

Chromosome analysis of workers occupationally exposed to radiation at the Sellafield nuclear facility

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Abstract.

Purpose: To investigate the relationship between stable chromosome aberration frequency in peripheral blood lymphocytes and occupational cumulative radiation exposure.

Materials and methods: Cytogenetic analysis using G-banding was performed on peripheral blood lymphocyte cultures from 104 workers from the British Nuclear Fuels PLC facility at Sellafield, UK. The study group comprised 61 men with lifetime cumulative doses > 500 mSv, 39 men with minimal exposure (i.e. < 50 mSv) who formed a control group and 4 men with intermediate doses.

Results: The slope of the dose–response, adjusted for smoking status, for translocations and insertions was $0.55 \pm 0.31 \times 10^{-2}$ /cell/Sv. Consideration of chromosome breakpoints for all aberrations combined in the radiation workers revealed an excess in the C group chromosomes and a deficit in the F group chromosomes with breakpoints being concentrated in the terminal regions whereas the distribution in the control group did not deviate from expectation.

Conclusions: The dose–response was not significantly different from the parallel FISH analysis (Tucker *et al.* 1997) and confirms that chronic radiation exposure appears to be substantially less effective at inducing stable chromosome aberrations in comparison with acute exposure.

1. Introduction

Chromosome aberration analysis is a well established technique for estimating radiation dose in cases of recent exposure, since the dose–response *in vivo* and *in vitro* is similar and well defined for peripheral blood lymphocytes sampled a short time afterwards (Lloyd 1984, Bender *et al.* 1988, Bauchinger 1995, Edwards 1997). Traditionally radiation biodosimetry has relied on the frequency of dicentrics in peripheral blood lymphocytes. The dicentric has the advantage of being easily identified

using a conventional block staining technique and has a low background frequency in the general population (Lloyd *et al.* 1980, Tawn 1987, Bender *et al.* 1988, Bauchinger 1995). It is, however, an unstable aberration which encounters mechanical difficulties when passing through cell division, and cells with dicentrics are eliminated from the peripheral blood lymphocyte population with a half life of about three years (Lloyd *et al.* 1980, Tawn and Binks 1989). Dicentric frequencies, whilst a good indicator of recent exposure, are therefore of little use as a marker of historical or chronic exposure. The dicentric is an asymmetrical interchange between two chromosomes. The equivalent symmetrical rearrangement, the translocation, is stable and able to replicate with fidelity. Studies of the Japanese A-bomb survivors and patients receiving radiotherapy have shown translocations to persist in peripheral blood lymphocytes many years after exposure (Awa 1983, 1991, Buckton 1983, Kleinerman *et al.* 1989, 1990, 1994) and repeated cytogenetic analyses have also indicated that the frequencies of cells with translocations remain unchanged (Buckton 1983, Lloyd *et al.* 1998). They are thus potentially a better indicator of cumulative dose. This persistence must reflect the induction of aberrations in stem cells with subsequent constant replenishment of the mature lymphocyte pool.

Detection of translocations by conventional block staining techniques will only identify those with obvious length changes and is therefore inefficient and probably subject to scorer bias. Fluorescence *in situ* hybridisation (FISH) using whole chromosome paints enables the detection of translocations involving selected chromosomes. A number of studies, using different combinations of painted chromosomes, have adopted the approach of Lucas *et al.* (1989) and extrapolated to the whole genome on the assumption that radiation-induced exchanges are produced randomly. Analysis using G-banding, although more time consuming, allows the identification of rearrangements involving any chromosome in the genome and also the location of breakpoints

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within each chromosome. Therefore, the data can be also examined for excesses and deficits in the involvement of specific chromosomes or chromosome regions in rearrangements.

The risk to health of exposure to low doses of ionizing radiation is a subject of continuing debate that is unlikely to be realized entirely by classical epidemiological methods. Extrapolation from high-dose studies requires assumptions about the shape of the dose-response relationship and also the mechanisms of carcinogenesis. The role of translocations in oncogenesis is well established (Heim and Mitelman 1995, Rabbitts 1994, Tawn 1997) and therefore the study of translocation frequency in occupationally exposed radiation workers with well documented radiation dose histories will give data that not only can provide dose-response relationships for use in biological dosimetry, but can also be used in comparisons with data from acutely exposed populations to give an insight into the risks of different types of exposure.

The British Nuclear Fuels PLC (BNFL) Sellafield nuclear reprocessing facility, formally Windscale and Calder Works, in West Cumbria, UK, began operations in 1950 and because there has been little general workforce mobility in the area there has been opportunity, albeit within regulatory limits, for some individuals to accumulate relatively large doses of radiation thus making them an important group for the study of the effects of chronic exposure to low doses of ionizing radiation. In this report the results of a study using G-banded chromosome analysis on a group of current workers are presented. The work was part of a collaborative project set up to study a number of somatic genetic endpoints from a single blood sample thus avoiding the necessity for repeated sampling. Results of HPRT and GPA mutation assays and chromosome painting analysis have already been reported (Cole *et al.* 1995, Tucker *et al.* 1997).

2. Materials and Methods

2.1. Study Population

Full details of the selection of individuals have been previously reported (Tucker *et al.* 1997). Of the 170 men originally identified as having accumulated lifetime doses in excess of 500 mSv, 87 provided blood samples. Likewise 50 control individuals (not 49 as originally reported by Tucker *et al.* 1997) with doses thought to be less than 50 mSv also provided blood samples. Data on age and smoking habits were obtained by a questionnaire. Exposures were measured by film badge dosimetry (Kite and Britcher 1996). When individual dosimetry records were

examined three of the 87 thought to have doses greater than 500 mSv were actually found to have accumulated doses of only 338 mSv, 449 mSv and 497 mSv, respectively and three of the 50 control individuals had doses greater than 50 mSv, i.e. 142 mSv, 173 mSv and 187 mSv. The group used for the G-banded analysis study which is the subject of this report comprised a total of 104 men, 60 having doses > 500 mSv, 39 with doses < 50 mSv and five of the individuals with intermediate doses described above, namely 142 mSv, 187 mSv, 338 mSv, 449 mSv and 497 mSv. All but 14 of the 81 men studied using FISH with whole chromosome painting probes (Tucker *et al.* 1997) are included in the G-banding study and an additional 37 men, not studied with FISH, were analysed with G-banding.

2.2. Cell culture and chromosome analysis

Peripheral blood lymphocytes were cultured for 48 h at 37°C using Eagles minimal essential medium supplemented with 15% foetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin and 2% phytohaemagglutinin. Colcemid was added for the last 4 h of culture at a final concentration of 0.1 µg/ml. Bromodeoxyuridine was present throughout culture at a concentration of 10 µM and subsequent fluorescence + Giemsa staining of a number of samples indicated that > 95% of the cells were in their first mitosis. Harvesting involved treatment with 75 mM KCl and repeated fixation with methanol and acetic acid in the ratio 3:1.

Metaphases were G-banded with trypsin to a resolution of approximately 400 bands per cell (Mitelman 1995) and 100 cells from each individual were analysed for all observable aberrations, i.e. translocations, inversions, insertions, dicentrics, centric rings and acentrics, with the latter category including recognizable interstitial and terminal deletions and excess fragments of unknown origin. Complex rearrangements involving more than two chromosomes were broken down into the equivalent number of simple interchanges, i.e. translocations and dicentrics, (Savage 1975). In order to make direct comparisons with the chromosome painting analysis (Tucker *et al.* 1997) aberration frequencies were derived for translocations plus insertions, with insertions being classed as one symmetrical aberration, and for dicentrics. Acentric frequencies were also calculated.

For breakpoint analysis chromosome aberrations were classified into two groups, symmetrical, i.e. translocations, insertions, inversions and symmetrical complex exchanges, and asymmetrical plus acentrics, i.e. dicentrics, centric rings, interstitial deletions,

asymmetrical complex exchanges and terminal deletions. Because the dataset is relatively small the chromosomes were grouped into seven categories (A–G) according to the Denver classification (Mitelman 1995) and observed numbers of breakpoints compared to those expected based on established whole chromosome lengths (Savage and Papworth 1982). Two apparently identical translocations found in one individual were considered only once in this analysis and acentric fragments of unknown origin were excluded.

Statistical analyses on aberration frequencies were performed using the Generalized Interactive Modelling System (GLIM, Royal Statistical Society UK) and were analysed using a Poisson linear model, with adjustment for overdispersion based on the normalized residual deviance (McCullagh and Nelder 1989). Two-sided *p*-values are reported for all analyses. Statistical analyses of breakpoints were carried out using the likelihood-ratio χ^2 test.

3. Results

3.1. Aberration Frequencies

Chromosome aberration data are presented in similar categories to those defined by Tucker *et al.* (1997) (table 1), these being chosen originally to give

five groups of approximately equal size in the chromosome painting study. Dividing the data into more conventional dose groups (table 2) gives a more regular increase in translocation plus insertion frequencies, although there is not a great difference between the two summaries. However it does illustrate that different impressions can sometimes result depending on how data are grouped.

Univariate analysis of translocations plus insertions with cumulative dose as a continuous variable gave a slope (\pm standard error) of $0.57 \pm 0.33 \times 10^{-2}/\text{cell/Sv}$ ($p=0.086$) (figure 1). Comparison of ever-smokers with never-smokers revealed a higher frequency of translocations plus insertions in ever-smokers ($p=0.006$). Analysis restricted to never-smokers gave a slope for cumulative dose of $0.9 \pm 0.4 \times 10^{-2}/\text{cell/Sv}$ ($p=0.034$). Analysis restricted to ever-smokers gave a slope of $0.34 \pm 0.43 \times 10^{-2}/\text{cell/Sv}$ ($p=0.43$) indicating no significant association for cumulative dose. Age was significantly associated with translocation plus insertion frequencies in a univariate analysis ($p=0.021$) but was not significantly associated when adjusted for ever-smoking and cumulative dose ($p=0.18$). In a regression with both ever-smoking status and dose the slope for dose was $0.55 \pm 0.31 \times 10^{-2}/\text{cell/Sv}$ ($p=0.081$).

Acentric frequencies were not significantly associ-

Table 1. Data on age, smoking, radiation exposure and chromosome aberrations by worker groups defined by Tucker *et al.* (1997).

	Dose group				
	1	2	3	4	5
Dose range, mSv	< 50	140–560	564–655	655–760	> 760
Number of subjects	39	17	17	17	14
Number of cells analysed	3900	1700	1700	1700	1400
Mean age, years (range)	53 (41–72)	51 (39–61)	55 (45–62)	54 (43–61)	58 (52–64)
Number of smokers	25	10	14	13	12
Translocations	30	12	15	11	22
Inversions	8	3	5	0	5
Insertions	2	0	0	0	1
Translocations + insertions	32	12	15	11	23
Dicentric	4	1	2	5	3
Rings	0	0	1	1	0
Acentrics	8	5	3	3	1
Complex	0	2 ^a	1 ^b	1 ^c	1 ^d
Translocations + insertions per 100 cells \pm S.E. ^e	0.82 ± 0.15	0.88 ± 0.23	1.00 ± 0.24	0.77 ± 0.21	1.86 ± 0.36
Dicentric per 100 cells \pm S.E. ^f	0.10 ± 0.05	0.12 ± 0.08	0.12 ± 0.08	0.29 ± 0.13	0.21 ± 0.12
Acentric per 100 cells \pm S.E.	0.21 ± 0.07	0.29 ± 0.13	0.18 ± 0.10	0.18 ± 0.10	0.07 ± 0.07

^aOne cell with three-way translocation (classed as two translocations) and one cell with a complex rearrangement classed as one translocation and one dicentric.

^bOne cell with three-way translocation (classed as two translocations).

^cOne complex defined as one translocation and one insertion.

^dOne four way translocation (classed as three translocations).

^eIncludes translocation and insertion equivalents derived from complex data.

^fIncludes dicentric equivalents derived from complex data.

Table 2. Data on age, smoking, radiation exposure and chromosome aberrations by conventional dose groups.

	Dose group				
	1	2	3	4	5
Dose range, mSv	< 500	500–599	600–699	700–799	> 800
Number of subjects	44	19	13	19	9
Number of cells analysed	4400	1900	1300	1900	900
Mean age, years (range)	53 (41–72)	53 (39–62)	53 (43–62)	56 (46–64)	58 (52–61)
Number of smokers	30	12	8	16	8
Translocations	32	14	12	19	13
Inversions	8	4	4	2	3
Insertions	2	0	0	0	1
Translocations + insertions	34	14	12	19	14
Dicentrics	4	1	2	6	2
Rings	0	0	1	1	0
Acentrics	9	4	3	3	1
Complex	0	3 ^a	0	1 ^b	1 ^c
Translocations + insertions per 100 cells ± S.E. ^d	0.77 ± 0.13	1.00 ± 0.23	0.92 ± 0.27	1.11 ± 0.24	1.89 ± 0.46
Dicentrics per 100 cells ± S.E. ^e	0.09 ± 0.05	0.11 ± 0.07	0.15 ± 0.11	0.32 ± 0.13	0.22 ± 0.16
Acentrics per 100 cells ± S.E.	0.20 ± 0.06	0.21 ± 0.11	0.23 ± 0.13	0.16 ± 0.09	0.11 ± 0.11

^aTwo cells with three-way translocation (classified as two translocations) and one cell with a complex rearrangement classified as one translocation and one dicentric.

^bOne complex defined as one translocation and one insertion.

^cOne four way translocation (classified as three translocations).

^dIncludes translocation and insertion equivalents derived from complex data.

^eIncludes dicentric equivalents derived from complex data.

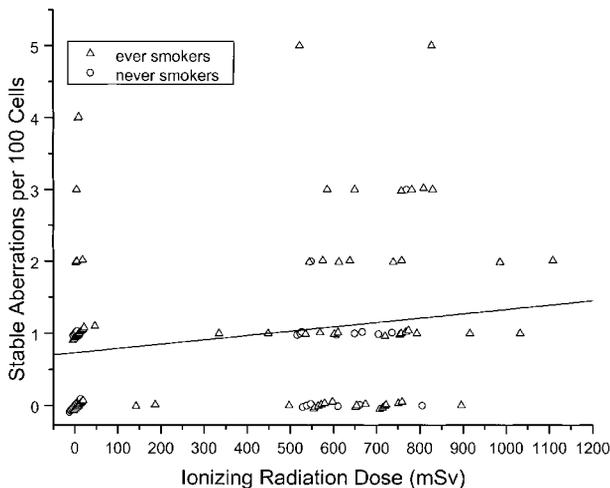


Figure 1. Frequency of stable aberrations plotted by dose of ionizing radiation. The straight line is the result of a linear regression analysis treating dose as a continuous variable without adjustment for smoking status.

ated with any of the variables ($p > 0.1$). In fact, acentric frequencies decreased slightly with increasing cumulative dose, with increasing age, and were slightly lower in ever or current smokers.

Univariate analysis of dicentrics with cumulative dose gave a slope of $0.16 \pm 0.09 \times 10^{-2} / \text{cell/Sv}$ ($p = 0.081$). Dicentrics were slightly elevated in ever smokers ($p = 0.67$), and adjustment for ever

smoking gave a slope for cumulative dose of $0.17 \pm 0.09 \times 10^{-2} / \text{cell/Sv}$ ($p = 0.052$). Dicentric frequencies were unrelated to age ($p = 0.70$).

For comparison, data on the 67 men who were studied with fluorescence *in situ* hybridization by Tucker et al (1997) and also by G-banding are presented in table 3. A summary table of the dose-response relationships from the two studies in relation to smoking is provided in table 4.

3.2. Breakpoint analysis

Data on the breakpoints involved in chromosome rearrangements are presented in figure 2 and table 5. The men were considered in two groups, controls with cumulative exposure < 50 mSv (table 1, Group 1) and exposed radiation workers with cumulative exposure > 50 mSv (table 1, Groups 2–5).

Analysis of breakpoints involved in symmetrical aberrations revealed no deviation from expectation based on chromosome length ($p > 0.9$), but significant departure from expectation for radiation workers ($p = 0.016$). After removal of aberrations involving solely chromosomes 7 and 14 (i.e. *inv*(7), *inv*(14), *t*(7;14), *t*(7;7), *t*(14;14)) which are known to arise *in vivo* during immunological development (Prieur *et al.* 1988, Tawn 1988), the distribution of aberrations in radiation workers did not differ significantly from

Table 3. Stable aberration frequencies^a for 67 workers with dual analysis using fluorescence *in situ* hybridization and G-banding.

	Dose groups				
	1	2	3	4	5
Dose range, mSv	< 500	500–599	600–699	700–799	> 800
Number of subjects	22	14	10	14	7
FISH	0.78 ± 0.14	0.82 ± 0.10	0.91 ± 0.24	1.33 ± 0.26	1.33 ± 0.42
G-banding	0.73 ± 0.23	1.00 ± 0.39	0.80 ± 0.33	1.07 ± 0.29	1.86 ± 0.67
Combined ^b	0.78 ± 0.13	0.90 ± 0.13	0.89 ± 0.20	1.28 ± 0.20	1.46 ± 0.46

^aTranslocations + insertions per 100 cells ± S.E.

^bThe combined aberration frequency was defined as the total number of aberrations identified by either FISH or G-banding divided by the total number of cell equivalents examined by FISH plus the total number of cells examined with G-banding.

Table 4. Dose–response data.

	Stable aberrations × 10 ⁻² /cell/Sv		
	All subjects	Ever smokers	Never smokers
FISH (Tucker <i>et al.</i> 1997)	0.79 ± 0.22	0.11 ± 0.40	1.04 ± 0.25
G-banding (present study)	0.55 ± 0.31	0.34 ± 0.43	0.9 ± 0.4
Dual analysis			
FISH	0.45 ± 0.24	0.33 ± 0.27	1.01 ± 0.55
G-banding	0.66 ± 0.41	0.47 ± 0.57	1.03 ± 0.61
FISH and G-banding combined	0.49 ± 0.23	0.35 ± 0.27	1.05 ± 0.46

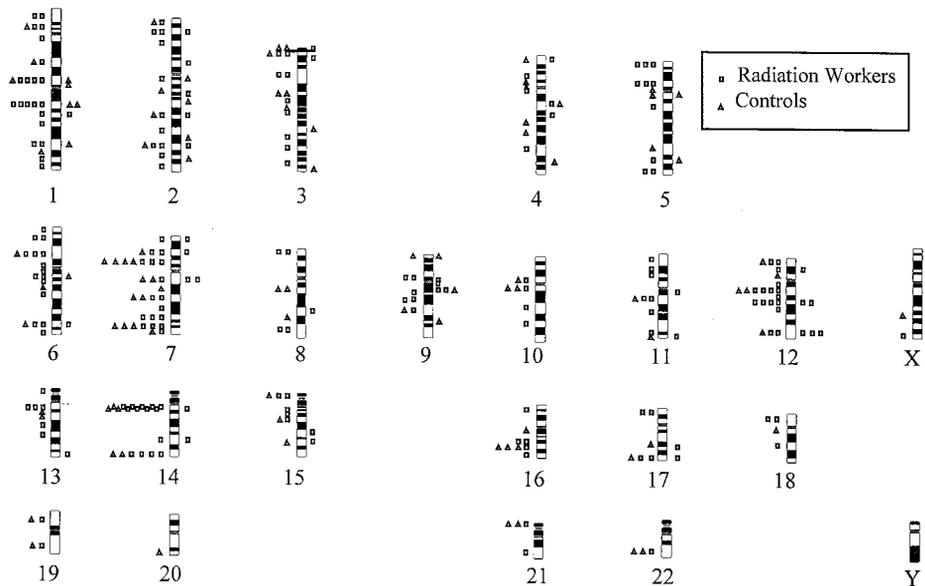


Figure 2. Distribution of chromosome breakpoints in symmetrical exchanges (left) and asymmetrical exchanges plus acentric fragments (right).

expectation ($p=0.053$). Control data remained distributed according to expectation after removal of chromosomes 7 and/or 14 rearrangements ($p>0.9$). Consideration of asymmetrical aberrations plus acentrics revealed no significant difference between observed and expected distributions for radiation workers ($p=0.53$), but a significant departure from

expectation for controls ($p=0.005$). This departure from expectation in controls was due to an excess of aberrations in the longer chromosomes (group A). Closer examination did not indicate preferential involvement of any particular chromosome. Combining data for all aberrations resulted in a deviation from expectation in radiation workers, irrespective

Table 5. Observed and expected frequencies of breaks by chromosome groups.^a

	Chromosome groups	Controls		Radiation workers	
		Observed	Expected	Observed	Expected
Symmetrical aberrations	A	17	18.56	41	37.58
	B	8	9.79	13	19.82
	C	33	29.59	71	59.91
	D	10	8.62	24	17.45
	E	7	7.46	12	15.11
	F	3	4.06	2	8.22
	G	4	3.92	3	7.93
			$p > 0.9$		$p = 0.016$
Symmetrical aberrations minus 7 and/or 14 rearrangements	A	17	15.85	41	34.41
	B	8	8.36	13	18.15
	C	25	25.26	67	54.86
	D	6	7.36	14	15.98
	E	7	6.37	12	13.83
	F	3	3.47	2	7.52
	G	4	3.35	3	7.27
			$p > 0.9$		$p = 0.053$
Asymmetrical aberrations plus acentrics	A	11	4.3	8	8.38
	B	4	2.27	3	4.42
	C	4	6.86	18	13.35
	D	0	2.00	5	3.89
	E	0	1.73	2	3.37
	F	0	0.94	0	1.83
	G	0	0.91	1	1.77
			$p = 0.005$		$p = 0.53$

^aDenver classification (Mitelman 1995).

of whether chromosome 7 and/or 14 rearrangements were included ($p=0.003$) or excluded ($p=0.013$) from analyses. The departure from expectation was primarily due to an excess of aberrations in C group chromosomes and a deficit in F group chromosomes. In contrast, the distribution for all aberrations in the control group did not deviate from expectation based on length regardless of whether chromosome 7 and/or 14 rearrangements were included in analyses ($p > 0.5$).

The distribution of breakpoints within three major chromosome regions was also examined (table 6). These regions were defined as centromeric (i.e. involving bands adjacent to the centromere), terminal (i.e. bands at the ends of chromosome arms), and interstitial (i.e. the remaining bands). The observed numbers of aberrations in these regions were compared to the expected numbers based on the relative lengths of all bands in each region (Yu *et al.* 1978). When all aberrations were considered, there was a significant deviation from expectation for the radiation workers ($p=0.020$), with an excess of breakpoints in the terminal regions. After removal of the chromosome 7 and/or 14 rearrangements, which predominately involve centromeric and interstitial regions, the devi-

ation from expectation became more marked ($p=0.005$). The control breakpoint data was distributed as expected based on chromosome region length, both with ($p=0.19$), and without ($p=0.22$), chromosome 7 and/or 14 aberrations in the analysis.

4. Discussion

Because translocation frequency is a cumulative measure of clastogenic exposure, lifestyle factors which result in exposure to chromosome breaking agents need to be assessed in any population study aimed at evaluating the effect of a particular agent. Two important factors are age and smoking (Tawn and Cartmell 1989, Tucker and Moore 1996). When adjusted for smoking and cumulative dose no effect of age was found on translocation plus insertion (i.e. stable aberrations) frequencies in this study. Most of the men in this study were between the ages of 48 and 60 (the youngest was 39 and the oldest 72 years), and this restrictive age range limits the power to detect an age effect. The effect of smoking is difficult to quantify because of the range of smoking habits. However a stronger radiation dose-response for translocations plus insertions was found for the non-

Table 6. Comparison of breaks within chromosomes.^a

	Chromosome area	Controls		Radiation workers	
		Observed	Expected	Observed	Expected
Symmetrical and asymmetrical aberrations combined	Centromeric	18	12.97	21	25.81
	Terminal	21	18.12	51	36.06
	Interstitial	62	69.9	129	139.11
			$p = 0.19$		$p = 0.020$
Symmetrical and asymmetrical aberrations combined without 7 and 14 aberrations	Centromeric	15	11.43	14	24.01
	Terminal	20	15.97	48	33.55
	Interstitial	54	61.6	125	129.42
			$p = 0.22$		$p = 0.005$

^aRegions defined by Yu *et al.* (1978).

smokers ($0.9 \pm 0.40 \times 10^{-2}$ /cell/Sv) compared to the ever-smokers ($0.34 \pm 0.43 \times 10^{-2}$ /cell/Sv), a finding in line with previous analysis of the chromosome painting (FISH) data (Tucker *et al.* 1997).

Consideration of dicentrics revealed only slight positive correlations with smoking and cumulative dose which were not significant. These unstable aberrations will not continue to accumulate in the peripheral blood over time and their frequency will only reflect recent clastogenic exposure. The study of dicentric frequency is therefore of limited value in cases of low dose chronic exposure or where exposures have occurred many years previously.

Studies of the dose-response relationships for translocation induction where the radiation dose has been well documented are limited. Populations exposed to partial body radiotherapy many years previously have been analysed for chromosome aberrations in peripheral blood lymphocytes in a series of studies by Kleinerman and colleagues (Kleinerman *et al.* 1989, 1990, 1994). Using total bone marrow doses averaged over the whole body, positive dose-responses for stable aberrations determined by block-staining were found for patients who had received radiotherapy approximately 20 years earlier for cervical cancer (Kleinerman *et al.* 1989) and more than 30 years previously for enlarged thymus, enlarged tonsils and tuberculosis (Kleinerman *et al.* 1990), although the magnitude of the response varied. It was recognized that dose-response relationships will be influenced by the size of the area of the body exposed and that it is difficult to make direct comparisons of data obtained from different regimes of partial body exposure because of the different proportions of bone marrow exposed and the problems of assessing average marrow doses. Additionally some regimes, notably those for cervical cancer, involved mean doses of 8 Gy and this will have resulted in cell killing which will have influenced the dose-response.

This was highlighted in a more recent study in which the cervical cancer group was compared to a group of women who had received radiotherapy for benign gynaecological disease (Kleinerman *et al.* 1994). Despite a ten-fold difference in dose the rates of stable chromosome aberrations were similar in the two groups. The very high doses received by the haemopoietic stem cells in the radiation field for cervical cancer therapy will, in the main, have been cell lethal and thus will not have contributed to the late effects. The two groups of women also had comparable leukaemia risks. Such studies, together with the earlier work on ankylosing spondylitis patients who received radiotherapy to the spine (Buckton 1983), have therefore indicated that dose-related increases in stable chromosome aberrations can be detected many years after exposure although because of the nature of the exposure they may be more appropriate as biomarkers of effective risk rather than of total radiation dose received.

The survivors of the Japanese A-bombs, however, received relatively homogeneous whole body exposures so that all haemopoietic cells and organs will have received approximately the same dose. The most comprehensive data on stable aberrations comes from block-stained analysis of blood samples collected and cultured between 1968 and 1980 from 788 individuals in Hiroshima and 381 in Nagasaki (Awa *et al.* 1988, Awa 1991). Using the DS86 kerma doses (Fry and Sinclair 1987) the dose-response for cells containing at least one stable aberration, applying a linear fit, was $5.6 \pm 0.3 \times 10^{-2}$ /cell/Sv for Hiroshima survivors and $4.0 \pm 0.4 \times 10^{-2}$ /cell/Sv for Nagasaki (Awa *et al.* 1988, Awa 1991). The difference between the two cities has been attributed to the different proportions of neutrons contributing to the radiation dose (Straume *et al.* 1992, Stram *et al.* 1993) and it also appears that some doses in Nagasaki may have been overestimated (Preston *et al.* 1997). More

recently Ohtaki (1992) has reported dose-response data from a G-banded analysis of 63 Hiroshima survivors. Examination of aberrant cells revealed a slope of $15.2 \times 10^{-2}/\text{cell}/\text{Sv}$ for cells carrying stable aberrations. Ohtaki (1992) also presented data on different aberration types. Reciprocal translocations predominated with a dose-response of $11.7 \times 10^{-2}/\text{cell}/\text{Sv}$, insertions contributed a further $0.26 \times 10^{-2}/\text{cell}/\text{Sv}$, complex translocations $0.48 \times 10^{-2}/\text{cell}/\text{Sv}$ and complex exchanges $0.52 \times 10^{-2}/\text{cell}/\text{Sv}$ bringing the total for stable interchange aberrations to approximately $13 \times 10^{-2}/\text{cell}/\text{Sv}$. Including complex rearrangements, inversions and terminal and interstitial deletions the dose-response for all aberrations is $18.98 \times 10^{-2}/\text{cell}/\text{Sv}$. These data contrast with the earlier data using conventional analysis described above which indicate about a factor of 3 lower dose-response for stable cells. However, in a parallel study using conventional analysis the frequencies of stable cells were only 70% of those determined by G-banding. Comparative studies on a few A-bomb survivors indicate that aberration frequencies determined by G-banding and FISH are similar (Lucas *et al.* 1992, Awa 1997) but as yet no comprehensive dose-response data using FISH have been reported.

The importance of accurate characterization of chromosome aberrations, particularly for making comparisons of data obtained using different techniques, is highlighted in a recent study of a small group of Chernobyl liquidators (Pilanskaya 1996) which utilized conventional staining, G-banding and FISH. The frequency of atypical monocentric chromosomes which for G-banding included translocations, inversions, deletions and duplications was 15 times greater than for abnormal monocentrics determined by conventional staining. In one individual with an estimated dose of 4.5 Gy the frequency of abnormal monocentrics by conventional analysis was $2 \times 10^{-2}/\text{cell}$, compared with a frequency for terminal and interstitial deletions of $32 \times 10^{-2}/\text{cell}$ and for reciprocal translocations and inversions of $28 \times 10^{-2}/\text{cell}$, giving a combined frequency for abnormal monocentrics of $60 \times 10^{-2}/\text{cell}$. In contrast the FISH analysis which predominantly identified translocations revealed a frequency of $45.9 \times 10^{-2}/\text{cell}$. The authors suggest that the discrepancy between the G-banding and FISH data could be due to the inclusion of some dicentrics with translocations in the FISH analysis and the categorization of small translocations as deletions in the G-banding analysis. Indeed, the latter seems quite probable since it is acknowledged that most of the terminal and interstitial deletions had no accompanying acentric fragment. Such misclassification is very unlikely to have

happened in the study of Sellafield workers since for the FISH analysis, although a centromeric probe was not applied, only cells containing chromosomes with well defined centromeres were analysed (Tucker *et al.* 1997) and in the G-banding study reported here it is notable that the frequencies of acentrics (which comprised terminal and interstitial deletions) were not raised in the radiation workers.

In this data analysis the complex aberrations were broken down into equivalent numbers of translocations and insertions (Savage 1975). Our analysis criteria should allow direct comparison of the G-banding data with the previously reported FISH data (Tucker *et al.* 1997). The smoking adjusted dose-response for stable aberrations of $0.55 \pm 0.31 \times 10^{-2}/\text{cell}/\text{Sv}$ is lower than the value of $0.79 \pm 0.22 \times 10^{-2}/\text{cell}/\text{Sv}$ obtained from the FISH analysis but the two are statistically compatible. Direct comparison of G-banding and FISH aberration data on the 67 men for whom both staining techniques were applied (table 3) gives a correlation coefficient of 0.25 ($p=0.041$) and smoking adjusted dose-responses from FISH and G-banding of $0.45 \pm 0.24 \times 10^{-2}/\text{cell}/\text{Sv}$ and $0.66 \pm 0.41 \times 10^{-2}/\text{cell}/\text{Sv}$ respectively.

The report of the FISH analysis highlighted the much lower dose-response in these occupationally exposed workers in comparison to those obtained from conventional and G-banded studies of the Japanese A-bomb survivors (Awa *et al.* 1988, Awa 1991, Ohtaki 1992) and this decrease is emphasised by the lower value found in the G-banding study. In view of the importance attached to the dose-response data from the epidemiological studies on malignancy conducted on the Japanese A-bomb survivors and its use in extrapolating to low dose risks it is of prime importance that the differences in response observed for the induction of chromosome aberrations in a range of radiation-exposed populations be further examined.

The use of G-banding allowed analysis of the distribution of breakpoints in the chromosome aberrations. In the radiation workers no deviations from expectations based on length attributable to radiation were detected when the symmetrical aberrations and asymmetrical aberrations plus acentrics were considered separately, but combining the data revealed an excess of breakpoints in C group chromosomes and a deviation in the F group (table 5). A considerable number of reports have been published on the distribution of breakpoints involved in radiation-induced chromosome aberrations, but this review will concentrate on *in vivo* irradiation of human lymphocytes. In an early study using Q-banding, San Roman and Bobrow (1973) found an increased number of breaks

in chromosome 3 and a deficit in chromosome 16 in lymphocytes from patients treated with radioactive isotopes. Buckton (1978) found a difference in the distribution of breakpoints in ankylosing spondylitis patients studied three months and four years after radiotherapy. At three months an excess of breaks was found in chromosomes 12 and 17 and a deficit in chromosomes 1, 2, 3 and 19 but after four years chromosomes 9, 10 and 11 had an excess and chromosomes 3, 19 and 22 a deficit. In a later report Buckton (1983) confirmed a deficit in the A, B and F group chromosomes and an excess in the C (particularly chromosomes 9 and 10) and also noted an excess in the D group chromosomes. A deficit of breaks in chromosomes 1 and 2 was also observed in the study by Tanaka *et al.* (1983) of the Japanese A-bomb survivors together with a significant excess of breaks in chromosomes 15, 18 and 22 and a deficit in the X chromosome. In contrast, three studies have found an excess of breaks in chromosome 1. In a further study of the A-bomb survivors Lucas *et al.* (1992) noted an excess in chromosome 1 and in a study of patients receiving radiotherapy for a range of malignancies Barrios *et al.* (1989) observed an excess in chromosomes 1, 3 and 7 and a deficit in the D and G group chromosomes shortly after radiotherapy. Chromosome analysis on a man accidentally exposed 25 years previously revealed chromosomes 1 and 11 to be involved in aberrations more than expected based on relative chromosome lengths (Maes *et al.* 1993). A recent report from this laboratory (Whitehouse *et al.* 1998) examined the chromosome breakpoints in occupational radiation workers with intakes of plutonium and found the distribution according to chromosome lengths to be random amongst the Denver chromosome groups thus confirming an earlier study (Tawn *et al.* 1985). The plutonium workers had also been exposed to external radiation and so a group of workers with little or no plutonium intakes but similar histories of external, primarily gamma, radiation exposure was also studied. Overall the breakpoints in the chromosome aberrations identified in this externally exposed group were randomly distributed but when asymmetrical aberrations plus acentrics were considered as a separate group an excess of aberrations involving the A group chromosomes was observed. This excess was evenly distributed amongst all three A group chromosomes, i.e. 1, 2 and 3. The range in chromosome breakpoint distributions reported to date, therefore, make it difficult to draw reliable conclusions on any preferential involvement or exclusion of particular chromosomes in radiation-induced aberrations.

The distribution of breakpoints within the chromosomes was concentrated in the terminal regions in

the radiation workers in the present study (table 6). This has been a more consistent finding following *in vivo* irradiation being observed in radiotherapy patients (San Roman and Bobrow 1973, Barrios *et al.* 1989), A-bomb survivors (Tanaka *et al.* 1983) and occupational radiation workers with intakes of plutonium (Tawn *et al.* 1985, Whitehouse *et al.* 1998). However in the ankylosing spondylitis patients studied by Buckton (1978) the breakpoints were concentrated at the centromeres and in the occupationally exposed group with external irradiation studied in parallel with plutonium workers (Whitehouse *et al.* 1998) the breakpoints were found to be randomly distributed within the chromosome regions.

Because the dataset in the present study is relatively small, distribution analysis has been confined to the Denver groupings, although individual data is presented in figure 2. The application of G-banding for aberration frequency analysis allows the identification of breakpoints in individual chromosomes and whereas the data in any particular study is sparse, the presentation of this information should eventually result in any patterns of chromosome involvement being identified.

This is the final report of a multi-endpoint study of current radiation workers with cumulative lifetime doses in excess of 500 mSv. No significant correlation with dose was found for the two gene mutation assays, HPRT mutants in T lymphocytes (Cole *et al.* 1995) and GPA mutants in erythrocytes (Tucker *et al.* 1997), although a weak positive correlation with dose was found in the GPA study. However, the two cytogenetic studies, using different techniques to study chromosome aberrations in peripheral blood lymphocytes, both found a significant positive dose-response for stable chromosome aberrations, suggesting that these may be a more sensitive indicator of low dose occupational exposure to radiation. Chromosome analysis of radiation exposed populations, using techniques to identify stable aberrations, offers the opportunity to examine the relative effectiveness of different exposure conditions and is of particular relevance since chromosome rearrangements are of prime importance in the process of carcinogenesis. Such studies should therefore aid the understanding of the risks associated with radiation exposure.

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