NZ Air Force Ground crew), the NZ Police Force, or some form of compulsory military training. The main aim of this criterion was to control for the *healthy soldier effect.* This involves the expectation that, due to strong demands for physical fitness and mental toughness in military and police selection and subsequent service, those who have been in such service will generally be fitter and healthier than civilians (Medical Follow-up Agency, 1995, cited in MacDonald, 1997).

All of the exclusion criteria for the Experimental group also applied to the Controls. Additional exclusion criteria were:

1) Service in HMNZ Navy (due to possible ship contamination)
2) Inability to match for age in a particular geographic region
3) Too high an education level (e.g., a surgeon was excluded)
4) Recent immigration to New Zealand (attempting to control for variable background radiation levels)
5) Service without compulsory military training

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1. These included asbestos, tanilised timber, oil/petrol fumes, microwave radiation, road transport (dust and chemicals), and radiography work.

Sample Selection

To control for any variation in background radiation exposure, a stratified sampling procedure was employed. Potential Experimental group members were identified as belonging to one of 5 North Island regions: Wellington/Kapiti, Central North Island, Bay of Plenty/Waikato, Auckland, and Northland.

Table 2 shows how the 88 acceptable volunteers for the Experimental group membership were distributed by region. The 50 Experimental group members were randomly selected from the pool of potential volunteers, with the restriction that the proportion of members selected from each geographic region reflected the original distribution of 88 potential participants. For various reasons, 6 participants withdrew from the study. These men were replaced with another 6 drawn from the remaining acceptable volunteers.

Table 2. Number of Experimental Volunteers, Potential Participants, and Selected Participants, including mean Age, by Region

Code	Region	Volunteers	Potential	Selected	Mean Age
A	Wellington/Kapiti	13	5	3	67.0
B	Central North Island	16	12	9	65.6
C	Bay of Plenty/Waikato	17	13	7	70.0
D	Auckland	70	46	25	66.2
E	Northland	13	12	6	65.0
	Total	**129**	**88**	**50**	

Overall, 135 Controls volunteered, but 83 of these failed to meet the inclusion criteria, leaving a pool of only 52 from which to select. As Table 2 shows, it was not possible to obtain the ideal number of Controls from the Auckland and Northland regions in the time available. The shortfall was made up from extra Central North Island recruits (who were within easiest reach of the research team).

Table 3. Number of Control Volunteers, Potential Participants, and Selected Participants, including mean Age, by Region

Code	Region	Volunteers	Potential	Selected	Mean Age
A	Wellington/Kapiti	4	3	3	65.7
B	Central North Island	35	17	16	64.9
C	Bay of Plenty/Waikato	19	8	7	66.4
D	Auckland	61	19	19	67.3
E	Northland	16	5	5	66.0
	Total	**135**	**52**	**50**	

(2) Lymphocyte cultures

Two culture tubes were established for each participant and used for all of the 3 assays in this Report: G2 assay, micronucleus assay and mFISH. Each tube contained 5 ml of Medium-199 (GibcoBRL, Cat. No. 31100-035), 1 ml of fetal bovine serum, (GibcoBRL, Cat. No. 10093-136), and 0.1 ml of phytohaemagglutinin (PHA) M form (GibcoBRL, Cat. No.10576-015). Using the WBC count, calculations were made to obtain 3.25-million cells/per culture tube by adding approximately 0.3-0.6 ml of blood. Unless stated differently in the protocols for each of the assays below, all the tubes were incubated at 37 C for 72 h which included pretreatment with 0.05% colchicine for 1 h immediately prior to conventional harvesting for chromosome analysis.

(3) G2 assay

Peripheral blood lymphocytes were cultured as in *(2)* above, except for one variation in the protocol: tubes of cultured lymphocytes were irradiated (1Gy) 30 minutes prior to colchicine pre-treatment. Fifty c-metaphase complements were observed and scored for chromatid breaks and number of fragments in each participant. All c-metaphase chromosomes were in late G2 at the time of irradiation (1.5 h previously), hence the derivation of the name of this assay.

(4) Micronucleus (MN) assay

Peripheral blood lymphocyte cultures were initially established as in *(2)* above but without the mitogen phytohaemagglutinin. The tubes were placed in a water bath for one h at 37 C before being irradiated with X-rays (3.5 Gy), then placed back into a 37 C incubation chamber. Six hours after irradiation, phytohaemagglutinin (final concentration 10µg ml^{-1}) was added to each culture. 24 h after PHA stimulation, the cytokinesis-blocking agent, cytochalasin-B (final concentration 6µg ml^{-1}) was added to arrest the dividing cells undergoing cytokinesis. First generation post-mitotic cells could subsequently be identified as binucleated cells. Harvesting of cells and slide preparation were accomplished by the modified method of Fenech and Morley (1985), and Scott et al. (1998). Approximately 1000 cells were scored in each participant.

(5) Multicolour Fluorescent in situ Hybridisation (mFISH)

Peripheral blood lymphocyte cultures were established from a fresh blood sample. The culture tubes were incubated and harvested as in *(2)* above.

Preparation of probe mixture and in situ hybridisation

Fixed peripheral blood lymphocyte cultures were sent in 2ml Eppendorf tubes to Dr Ilse Chudoba at Metasystems GmBH, Germany, who performed the preparation of the probe mixture for all 46 chromosomes and *in situ* hybridisation (mFISH). C-metaphases were automatically located using a metaphase finder Metafer, and captured with an image analyzer and stored on disc for analysis in our laboratory with the ISIS programme, Metasystems.

Scoring criteria

The scoring criteria of Whitehouse et al. (2005) were followed. Aberrations were classified according to the PAINT nomenclature of Tucker et al. (1995), and classification of complete exchange aberrations was performed according to S&S nomenclature (Savage and Simpson, 1994). Analysis was conducted on intact metaphases where all 46 painted chromosomes could be identified. Cells with 45 chromosomes which exhibited balanced translocations were also recorded.

Several researchers have recognized that long after an exposure only stable aberrations remain. Stable cells in this context are those that do not contain unstable aberrations, i.e. dicentric, acentric or ring chromosomes involving any chromosomes. In this study, all translocations were recorded in stable cells only, including reciprocal translocations, one way translocations and

Robertsonian translocations. Reciprocal exchanges were designated as t(Ab) + t(Ba) and one way translocations as t(Ab) and a deleted chromosome.

Translocations were not scored if they occurred in complex cells (defined as three or more breaks on two or more chromosomes) or in any unstable cell. Each reciprocal (two way) translocation was counted as one translocation. Insertions were not included in the total translocation count. Robertsonian translocations were cytologically distinct from satellite fusions; in the latter the satellites are adjacent to each other but the chromosomes are not fused. Aneuploidy was scored when all the chromosomes could be identified, but many complex cells from the Experimentals with apparent aneuploids were excluded because the individual chromosomes could not be distinguished. For this reason the aneuploid data are unreliable for quantitative purposes.

The high number of extraordinarily complex cells with many translocations, apparently multicentric chromosomes, deletions, centric and acentric fragments, coupled with a lack of resources and time to invest in detailed study of these aberrations, restricted the amount of meaningful data we could gain from unstable cells.

(6) Dosage Reconstruction

The analysis of dicentric chromosomes in peripheral blood lymphocytes has been used for biological dosimetry of radiation exposure for decades (Lindholm et al., 1996). The dose to an individual is determined by comparing the aberration yield with an appropriate calibration curve produced *in vitro* (IAEA 2001). The basic dicentric method is most reliable in a situation in which the exposure is acute (delivered in less than 0.5 h). Because we were studying the possible consequences of an event that took place 50 years ago, we concentrated on gathering data in stable cells, although dicentric frequencies, acentric and centric fragment frequencies, in unstable cells with less than 5 aberrant events, were recorded. Aneuploids, or any other unusual aberrations, were also noted.

Radiation exposure and lymphocyte culture

Blood samples from 3 healthy donors (mean age 40.5) were irradiated with 60 Co at a dose rate 0.835Gy/min to different doses (0, 0.2, 0.5, 0.75, 1, 2 Gy). During irradiation the blood samples were kept at 37 C. After irradiation, lymphocytes were then cultured. Whole blood was added to the culture medium with phytohemagglutinin for 96 h. Blood was incubated at 37 C for 2 h in the presence of Colcemid (0.1 µg/ml) before harvesting. Slides were prepared after standard methanol/acetic (3/1,v/v) fixation. They were stored at - 20°C until use (M’kacher et al., 2003).

Fluorescent Plus Giemsa (FPG) staining was applied to the slides on which metaphases were spread. Only complete metaphases (46 centromeres) were scored for dicentrics, rings and breaks under a light microscope.

Stable chromosomal abnormalities were evaluated by FISH using a combination of standard procedures of the protocols recommended for chromosome analysis. Two hundred to four hundred c-metaphases were scored per irradiated dose. Painting of chromosomes 1, 3 and 4 was performed in order to score stable chromosomal aberrations, translocations, insertions and deletions. A complete (two way) translocation was scored when two bicoloured monocentric chromosomes were present in a stable cell (without unstable chromosomal abnormalities).

Since chromosomes 1, 3 and 4 in human lymphocytes represent 20.4% of the total genome, the results were extended to cover the complete genome. The correction factor is 34%.

❖ RESULTS

The investigation performed in this study employed 3 assays to ascertain whether or not the New Zealand nuclear test veterans incurred long-term genetic damage as a consequence of participating in Operation Grapple. Those assays were 1) the G2 assay, 2) the micronucleus assay, and 3) mFISH.

G2 Assay

Table 5 over the page lists for each participant the total number of chromatid breaks in c-metaphase chromosomes following irradiation in G2 and individual means per number of cells scored. Analysis of the group means is also shown below in Table 4. There was a small trend towards a greater number of breaks in the Controls (Mean = 2.81) compared to the Experimentals (Mean = 2.66), but this difference was not statistically significant using a Wilcoxon rank sum test (with continuity correction), $W = 1368$, $p = 0.11$.

					95% CI for Mean	
	n	mean	sd	sem	Lower Bound	Upper Bound
Experimentals	49	2.66	0.68	0.09	2.46	2.85
Controls	49	2.81	0.52	0.07	2.66	2.96

Table 4. Plot of means, standard deviation (sd), standard error of the mean (sem) and 95% confidence intervals (CI) for the Experimentals and the Controls of chromatid breaks at c-metaphase following G2 irradiation.

Fig. 1 illustrates the distribution of the mean frequencies of breaks in the Experimentals and the Controls. Fifty percent of the mean values in both the Experimentals and the Controls fell within a comparatively narrow band. The number of individuals that could possibly be called outliers was 2 in the Experimentals and 1 in the controls.

CONTROLS

Part.	# Breaks	Mean	sd	# Cells
NZTV002	152	2.9	2.4	51
NZTV005	158	2.8	2.4	56
NZTV006	141	2.6	2.2	54
NZTV007	124	2.2	1.4	55
NZTV008	199	3.6	2.2	54
NZTV009	166	2.9	2	56
NZTV012	112	2	1.7	54
NZTV027	120	2.1	1.6	56
NZTV028	141	3.2	2.5	43
NZTV029	166	3.2	1.9	51
NZTV030	173	3.3	1.9	51
NZTV031	193	3.8	2.7	50
NZTV032	141	2.8	2	50
NZTV035	139	2.6	2.1	53
NZTV036	114	2.5	1.8	45
NZTV040	138	3.1	2.4	44
NZTV059	141	2.8	2.4	49
NZTV063	116	2.4	1.7	47
NZTV067	133	2.6	3.2	51
NZTV071	127	2.5	2.1	49
NZTV077	112	2.4	2.4	45
NZTV078	88	2.1	1.7	41
NZTV080	149	3.3	2.7	45
NZTV081	147	3	2	49
NZTV082	94	2	1.9	46
NZTV083	105	2.1	1.5	50
NZTV084	130	2.8	1.9	45
NZTV085	146	3.5	3.8	41
NZTV086	132	2.9	2	45
NZTV087	130	2.6	2	50
NZTV088	119	2.5	1.8	47
NZTV089	212	4.6	2.5	46
NZTV090	161	2.9	2	55
NZTV091	168	3	1.7	56
NZTV092	140	2.5	1.8	55
NZTV093	107	2.6	1.7	40
NZTV094	96	2.9	1.8	33
NZTV095	62	2	1.6	31
NZTV096	86	2.2	1.7	38
NZTV098	147	2.7	2	53
NZTV099	179	3.2	2.1	55
NZTV100	111	2.3	2	47
NZTV101	138	2.5	1.7	54
NZTV102	145	3	2.2	47
NZTV103	160	3	2	53
NZTV104	172	3.5	2.5	48
NZTV105	120	2.1	1.9	56
NZTV107	147	2.7	1.6	53
	T=6597	**Av=2.81**		T=2343

EXPERIMENTALS

Part.	# Breaks	Mean	sd	# Cells
NZTV001	142	3.3	3.8	43
NZTV003	135	2.5	2.2	52
NZTV010	149	2.8	2	53
NZTV011	141	2.4	1.8	58
NZTV017	108	2.2	1.8	49
NZTV018	88	1.8	1.7	48
NZTV019	153	2.8	5.1	53
NZTV020	136	2.6	2.4	51
NZTV024	118	2.8	1.9	42
NZTV025	136	2.5	2	54
NZTV026	104	2.5	1.7	41
NZTV033	204	3.6	2.3	56
NZTV034	236	4.2	3.5	55
NZTV037	169	3	2.3	55
NZTV039	156	3.1	2.3	50
NZTV041	145	2.9	1.9	50
NZTV042	130	2.9	2.05	44
NZTV043	116	2.3	2.1	50
NZTV044	83	1.6	1.7	51
NZTV045	187	3.8	2.6	49
NZTV046	80	1.7	1.9	46
NZTV047	68	1.3	1.3	49
NZTV048	116	2.2	1.9	52
NZTV049	98	2.3	1.6	41
NZTV050	121	2.6	2.8	46
NZTV051	122	2.4	1.9	49
NZTV052	110	2.2	2.1	50
NZTV053	117	2.3	2.3	50
NZTV055	135	2.9	2.2	46
NZTV057	150	3.1	2.7	47
NZTV056	118	2.7	2.1	43
NZTV058	158	3	2.6	52
NZTV060	134	2.7	1.8	48
NZTV061	122	2.4	2	49
NZTV062	75	1.5	1.5	48
NZTV064	112	2.4	2.1	46
NZTV065	150	3.6	13.5	41
NZTV066	75	2.1	2.2	35
NZTV068	95	1.9	2.1	50
NZTV069	90	2	2.1	43
NZTV070	138	3.4	4.1	40
NZTV072	241	4.9	16.6	49
NZTV073	117	2.2	2.1	52
NZTV074	98	2.1	1.7	46
NZTV075	111	2.2	1.8	50
NZTV076	116	2.7	2.5	42
NZTV079	145	2.9	2.2	50
NZTV097	68	2.3	1.5	29
	T= 6116	**Av=2.66**		T=2293

Table 5. List of the total number of chromatid breaks in c-metaphase chromosomes in each of the Control and Experimental participants following irradiation in G2, and their individual mean/cell.
Part. = participant; # Breaks = total no. of breaks scored per individual; sd = standard deviation; # cells = total no. of cells scored per individual

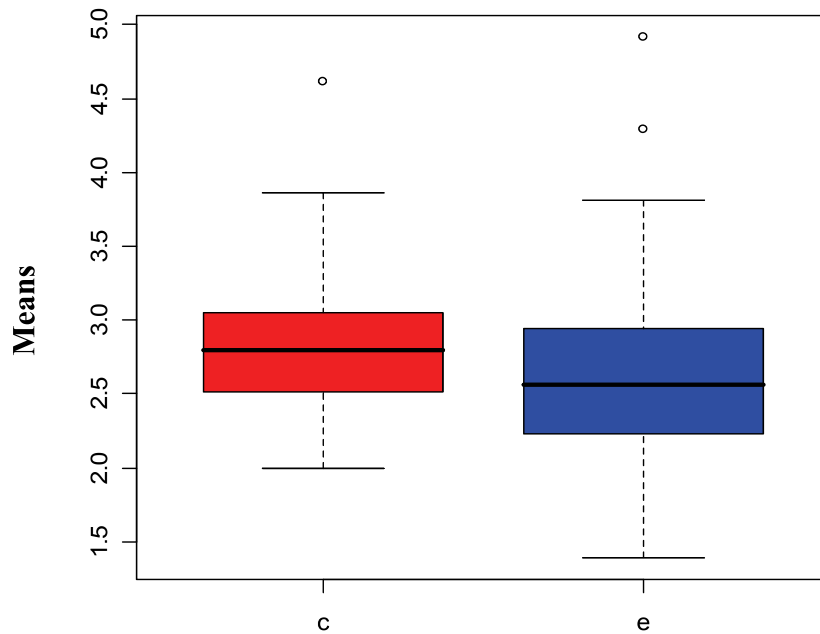

Fig. 1. Plot showing the distribution of the means for the number of breaks in the Experimentals (e) and the Controls (c) following irradiation of lymphocytes in G2. Coloured bars represent 50% of the distribution of the means. Upper and lower limits include 95% of the range of means. Small circles = outliers.

Fig. 2 shows the distribution of G2 fragments in both the Experimentals and the Controls. The vast majority of samples, irrespective of whether they were drawn from the Experimentals or the Controls, yielded a very low fragment count (0 - 4). There was a small trend towards a larger mean difference in fragment counts in the Experimental group (Mean = 5.58) compared to the Controls (Mean = 4.48), but this difference was not statistically significant, using a Welch two sample *t*-test, $t(79.9) = 0.60$, $p = 0.55$.

Fig. 2. Fragment distribution in the Experimentals and Controls following G2 irradiation of lymphocytes

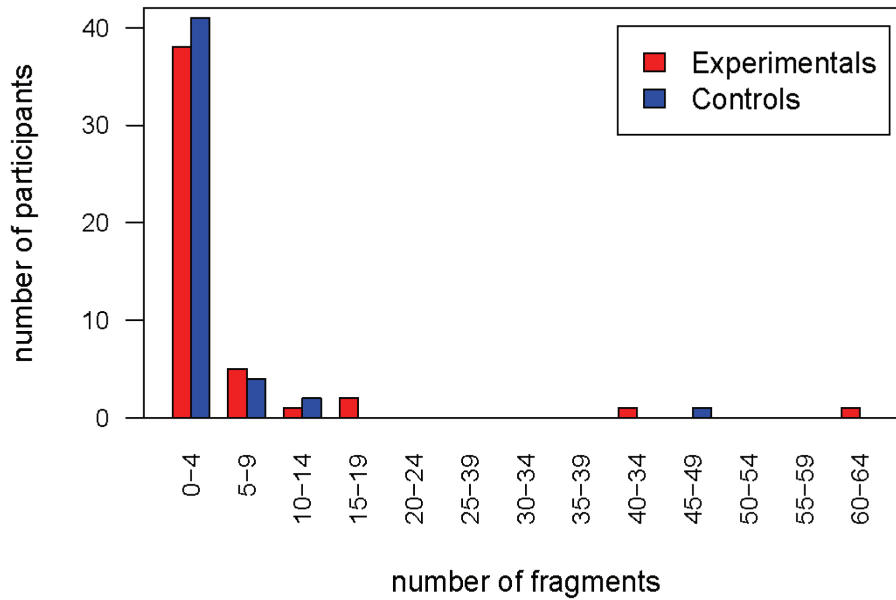

Table 6 lists the number of fragments observed in all participants. Both the Experimentals and the Controls show outliers, although only 3 controls had a total number of fragments in double figures, as opposed to 6 Experimentals. Furthermore, one could argue descriptively that one Control participant with a total of 47 fragments distorted the results, but then one could equally argue that the highest or 2 highest Experimentals did the same.

CONTROLS			EXPERIMENTALS		
Part.	**Frag. Freq.**	**# Cells**	**Part.**	**Frag. Freq.**	**# Cells**
NZTV002	5	51	NZTV001	1	43
NZTV005	1	56	NZTV003	2	52
NZTV006	7	54	NZTV010	2	53
NZTV007	0	55	NZTV011	3	58
NZTV008	5	54	NZTV017	1	49
NZTV009	1	56	NZTV018	2	48
NZTV012	2	54	NZTV019	18	53
NZTV027	3	56	NZTV020	3	51
NZTV028	47	43	NZTV024	3	42
NZTV029	2	51	NZTV025	4	54
NZTV030	2	51	NZTV026	1	41
NZTV031	8	50	NZTV033	10	56
NZTV032	1	50	NZTV034	17	55
NZTV035	0	53	NZTV037	7	55
NZTV036	2	45	NZTV039	0	50
NZTV040	9	44	NZTV041	3	50
NZTV059	1	49	NZTV042	3	44
NZTV063	5	47	NZTV043	2	50
NZTV067	5	51	NZTV044	2	51
NZTV071	13	49	NZTV045	3	49
NZTV077	3	45	NZTV046	2	46
NZTV078	1	41	NZTV047	1	49
NZTV080	0	45	NZTV048	3	52
NZTV081	4	49	NZTV049	1	41
NZTV082	3	46	NZTV050	1	46
NZTV083	3	50	NZTV051	4	49
NZTV084	2	45	NZTV052	0	50
NZTV085	13	41	NZTV053	1	50
NZTV086	4	45	NZTV055	0	46
NZTV087	5	50	NZTV057	0	47
NZTV088	5	47	NZTV056	2	43
NZTV089	7	46	NZTV058	8	52
NZTV090	2	55	NZTV060	2	48
NZTV091	2	56	NZTV061	2	49
NZTV092	5	55	NZTV062	3	48
NZTV093	1	40	NZTV064	4	46
NZTV094	1	33	NZTV065	44	41
NZTV095	4	31	NZTV066	3	35
NZTV096	1	38	NZTV068	6	50
NZTV098	5	53	NZTV069	4	43
NZTV099	2	55	NZTV070	14	40
NZTV100	2	47	NZTV072	61	49
NZTV101	2	54	NZTV073	0	52
NZTV102	4	47	NZTV074	1	46
NZTV103	4	53	NZTV075	3	50
NZTV104	5	48	NZTV076	1	42
NZTV105	3	56	NZTV079	4	50
NZTV107	3	53	NZTV097	6	29

Table 6. Total fragment frequency observed at c-metaphase in each Experimental and Control participant following G2 irradiation.
Part. = participant; Frag. Freq. = fragment frequency; # Cells = no. of cells scored.

Micronucleus Assay

The MN assay is internationally recognized as a sensitive biomarker for measuring the sensitivity of chromosomes to ionizing radiation. The explanation for increased sensitivity is attributed to defects in the processing of radiation-induced DNA damage, namely deficiency in repair of DNA in cells that are in the G0 state. The technique is illustrated in Appendix III.

Tables 7 and 8 show the number of radiation-induced micronuclei obtained for the Experimental group and Control group, respectively. The parameters that are compared using this assay are the number of micronucleated cells per 100 binucleated cells (column labelled “a” in both tables) and the number of micronuclei per 100 binucleated cells (column labelled “b” in both tables). The average value of “a” was 45.7 in the Experimentals and 45.3 in the Controls. The average value of “b” was 65.2 in the Experimentals and 64.0 in the Controls. These differences are very small and not statistically significant.

Fig. 3 illustrates the pooled data of the average number of cells in the Experimentals and Controls with a defined number of micronuclei per cell. The results show an almost identical distribution of micronuclei frequency in both groups. We also analyzed the data according to age strata (under 65 years, between 65-69 years, and over 70) to ascertain whether there was a difference between the Experimentals and the Controls within different age bands. No statistical difference was found between the Experimentals and the Controls for any band.

Participant	# of CB cells scored	# of CB cells with MNi	Total # of MNi	a	b	# of cells with 0 to 14 micronuclei per cell									
						0	1	2	3	4	5	6	7	8	14
NTV-001	50	289	354	578.00	708.00	755	233	50	4	1	1	0	0	0	0
NTV-003	1044	274	361	26.25	34.58	763	206	52	13	3	0	0	0	0	0
NTV-010	1037	515	750	49.66	72.32	609	339	134	32	6	2	1	1	0	0
NTV-011	1124	424	591	37.72	52.58	655	302	87	27	7	0	1	0	0	0
NTV-017	1079	477	685	44.21	63.48	552	312	131	29	2	2	1	0	0	0
NTV-018	1029	620	988	60.25	96.02	440	349	194	60	15	1	1	0	0	0
NTV-019	1060	669	1023	63.11	96.51	360	410	188	55	10	5	0	1	0	0
NTV-020	1029	539	815	52.38	79.20	554	341	139	48	4	6	1	0	0	0
NTV-021(4)	1093	153	246	14.00	22.51	186	93	42	11	3	2	1	0	1	0
NTV-023	339	525	911	154.87	268.73	487	229	230	47	14	5	0	0	0	0
NTV-024	1012	638	1012	63.04	100.00	382	370	181	70	15	2	0	0	0	0
NTV-025	1020	636	1035	62.35	101.47	421	353	203	65	10	3	2	0	0	1
NTV-026	1058	406	720	38.37	68.05	207	197	145	40	11	9	4	0	0	0
NTV-033	613	569	880	92.82	143.56	442	343	159	49	18	0	0	0	0	0
NTV-034	1011	406	538	40.16	53.21	652	304	77	20	5	0	0	0	0	0
NTV-037	1058	629	954	59.45	90.17	377	371	203	45	9	0	1	0	0	0
NTV-038	1006	541	757	53.78	75.25	488	354	163	19	5	0	0	0	0	0
NTV-039(49)	1029	382	511	37.12	49.66	660	276	88	17	0	0	0	0	1	0
NTV-041	1042	304	386	29.17	37.04	717	229	69	5	1	0	0	0	0	0
NTV-042	1021	576	865	56.42	84.72	460	353	171	38	14	0	0	0	0	0
NTV-043	1036	540	781	52.12	75.39	493	359	133	40	5	2	1	0	0	0
NTV-044	1033	326	434	31.56	42.01	774	239	71	11	5	0	0	0	0	0
NTV-045	1100	368	474	33.45	43.09	633	274	82	12	0	0	0	0	0	0
NTV-046	1001	345	457	34.47	45.65	667	258	69	14	2	1	1	0	0	0
NTV-047	1012	225	264	22.23	26.09	793	190	33	1	0	1	0	0	0	0
NTV-048	1018	461	620	45.28	60.90	575	339	90	28	3	1	0	0	0	0
NTV-050	1036	537	739	51.83	71.33	505	385	111	33	7	1	0	0	0	0
NTV-051	1042	559	781	53.65	74.95	516	381	142	30	4	2	0	0	0	0
NTV-052	1075	506	703	47.07	65.40	520	356	109	35	6	0	0	0	0	0
NTV-053	1026	228	268	22.22	26.12	803	190	36	2	0	0	0	0	0	0
NTV-055	1031	354	428	34.34	41.51	695	286	63	4	1	0	0	0	0	0
NTV-056	1049	460	619	43.85	59.01	561	337	93	24	6	0	0	0	0	0
NTV-057	1021	357	467	34.97	45.74	664	271	65	18	3	0	0	0	0	0
NTV-058	1021	296	377	28.99	36.92	717	230	52	13	1	0	0	0	0	0
NTV-060	1013	594	854	58.64	84.30	466	394	153	36	9	2	0	0	0	0
NTV-061	1060	433	591	40.85	55.75	607	315	89	22	5	0	2	0	0	0
NTV-062	1040	699	1091	67.21	104.90	411	417	196	67	14	5	0	0	0	0
NTV-064	1110	549	787	49.46	70.90	493	371	139	24	11	2	2	0	0	0
NTV-065	1042	549	772	52.69	74.09	527	371	139	33	6	0	0	0	0	0
NTV-066	1076	532	758	49.44	70.45	503	363	131	26	7	3	2	0	0	0
NTV-068	1035	351	427	33.91	41.26	724	285	56	10	0	0	0	0	0	0
NTV-069	1075	521	703	48.47	65.40	566	369	126	23	2	1	0	0	0	0
NTV-070	1087	434	560	39.93	51.52	553	328	87	18	1	0	0	0	0	0
NTV-072	987	165	199	16.72	20.16	260	135	26	4	0	0	0	0	0	0
NTV-073	425	475	623	111.76	146.59	570	355	96	20	4	0	0	0	0	0
NTV-074	1045	566	805	54.16	77.03	446	368	162	31	5	0	0	0	0	0
NTV-075	1012	472	624	46.64	61.66	611	349	98	21	4	0	0	0	0	0
NTV-076	1083	479	616	44.23	56.88	595	371	87	16	2	3	0	0	0	0
NTV-079	1074	392	530	36.50	49.35	645	286	78	25	2	1	0	0	0	0
NTV-097	1037	651	1004	62.78	96.82	386	382	200	57	9	3	0	0	0	0
	T = 50443			**Av=45.7**	**Av=65.2**										

T = total
a= Number of micronucleated cells per 100 binucleated cells.
b= Number of micronuclei per 100 binucleated cells.
CB = cytokinesis-blocked cells

Table 7. List of raw data and analysis of the Experimentals following application of the Micronucleus Assay.

Participant	# of CB cells scored	# of CB cells with MNi	Total # of MNi	a	b	# of cells with 0 to 12 micronuclei per cell										
						0	1	2	3	4	5	6	7	8	10	12
NTV-002	1027	310	426	30.19	41.48	717	226	60	18	4	2	0	0	0	0	0
NTV-006	1043	289	380	27.71	36.43	754	207	73	9	0	0	0	0	0	0	0
NTV-012	1022	386		37.77	53.42	636	273	79	25	6	2	1	0	0	0	0
NTV-013(5)	1157	707	1122	61.11	96.97	450	405	218	61	19	3	0	1	0	0	0
NTV-014(7)	1127	682	1086	60.51	96.36	445	410	177	69	19	4	2	1	0	0	0
NTV-015(9)	1131	594	887	52.52	78.43	537	390	145	38	14	5	2	0	0	0	0
NTV-016(8)	961	336	398	34.96	41.42	625	285	44	3	4	0	0	0	0	0	0
NTV-022	1004	460	650	45.82	64.74	544	311	117	23	9	0	0	0	0	0	0
NTV-027	1026	268	338	26.12	32.94	758	206	55	6	1	0	0	0	0	0	0
NTV-028	1010	551	783	54.55	77.52	460	377	136	28	7	2	0	0	0	1	0
NTV-029	1028	490	709	47.67	68.97	538	329	115	35	10	1	0	0	0	0	0
NTV-030	1076	625	944	58.09	87.73	451	385	176	53	8	2	1	0	0	0	0
NTV-031	1018	517	720	50.79	70.73	501	362	119	27	6	3	0	0	0	0	0
NTV-032	1041	337	434	32.37	41.69	704	258	63	14	2	0	0	0	0	0	0
NTV-035	1001	576	775	57.54	77.42	425	398	166	4	7	1	0	0	0	0	0
NTV-036	1017	478	697	47.00	68.53	539	313	125	29	9	1	1	0	0	0	0
NTV-040	1035	656	1033	63.38	99.81	379	384	193	59	15	4	1	0	0	0	0
NTV-059	1018	399	515	39.19	50.59	619	301	83	13	1	1	0	0	0	0	0
NTV-063	1083	479	634	44.23	58.54	604	352	102	22	3	0	0	0	0	0	0
NTV-067	1042	623	881	59.79	84.55	419	415	164	38	6	0	0	0	0	0	0
NTV-071	1014	462	619	45.56	61.05	552	332	107	19	4	0	0	0	0	0	0
NTV-077	1018	467	588	45.87	57.76	551	363	92	9	2	0	1	0	0	0	0
NTV-078	1022	369	496	36.11	48.53	653	267	80	19	3	0	0	0	0	0	0
NTV-080	1066	511	698	47.94	65.48	555	360	119	28	4	0	0	0	0	0	0
NTV-081	1006	310	392	30.82	38.97	696	239	61	9	1	0	0	0	0	0	0
NTV-082	1023	538	792	52.59	77.42	485	352	134	38	12	2	0	0	0	0	0
NTV-083	1104	526	715	47.64	64.76	578	373	123	25	4	1	0	0	0	0	0
NTV-084	1043	414	551	39.69	52.83	629	301	91	20	2	0	0	0	0	0	0
NTV-085	1024	502	674	49.02	65.82	522	369	110	17	3	2	0	0	0	0	1
NTV-086	1041	684	1124	65.71	107.97	357	388	199	65	20	9	3	0	0	0	0
NTV-087	1049	615	913	58.63	87.04	434	392	162	50	8	3	0	0	0	0	0
NTV-088	1051	469	666	44.62	63.37	582	316	116	30	7	0	0	0	0	0	0
NTV-089	1024	535	743	52.25	72.56	489	371	125	35	3	1	0	0	0	0	0
NTV-090	1063	397	522	37.35	49.11	666	300	77	15	2	3	0	0	0	0	0
NTV-091	1019	490	709	48.09	69.58	529	320	132	30	6	1	1	0	0	0	0
NTV-092	1033	512	735	49.56	71.15	521	351	114	35	10	1	1	0	0	0	0
NTV-093	1023	561	826	54.84	80.74	462	369	137	40	12	3	0	0	0	0	0
NTV-094	1009	616	939	61.05	93.06	393	367	189	48	10	2	0	0	0	0	0
NTV-095	510	278	395	54.51	77.45	232	190	63	21	4	0	0	0	0	0	0
NTV-096	1035	704	1094	68.02	105.70	331	420	209	52	18	3	1	1	0	0	0
NTV-098	1008	458	657	45.44	65.18	550	304	122	24	6	0	1	1	0	0	0
NTV-099	1020	441	613	43.24	60.10	579	316	88	29	6	2	0	0	0	0	0
NTV-100	518	199	279	38.42	53.86	319	142	41	10	5	1	0	0	0	0	0
NTV-101	1024	463	670	45.21	65.43	561	311	106	38	7	1	0	0	0	0	0
NTV-102	519	150	182	28.90	35.07	369	122	25	2	1	0	0	0	0	0	0
NTV-103	311	63	69	20.26	22.19	248	58	4	1	0	0	0	0	0	0	0
NTV-104	517	184	231	35.59	44.68	333	146	31	5	2	0	0	0	0	0	0
NTV-105	1016	303	379	29.82	37.30	713	240	51	11	1	0	0	0	0	0	0
NTV-106	524	86	106	16.41	20.23	438	67	18	1	0	0	0	0	0	0	0
NTV-107	1032	429	610	41.57	59.11	603	294	100	27	6	1	1	0	0	0	0
	T=48533			**Av=45.3**	**Av=64.0**											

T = total
a = Number of micronucleated cells per 100 binucleated cells.
b = Number of micronuclei per 100 binucleated cells.
CB cells = cytokinesis-blocked cells.

Table 8. List of raw data and analysis of the Controls following application of the Micronucleus Assay.

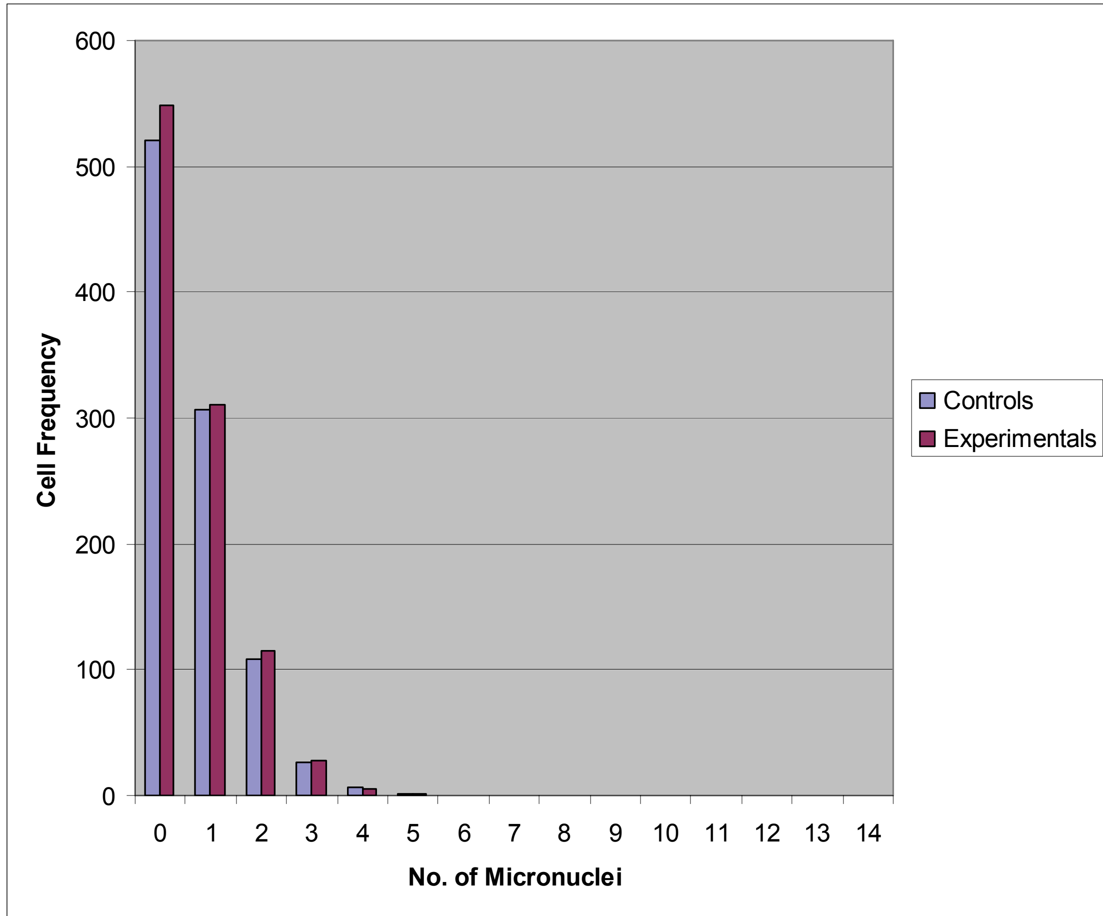

Fig.3 Graph showing the distribution of the total number of cells with micronuclei as a function of the number of micronuclei per cell in both the Experimentals and the Controls.

mFISH

Translocations were scored only in stable cells. Translocations were not scored if they occurred in complex cells (defined as three or more breaks on two or more chromosomes) or in any unstable cell. Table 9 shows that the mean number of translocations per 1000 cells was much higher for the Experimentals (Mean = 29.38, SD = 17.52) compared to the Controls (Mean = 10.05, SD = 8.86). Tables 10 and 11 show a summary list of translocation frequencies in the Experimental and Control groups, respectively. More data are provided in Appendix IVa,b.

					95% Confidence Interval for Mean	
Group	n	mean	sd	sem	Lower Bound	Upper Bound
Experimentals	49	29.38	17.52	2.50	24.08	34.15
Controls	50	10.05	8.86	1.25	7.29	12.32

Table 9. Data showing the mean average of total translocation frequencies in the Experimentals and the Controls as a function of number of translocations per 1000 cells. n = number of participants; sd = standard deviation; sem = standard error.

Fig. 4 shows the distribution of the total translocation frequencies as a function of the number of translocations per 1000 cells in the Experimentals and the Controls. The distributions are different between the two groups. The variance is greater in the Experimentals, with a range from 0 to 65 per 1000 cells in the Experimentals, and 0 – 35 per 1000 cells in the Controls. The Control group is heavily represented in the category of 0 – 10 translocations per 1000. The frequency distributions show that, whereas most Controls had no or few translocations, only a very small number of Experimentals fell into this category. A Wilcoxon two sample rank sum test revealed a highly significant increase in the number of translocations/cell for the Experimentals compared to the Controls, $W = 385$, $p < .0001$.

Participant	#cell k.	# t	# cells w/t	RT	rcp	1-way	dic	ace
NZTV-001	14	1	1	0	0	1	1	0
NZTV-003	203	8	4	0	2	6	1	13
NZTV-010	200	3	3	0	0	3	0	0
NZTV-011	200	4	4	0	1	3	0	2
NZTV-017	202	5	5	0	1	4	0	1
NZTV-018	201	3	2	0	0	3	0	4
NZTV-019	201	4	3	0	0	4	0	4
NZTV-020	200	7	7	1	4	2	0	0
NZTV-021	154	6	5	2	1	3	2	0
NZTV-023	201	5	5	2	3	0	0	1
NZTV-024	201	7	6	1	1	5	0	4
NZTV-025	204	8	7	2	3	3	0	10
NZTV-026	201	13	9	2	1	10	0	2
NZTV-033	203	10	8	2	2	6	0	3
NZTV-034	202	4	3	2	0	2	0	0
NZTV-037	No available data							
NZTV-039	200	7	4	1	1	5	1	1
NZTV-041	204	10	6	2	3	5	1	4
NZTV-042	201	9	9	0	7	2	1	0
NZTV-043	201	9	5	0	4	5	0	3
NZTV-044	203	8	7	1	3	4	1	1
NZTV-045	205	5	5	1	2	2	0	0
NZTV-046	203	3	1	0	1	2	0	0
NZTV-047	106	2	2	1	1	0	0	0
NZTV-048	188	7	5	1	2	4	0	1
NZTV-049	202	11	5	1	2	8	0	0
NZTV-050	201	4	4	0	4	0	0	0
NZTV-051	203	3	3	0	2	1	0	0
NZTV-052	202	1	1	0	0	1	0	0
NZTV-053	92	0	0	0	0	0	0	2
NZTV-055	209	9	9	0	2	7	0	1
NZTV-056	217	3	3	0	0	3	0	0
NZTV-057	167	1	1	0	0	1	0	0
NZTV-058	145	0	0	0	0	0	0	1
NZTV-060	164	0	0	0	0	0	0	0
NZTV-061	220	2	2	0	2	0	0	1
NZTV-062	200	13	9	1	6	6	0	0
NZTV-064	206	6	4	0	6	0	1	4
NZTV-065	182	2	2	0	2	0	0	0
NZTV-066	202	12	10	0	8	4	0	1
NZTV-068	208	4	3	0	3	1	0	1
NZTV-069	204	8	8	3	2	3	1	1
NZTV-070	205	5	7	3	0	2	0	0
NZTV-072	205	7	4	2	1	4	1	0
NZTV-073	214	3	3	2	0	1	0	1
NZTV-074	205	9	8	4	3	2	1	1
NZTV-075	203	7	8	4	1	2	0	5
NZTV-076	206	7	7	2	3	2	0	0
NZTV-079	199	4	4	2	1	1	0	0
NZTV-097	201	6	5	0	3	3	0	4
	9360	**275**	**226**	**45**	**94**	**136**	**12**	**77**

#cell k. = no. of cells karyotyped
# t = total no. of stable translocations (RT + rcp + 1-way)
# cells w/t = no. of cells with translocation(s)
RT = Robertsonian translocation
rcp = reciprocal translocation
1-way = one way translocation
dic = dicentric
ace = acentric

Table 10. List of raw data for translocation frequency and dicentic/acentric frequency in the Experimental group. (More information is provided in Appendix IVa)

Participant	#cell k.	# t	# cells w/t	RT	rcp	1-way	dic	ace
NZTV-002	86	0	0	0	0	0	0	2
NZTV-006	200	3	3	0	0	3	0	0
NZTV-012	203	2	2	0	2	0	0	1
NZTV-013	71	0	0	0	0	0	0	2
NZTV-014	150	1	1	0	0	1	0	0
NZTV-015	203	0	0	0	0	0	0	1
NZTV-016	201	1	1	0	0	1	0	1
NZTV-022	201	3	3	0	1	2	0	1
NZTV-027	201	2	1	0	1	1	0	1
NZTV-028	202	2	1	0	1	1	0	1
NZTV-029	203	0	0	0	0	0	0	2
NZTV-030	200	1	1	0	0	1	0	2
NZTV-031	202	2	2	0	1	1	0	1
NZTV-032	202	3	3	0	2	1	0	2
NZTV-035	202	0	0	0	0	0	0	0
NZTV-036	203	4	1	0	1	3	0	0
NZTV-040	202	4	3	0	2	2	0	4
NZTV-059	144	4	3	0	0	4	0	0
NZTV-063	200	4	4	2	0	2	0	0
NZTV-067	205	4	4	2	1	1	0	1
NZTV-071	202	2	2	0	2	0	0	1
NZTV-077	203	1	1	0	1	0	0	0
NZTV-078	203	0	0	0	0	0	0	2
NZTV-080	204	0	0	0	0	0	0	1
NZTV-081	203	1	1	0	1	0	1	0
NZTV-082	202	1	1	0	1	0	0	1
NZTV-083	203	2	2	0	1	1	0	0
NZTV-084	200	1	1	0	1	0	0	0
NZTV-085	203	3	3	0	3	0	0	1
NZTV-086	202	2	2	0	1	1	0	1
NZTV-087	201	0	0	0	0	0	0	1
NZTV-088	200	0	0	0	0	0	0	1
NZTV-089	201	2	2	0	1	1	0	1
NZTV-090	202	1	1	0	0	1	0	0
NZTV-091	203	0	0	0	0	0	0	0
NZTV-092	201	1	1	0	0	1	0	0
NZTV-093	106	0	0	0	0	0	0	0
NZTV-094	89	1	1	0	0	1	0	0
NZTV-095	203	1	1	0	1	0	0	4
NZTV-096	203	7	7	2	3	2	0	2
NZTV-098	200	1	1	0	1	0	0	1
NZTV-099	190	2	2	0	1	1	0	3
NZTV-100	201	6	6	0	4	2	0	0
NZTV-101	200	1	1	0	0	1	0	0
NZTV-102	203	3	3	0	3	0	0	1
NZTV-103	202	3	3	0	2	1	0	2
NZTV-104	229	2	2	0	1	1	0	2
NZTV-105	203	7	7	1	2	4	0	1
NZTV-106	202	2	2	0	0	2	0	0
NZTV-107	203	3	2	0	3	0	0	0
	9548	**96**	**88**	**7**	**45**	**44**	**1**	**48**

#cell k. = no. of cells karyotyped
# t = total no. of stable translocations (RT + rcp + 1-way)
# cells w/t = no. of cells with translocation(s)
RT = Robertsonian translocation
rcp = reciprocal translocation
1-way = one way translocation
dic = dicentric
ace = acentric

Table 11. List of raw data for translocation frequency and dicentic/acentric frequency in the Control group using mFISH. (More information is provided in Appendix IVb)

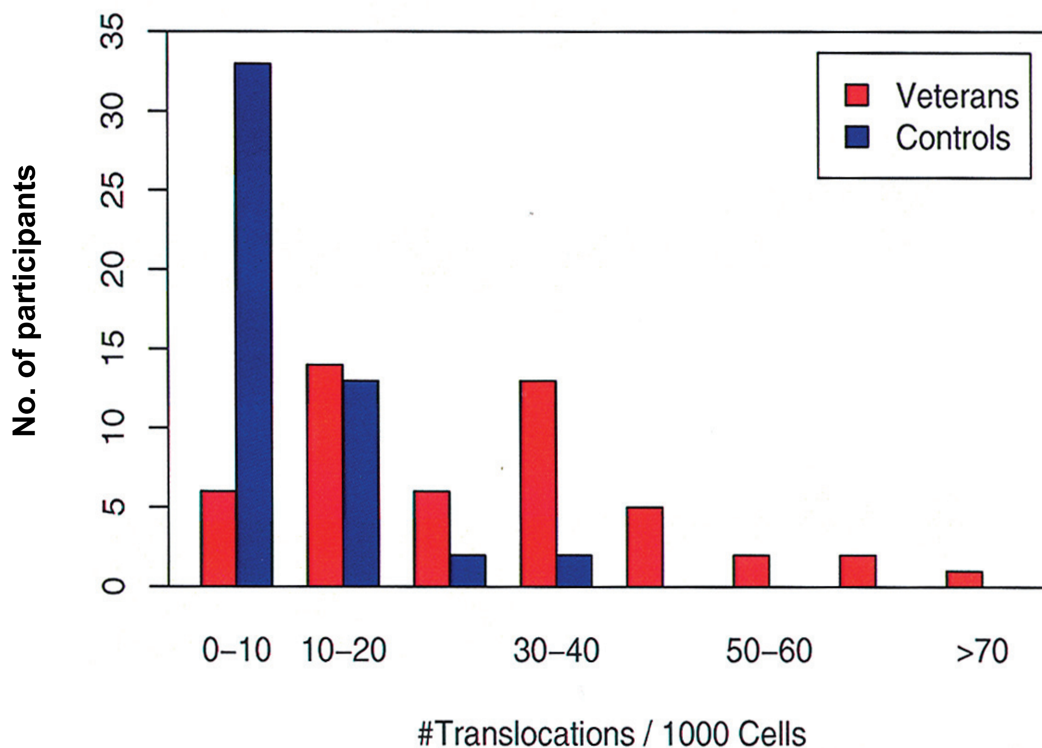

Fig.4. Graph showing the distribution of total translocation frequencies (RT, complete and incomplete) in the Experimentals (Veterans) and the Controls as a function of the number of translocations per 1000 cells.

The total number of number of two way (reciprocal) translocations to one way translocations that were scored was 94 and 136, respectively, in the Experimentals, and 45 and 44, respectively, in the Controls.

Appendix V(1 – 9) are illustrations of a normal karyotype, complex cells and translocations observed in one participant as an example of our scoring procedure. Translocations in complex cells were not scored, and neither were the number of dicentric chromosomes and acentric fragments. It follows, then, that these aberrations were scored only in cells with less than three or more breaks involving two or more chromosomes. This boundary was deliberately chosen as the variation in complexity of aberrations amongst complex cells was considerable and most often impossible to score. Thus, the dicentric and acentric scores listed in Table 10 are a gross underestimate.

Smoking

The mean total tobacco smoked over the lifetime was 192,596 units (see Appendix VI for definition of a unit) for the Experimental group, and 97,449 units for the Control group. Clearly, there was a large difference between the two groups in the amount of tobacco smoked in the past, with the Experimental group having smoked almost twice as much tobacco as the Control group. This result made it necessary to include smoking as a covariate. 17(34%) of the controls were never smokers and 12(24%) of the Experimentals were never smokers. There is no significant difference in current smoking consumption between the two groups. Nearly all participants were currently non-smokers.

						95% CI for Mean	
Group	Smoking status	n	mean	sd	sem	Lower Bound	Upper Bound
Experimentals	Smoker	37	28.21	14.37	2.36	23.41	32.99
	Never-smoker	12	31.94	25.54	7.37	15.7	48.16
Controls	Smoker	33	9.20	8.29	1.44	6.25	12.13
	Never-smoker	17	11.00	10.03	2.43	5.84	16.15

Table 12. Data comparing the mean average of total translocation frequency between smokers and never-smokers in the Experimentals and the Controls.
n = number of participants; sd = standard deviation; sem = standard error; CI = Confidence Interval

It is possible that the higher rate of smoking among the Experimentals as compared with the Controls is at least partially responsible for the higher translocation rates in the veterans. If so, then the translocation rates should be higher for the smokers in both the Experimental and Control groups, but as Table 12 shows, this clearly is not the case. A between-groups analysis of variance with Group (Experimentals and Controls) as one factor and Smoking (Smokers and Never-smokers) as the other, showed no effect of smoking, $F<1$. The mean number of translocations/cell in the Veterans group for smokers (Mean = 28.21, SD = 14.37) was in fact a little higher among the never-smokers (Mean = 31.94, SD = 25.54). Similarly for the Controls; the never-smokers (Mean = 11.00, SD = 10.03) had a slightly higher mean translocation rate than the smokers (Mean = 9.20, SD = 8.29). Thus, smoking was not a factor influencing translocation frequency.

Dose Reconstruction

The introduction of FISH has proved a valuable tool to establish the amount of past exposure to radiation in humans. An attempt was made in this study to reconstruct possible radiation dosage in the nuclear test veterans. The estimate is based on two way translocations involving chromosomes 1, 3 and 4 in stable cells, and also on dicentric chromosome frequency. Two way translocations, as previously stated, were scored only in stable cells. Since chromosomes 1, 3 and 4 in human lymphocytes represent 20.4% of the total genome, the results were extended to cover the complete genome. The correction factor is 34%.

Cells with dicentric chromosomes are unstable cells. Many cells amongst the Experimentals exhibited dicentric and multicentric chromosomes, also as previously noted (see Appendix V). But dicentric frequencies were scored only in cells that were not complex. It was necessary for us to adopt this strict boundary in our scoring criteria of dicentrics in order to gather some semblance of meaningful information amongst the Experimentals. We acknowledge that the dicentric and acentric scores observed in the Experimentals are grossly underestimated, which inevitably affects our attempt at dosage reconstruction when relying on dicentric data. Nevertheless, it is an attempt that we have made, accepting its limitations. In spite of unknown background, a dosimetric approach was made from both dicentric frequencies and two way translocations.

Dosimetry calculations

Table 13 shows the dose response curves for dicentrics and rings and Table 14 for translocations and insertions after *in vitro* irradiation of circulating lymphocytes of healthy donors. Using the curves from healthy donors it has been possible to calculate a dosimetric index for each Experimental participant.

Table 15 shows the estimated doses and 95% Confidence Intervals in the Experimentals after scoring of stable and unstable chromosomal abnormalities. The estimated doses could be classified into three categories: (1) 0 – 0.49Gy 37 individuals, (2) 0.5 – 0.99Gy 6 individuals, (3) >1Gy 5 individuals.

Individual dosage estimates calculated on reciprocal translocation frequencies are very high in 5 cases with an estimate of 1Gy. 6 received between 0.5 and 0.95Gy, and 16 received between 0.2 – 0.45Gy. There were no estimates for 15 veterans. 96 h of culture for the dose-response curves can slightly overestimate the dosimetric index but not the hierarchy of the risk.

Twelve dicentric chromosomes were scored in the Experimentals as opposed to one dicentric chromosome in the Controls. The dose-effect curve for dicentrics was established in normal donors. We acknowledge an underestimation of the dicentric score which means any dosimetric approach with the dicentrics is very debatable. However, we were able to corroborate the dosimetric approach by examining translocation frequencies, without the disadvantage of a background.

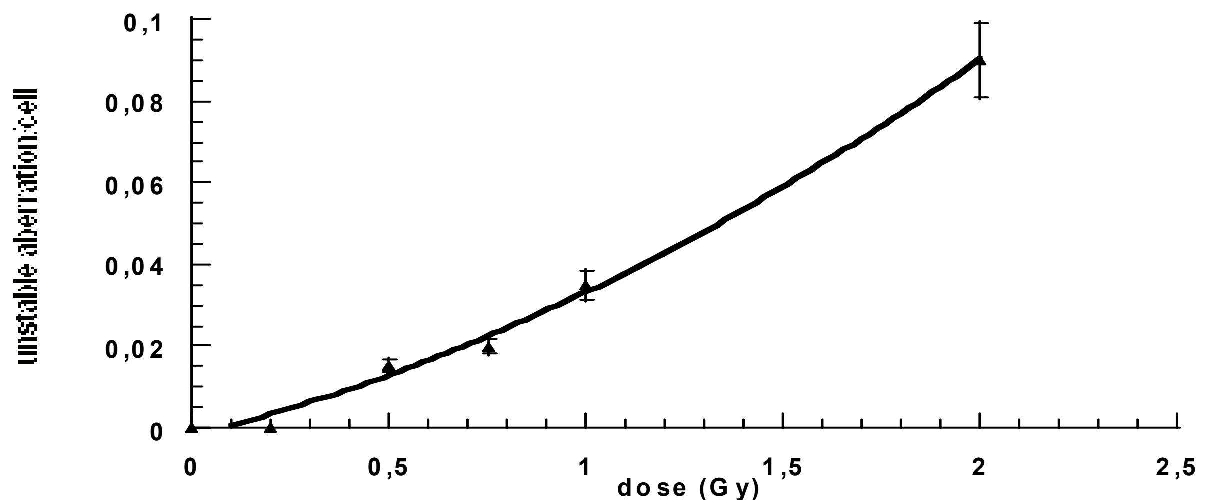

Table 13. Dose-response curve after *in vitro* irradiation of circulating lymphocytes of healthy donors. Unstable chromosomal abnormalities were scored by conventional cytogenetics methodology. Gy = Gray

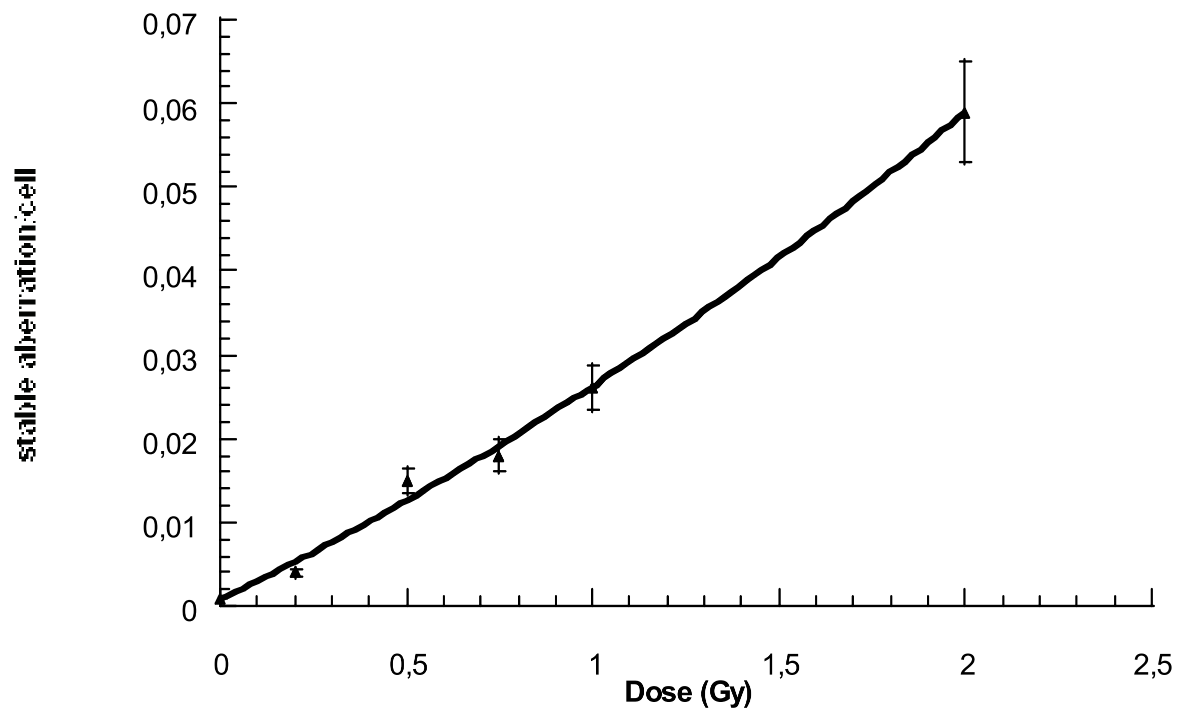

Table 14. Dose response curve after *in vitro* irradiation of circulating lymphocytes of healthy donors. Stable chromosomal abnormalities (complete translocation (two-way)) were scored by chromosome 1, 3 and 4 painting. Gy = Gray

Participant	#cells scored	Chr. #1,3,4	dose(Gy)	%95 CI (Gy)	dic	Dose(Gy)	%95 CI (Gy)
NZTV-001	14	**0**			1:(11,11)		
NZTV-003	203	**1**	0.18	0,03-0,98	1:(19,7)	0.3	0,15-0,80
NZTV-010	200	**0**	0	0-0,68	0	0	0-0,55
NZTV-011	200	**0**	0	0-0,68	0	0	0-0,55
NZTV-017	202	**3**	0.6	0,05-1,6	0	0	0-0,55
NZTV-018	201	**0**	0	0-0,68	0	0	0-0,55
NZTV-019	201	**2**	0.4	0,04-1,2	0	0	0-0,55
NZTV-020	200	**1**	0.18	0,03-0,98	0	0	
NZTV-021	154	**1**	0.28	0,01-1,2	2	0.4	
NZTV-023	201	**3**	0.6	0,05-1,6	0	0	0-0,55
NZTV-024	201	**0**	0	0-0,68	0	0	0-0,55
NZTV-025	204	**2**	0.4	0,04-1,2	0	0	0-0,55
NZTV-026	201	**8**	1.4	0,5-2,1	0	0	0-0,55
NZTV-033	203	**2**	0.4	0,04-1,2	0	0	0-0,55
NZTV-034	202	**1**	0.18	0,03-0,98	0	0	0-0,55
NZTV-039	200	**2**	0.4	0,04-1,2	1:(8,21)	0.3	0,2-0,8
NZTV-041	204	**6**	1.12	0,4-2)	1:(22,16)	0.3	0,2-0,8
NZTV-042	201	**2**	0.4	0,04-1,2	1:(2,9)	0.3	0,2-0,8
NZTV-043	201	**3**	0.6	0,05-1,6	0	0	0-0,55
NZTV-044	203	**5**	0.95	0,3-1,8	1:(3,17)	0.3	0,2-0,8
NZTV-045	205	**1**	0.18	0,03-0,98	0	0	0-0,55
NZTV-046	203	**0**	0	0-0,68	0	0	0-0,55
NZTV-047	106	**0**	0	0-1,1	0	0	0-0,65
NZTV-048	188	**2**	0.42	0,05-1,25	0	0	0-0,55
NZTV-049	202	**4**	0.78	0,18-1,15	0	0	0-0,55
NZTV-050	201	**2**	0.4	0,04-1,2	0	0	0-0,55
NZTV-051	203	**0**	0	0--0,68	0	0	0-0,55
NZTV-052	202	**1**	0.18	0,03-0,98	0	0	0-0,55
NZTV-053	92	**1**	0.4	0,01-2	0	0	0-0,72
NZTV-055	239	**8**	1.15	0,45-2,05	0	0	0-0,55
NZTV-056	217	**0**	0	0-0,62	0	0	0-0,55
NZTV-057	167	**0**	0	0-0,69	0	0	0-0,55
NZTV-058	145	**0**	0	0-0,72	0	0	0-0,55
NZTV-060	164	**0**	0	0-0,70	0	0	0-0,55
NZTV-061	220	**2**	0.35	0,03-1,15	0	0	0-0,55
NZTV-062	200	**4**	0.8	0,18-1,15	0	0	0-0,55
NZTV-064	206	**1**	0.18	0,03-0,98	1:(4,22)	0.3	0,2-0,8
NZTV-065	182	**1**	0.2	0,03-098	0	0	0-0,55
NZTV-066	202	**6**	1.15	0,4-2	0	0	0-0,55
NZTV-068	208	**2**	0.35	0,03-1,2	0	0	0-0,55
NZTV-069	204	**2**	0.35	0,03-1,2	1:(1,15)	0.3	0,2-0,8
NZTV-070	225	**0**	0	0-0,68	0	0	0-0,55
NZTV-072	205	**0**	0	0-0,69	1:(13,14)+1tric	0.55	0,25-1,1
NZTV-073	214	**2**	0.37	0,04-1,15	0	0	0-0,55
NZTV-074	205	**6**	1.14	0,4-2	1:(1,11)	0.3	0,2-0,8
NZTV-075	203	**1**	0.17	0,03-0,96	0	0	0-0,55
NZTV-076	206	**2**	0.35	0,03-1,15	0	0	0-0,55
NZTV-079	199	**2**	0.37	0,04-1,15	0	0	0-0,55
NZTV-097	201	**0**	0	0-0,68	0	0	0-0,55

Table 15. Radiation dosimetry estimates for the Experimental group, calculated on translocation frequency and dicentric frequency. CI = Confidence Interval; dic = dicentric

❖ DISCUSSION

The aim of the current study was to ascertain whether or not New Zealand naval personnel who participated in Operation Grapple incurred long-term genetic damage. The results of three assays are reported here (G2 assay, micronucleus (MN) assay and mFISH), with the results of two further assays having already been released into the public domain (SCE assay report to the New Zealand Department of Veterans Affairs, 2005, and an MSc thesis using the COMET assay (Johnson, 2004)). The reason for performing a number of different assays is because each test investigates different genetic processes. For instance, the MN assay indicates the radiosensitivity of an individual and is interpreted as measuring the efficiency of a person's DNA repair system at G1 in the cell cycle. The G2 assay is similar to the MN assay in measuring the radiosensitivity of an individual during G2 of the cell cycle. Individuals can vary in their response to either assay, and among different assays, so it was necessary to perform a range of tests.

In the present study we report that the New Zealand nuclear test veterans show no evidence of radiosensitivity as seen by applying either the G2 assay or MN assay. Neither of these assays showed any significant difference between the Experimentals and the Controls, from which we conclude that, in general terms, the DNA repair mechanisms of the veterans as a group are no more deficient than any normal group of men of similar age.

In the G2 assay, one notable outlier was observed in the Controls and 2 in the Experimentals for the mean number of chromosome breaks, but in fact the overall average number of breaks was marginally higher in the Controls than the Experimentals, although this did not reach significance ($p = 0.11$). Conversely, there was a trend towards a larger mean difference in fragment count in the Experimental group compared to the Controls. Again, this difference was not statistically significant ($p = 0.55$). As noted previously, one Control participant with an extraordinarily high total of 47 fragments distorted the results, but then one could equally argue that the highest or two highest Experimentals did the same.

The results of the MN assay, which is similar to the G2 assay as a technique to assess radiosensitivity, but prior to DNA synthesis, showed remarkable conformity between the veterans and the matched controls. The 2 main parameters that are compared using the MN Assay are the number of micronucleated cells per 100 binucleated cells (“a”) and the number of micronuclei per 100 binucleated cells (“b”). The average value of “a” was 45.7 in the Experimentals and 45.3 in the Controls. The average value of “b” was 65.2 in the Experimentals and 64.0 in the Controls. A pooling of the data shows an almost identical distribution of micronuclei frequency in both groups. As noted previously, the differences are not statistically significant. This reinforces our G2 findings that the veterans have incurred no long term impairment of their capacity to repair damaged DNA.

The mFISH procedure, however, showed a highly significant difference between the Experimentals and the Controls in total translocation frequencies. Our findings are quite clear. The Experimental group (veterans) have an extraordinarily high number of total stable translocations compared to the matched Control group (29.38 per 1000 cells and 10.05 per 1000 cells, respectively). This result is compatible with many studies which show high translocation frequencies in peripheral blood lymphocytes following exposure to radiation. Nevertheless, it is important to address key issues concerning both the gathering and interpretation of the data reported in this study.

First, methodology. The procedure for conducting FISH that we followed is detailed in IAEA (2001), except for one admittedly important difference, that being the length of lymphocyte culture time. To ascertain the frequency of stable aberrations many years after exposure, it is standard practice to culture lymphocytes for a finite period of 46-50 h to optimise the collection of cells in their first cell division in *in vitro* culture. In our investigation we performed several assays and were faced with making decisions on various aspects of lymphocyte culture to accommodate the different assays from each valuable blood sample. We acknowledge that the lymphocyte cultures used for mFISH analysis were harvested after 72 h and would thus have undergone 2 or 3 cell cycles. Because we were looking at possible long term exposure, we committed ourselves to

scoring only stable cells, with the probability of finding unstable cells with dicentric chromosomes being remote. If cells pass through more than one cell cycle then clonality becomes an issue when scoring translocation frequencies. We were particularly alert to this possibility, but in all our observations of translocations in each individual, we did not detect any clonal cells. According to Nakamura et al. (2004), the majority of clones are aberration free and hence are undetectable. They also disappear soon after exposure. Guerrerro-Carbajal et al. (1998) further established that there is no decrease in translocation yield after 3 to 4 divisions. In our judgement, the key point was to ensure that the procedures followed were exactly the same between the Experimentals and the Controls. We are confident that even if some clonal cells escaped our attention, the highly significant difference in translocation frequency observed between the 2 groups would alter negligibly and not affect our conclusions.

Secondly, we must address the issue of confounding factors possibly contributing to the translocation scores, particularly with respect to the high translocation frequency in the Experimentals. We realised early on in this investigation that the selection of our control group was going to be crucial in the interpretation of the data, especially if we discovered significant differences between the two groups. We offer the view that many studies similar to our own have placed too much faith in published data on what is considered to be a "normal" population for translocation frequencies. The only data that can be truly relied on is selecting matched controls against the target group under investigation. We went to extraordinary lengths to ensure that our control group was matched to the experimental group in order to isolate the variable of interest: one New Zealand group (the Experimentals) took part in Operation Grapple, on either the HMNZS Rotoiti or HMNZS Pukaki, and the other New Zealand group (the Controls) did not. The Experimental group was a well-identified target group. If a veteran lived in the far north of New Zealand, e.g. Kaitaia, we chartered a plane to fly there to interview and select a control living in the same town. The questionnaire we constructed (see Appendix II) details the information we sought from all participants. We eventually matched the controls with the veterans for all feasible confounding factors except for one: the veterans were heavier smokers in the past than the veterans.

All the Control subjects were ex-army personnel except for some ex-policemen. Ideally it would have been preferable to have selected ex-naval men, but New Zealand only had 2 frigates in the 1950s and they both went to Christmas Island. When they returned to New Zealand and subsequently manned by other crew, there was controversy as to how “clean” the boats were. We chose to exclude ex-naval men from our selection of participants in the Controls.

Third, cytogenetic damage accumulates in humans with age, either due to the prolonged exposure to oxidative damage, chemicals as well as occupational, therapeutic or accidental radiation (Ramsey et al. 1995). Apart from radiation exposure, age is a well-known predictor for increased translocation yield; close to zero in neonatal cord blood to 10 -13 in persons aged between 60 and 79. For this reason it was crucial to age-match the controls with the veterans. In our study, participant age ranged from 58 to 75, with the mean age for the veterans and control groups being 65.9 years (SD = 3.1) and 66.5 years (SD = 3.8), respectively. Therefore, age is not considered to be a confounding variable in our study. The Controls fitted the norm with an average stable translocation frequency of 10.05 translocations per 1000 cells.

Fourth, the one notable potentially confounding variable between the 2 groups was past smoking consumption, with the Experimentals being heavier smokers in the past. One should also note, however, that both groups were virtually identical for current smoking consumption – nearly all have been non-smokers for several years. Our analyses showed no statistical difference in translocation frequencies between the Experimentals and the Controls for smoking. In fact, in both groups never-smokers had a marginally higher total stable translocation frequency than smokers. Whitehouse et al. (2005) record the latest information on control levels of translocations in cultured human lymphocytes and conclude that “there is no obvious sign that smoking affects the control translocation yield.” They continue, “No lifestyle factor, other than age, has been identified in this study as contributing significantly to translocation yield.”

Another question that warrants addressing is the proportionally high frequency of one way translocations observed in the current study. Most *in vitro* studies on radiation-exposed peripheral blood lymphocytes, and also studies of people

accidentally exposed to high radiation doses, show a higher proportion of complete (also called reciprocal translocations or two way translocations) to incomplete (one way) translocations (Lindholm et al., 2002; Braselmann et al., 2005), in a very approximate ratio of 2:1. Our data show a higher ratio of one way translocations to two way translocations in the veterans (approximately 1.5:1), whereas the controls showed a ratio close to 1:1.

Pertinent to this question is an authoritative review of translocations detected by FISH conducted by Edwards et al. (2005). In combining the results from several studies, the authors agreed that, over time, one way translocations decrease in frequency more rapidly than two way translocations. If this is the case, we are faced with having to explain why it is that the veterans have such a high frequency of one way exchanges.

An important point to note here is that discrimination between complete and incomplete translocations depends on the resolution of the FISH technique, as noted by Natarajan (2002). The use of telomeric probes has shown that many cryptic translocations involve sub-telomeric regions that remain undetected by conventional chromosome painting. Kodama et al. (1997) estimate that by using chromosome painting the minimal detectable size of translocated chromosome segments is 11.1 Mb. Human telomeres are 5 to 15 kb long. Therefore, the presence or absence of a small terminal region of a chromosome involved in an exchange would not be resolved unless telomeres are detected. Interestingly, Wu et al. (1998), using telomeric probes, estimated that the rate of incomplete translocations in their study was as low as 3%. Boei et al. (1998) also concluded that the majority of incomplete aberrations arise from terminal exchanges that are unresolvable using FISH and not from incompleteness. More recently, Fomina et al. (2000, 2001), also using PNA telomeric probes, established that the true percentage of incomplete exchange patterns is approximately 5%. Whitehouse et al. (2005), in their major review of translocation yields in peripheral blood lymphocytes, go further and explicitly advise that “for retrospective dosimetry, it is all translocations in stable cells that should be recorded.”

From these studies, we estimate that our scoring of complete and incomplete exchanges is over weighted towards a high incomplete exchange frequency which could be attributed to a lack of resolution of the FISH technique; specifically, the inability to detect minute telomeric exchanges, especially in older men. It is indeed quite likely that we have underscored the number of complete translocations in our study and thus our results are conservative.

The comparatively higher frequency of dicentrics observed in the veterans compared to the controls is interesting and evokes the results found in French Polynesians with thyroid cancer (Violot et al., 2005). While the frequency of dicentrics is useful for biological dosimetry immediately after exposure, they are not usually scored for retrospective estimation after several months or years, because the frequency of lymphocytes carrying dicentrics decline after exposure. Unlike dicentrics, which are unstable, chromosome translocations are expected to be stable and their frequencies can be used in retrospective dosimetry (Edwards, 2000). Thus it came as a surprise to us to find a high frequency of dicentric chromosomes and acentric fragments in the veterans, many that were unable to be scored in very complex cells. This high frequency of dicentric chromosomes in the veterans, including those that were observed but not scored in very complex cells, is very evocative of irradiation and suggests to us that the veterans may have been contaminated and may have retained high-LET long half-life radionuclides in their bodies (Rowland et al., 2005).

After a single irradiation, the translocations should follow a Poisson distribution. This is not the case in the veterans, which is not surprising because multiple irradiations and moreover possible contaminations can result in great heterogeneity in the distribution of the pathologic translocations (IAEA 2001).

Another issue is whether it is possible to attribute translocations observed at the present to an event which occurred 50 years ago. Persistence of stable translocations over time is well known. Lindholm et al. (2002) performed an intercomparison of translocation and dicentric frequencies between laboratories in a follow-up of a radiological accident in Estonia. The general conclusion was that the half-time was about 8 years for two way translocations and around 6

years for all other translocations, and that cells containing complex rearrangements were few in number and disappeared with time. This would suggest that the translocations we observed in the veterans could not be attributed to their participation in Operation Grapple. However, as noted in the Introduction, past radiation exposure can leave a permanent signature in the genome (Hande et al. 2003), originating as far back as 1949, and even 1945 in Hiroshima atomic bomb survivors (Nakano et al., 2001; Kodama et al., 2001), as well as in more recent Chernobyl cleanup workers several years after the event (Lazutka, 1996; Slozina et al., 1997). In our view, the comparatively high frequency of dicentric chromosomes in the veterans would endorse the view that the chromosomal aberrations we observed are most likely attributable in large part to current radiation exposure. The dosimetries calculated by dicentric frequency and translocation frequency are not identical but descriptively well correlated. The differences are not surprising and could perhaps be explained by different half-lifes of a variety of radionuclides. We do not know the decrease of these elements over time, but in our opinion it was important to establish some estimate of exposure as it is the only quantified index of the risks for the veterans.

A notable feature amongst the veterans in our study was the high number of extraordinarily complex cells with many translocations, apparently multicentric chromosomes, deletions, centric and acentric fragments. Such chromosomally unstable cells were termed “rogue” cells by Awa and Neel (1986). Rogue cells are usually rare in comparison with the general level of chromosomally aberrant cells, so their high frequency in the veterans in our study is intriguing. Incidentally, a lack of resources and time to invest in detailed study of these aberrations, even to establish a sample estimate, restricted the amount of meaningful data we could gain from these unstable cells. Unfortunately, we have no precise record of their frequency, but the data are still available for future analysis. Nevertheless, descriptively the number of rogue cells observed in the Control group amongst the thousands of cells observed, amounted to less than 10. In contrast, the number of rogue cells observed in the Experimental group (but not scored) amounted to a few hundred.

Rogue cells in general remain a puzzle. Their aetiology has not been determined with any certainty, although current opinion favours the triggering of enzymatic activity, e.g. endonucleases, by viruses. They were first observed in the lymphocytes of Yanomama Indians in Venezuela (Bloom et al., 1970) and later in several other populations around the world. Rogue cells are usually rare in comparison with the general level of chromosomally aberrant lymphocytes, and follow-up studies have demonstrated that the frequency of rogue cells decreases rapidly after their initial appearance (Bloom et al., 1973; Tawn et al., 1985).

There is general agreement in the literature that there is no correlation between exposure to ionizing radiation *per se* and the occurrence of rogue cells, except possibly in one study on astronauts exposed to high-LET radiation (Mustonen et al., 1998). Lazutka (1996), however, reported that rogue cells are seen in patients after nuclear accidents concomitant with a stimulation of JC virus antibodies (JC virus is a polyoma virus designated by the initials of the patients from whom it was first isolated). It has also been established that the immune system is compromised by exposure to low dose radiation (Godekmerdan et al., 2004). In common with most observers, the high rogue cell count we observed in the veterans could be interpreted as a signature of immunodeficiency, arising perhaps initially as a consequence of radiation exposure. Viral infection alone, however, may not be sufficient to explain the extent of chromosome damage observed in some cells. A controversial suggestion, despite the above consensus, is that the complexity of anomalies observed may be explained by possible exposure to heterogeneity of past and possibly present irradiations (gamma-rays, protraction and multiplicity of exposures, ingested contaminations of long half-life radionuclides).

Chromosome analysis of human peripheral blood lymphocytes following radiation exposure is a well-established technique for dose estimations in cases of accidental over-exposure (IAEA 2001). The introduction of FISH has proved a valuable tool to establish the amount of past exposure to radiation in humans. An attempt was made in the current study to reconstruct possible radiation dosage in the Operation Grapple veterans. Possible exposure estimates are listed in Table 15. We acknowledge that there are many uncertainties surrounding this estimate,

which should be used only as a guide. The reconstruction was based on the translocation frequencies observed in chromosomes 1, 3 and 4. Individual estimates in 5 cases are very high with an estimate of 1Gy. 6 received between 0.5 and 0.95Gy, and 16 received between 0.2 – 0.45Gy. As noted, there were no estimates for 15 veterans. A dosage estimate was also attempted based on the dicentric count observed, which descriptively corroborates the translocation estimate.

❖ SUMMARY

Three assays were performed in this study, as part of a larger investigation, to ascertain the genetic status of New Zealand military personnel who took part in Operation Grapple in 1957-58. The results of two of these assays: the G2 assay and the micronucleus (MN) assay, show that the veterans do not exhibit any deficiency in their DNA repair mechanisms.

The third assay, mFISH, shows a very high frequency of total translocations in the veterans' chromosomes as opposed to a matched control group. This result is highly significant and we are faced with answering the question: what unifying factor could give rise to such high translocations amongst the veterans? Our carefully planned case-control study leads us to the view that this can be attributed to their participation in Operation Grapple.

This leads us to a second question: What, then, could cause these high translocation frequencies after such a long period of time since the event occurred?

Different environmental agents can cause chromosomal breakages, but 29 translocations per 1000 cells as observed in the veterans, compared to 10 for the controls, is a particularly high score. The causative agent must be a powerful inducer of chromosome breaks. Very strict exclusion/inclusion criteria were applied in the selection process of both the veterans and the controls to exclude possible confounding factors. A detailed analysis of the scientific literature on related studies involving high chromosome translocation frequencies was also conducted.

We submit the view that the probable cause of the veterans' elevated translocation frequencies is radiation exposure. This view is supported by the observation of a comparatively high dicentric chromosome score in the veterans, which is characteristic of radiation exposure.

The findings presented here are based on only 50 veterans from New Zealand who took part in Operation Grapple. We would encourage those in authority to initiate research to corroborate our findings by conducting a similar study on British and Fijian personnel who also took part in Operation Grapple.

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