PROCEEDINGS OF SCIENCE

Relative Biological Efficiency of 192 MeV Neutron Radiation for the Induction of Chromosome Aberrations in Human Lymphocytes of the Peripheral Blood

R. Nolte*, V. Dangendorf
Physikalisch-Technische Bundesanstalt
38116 Braunschweig, Germany
E-mail: ralf.nolte@ptb.de, volker.dangendorf@ptb.de

A. Buffler, F.D. Brooks
University of Cape Town
Rondebosch 7701, South Africa
E-mail: abuffler@science.uct.ac.za, brooks@science.uct.ac.za

J.P. Slabbert, F.D. Smit
iThemba Laboratory for Accelerator Based Sciences
Somerset West 7129, South Africa
E-mail: jps@tlabs.ac.za, smit@tlabs.ac.za

M. Haney
Institute of Radiobiology, GSF-National Research Center for Environment and Health
85764 Neuherberg, Germany
E-mail: mhaney@gsf.de

E. Schmid
Radiobiological Institute, University of Munich
80336 Munich, Germany
E-mail: eschmid@bfs.de

G. Stephan
Federal Office for Radiation Protection
85764 Oberschleissheim, Germany,
E-mail: gstephan@bfs.de

* Speaker

PoS(FNDA2006)082

The production of dicentric chromosomes in human lymphocytes by high-energy neutron radiation was studied at the neutron beam facility of iThemba Laboratory using a quasi-monoenergetic 192 MeV neutron beam. The linear yield coefficient of the linear dose-response relationship for dicentric chromosomes was measured to be (0.096 ± 0.020) Gy^{-1}. This confirms our earlier observations that the yield of dicentric chromosomes decreases with increasing neutron energy above 400 keV and is almost constant above 20 MeV. Using the linear-quadratic dose-response relationship for dicentric chromosomes established in blood of the same donor for ^{60}Co γ-rays as reference radiation, an average maximum low-dose RBE (RBE_M) of 9 ± 3 for 192 MeV quasi-monoenergetic neutrons, with a dose-weighted average energy of 162.1 MeV, was obtained. The yield of chromosome aberrations due to neutrons from the low-energy breakup continuum of the quasi-monoenergetic neutron spectrum was determined by an additional measurement at a neutron emission angle of 16° where the high-energy peak is strongly suppressed. Using this technique, a yield coefficient $\alpha = (0.121 \pm 0.026)$ Gy^{-1} and an RBE_M of 12 ± 4 for virtually monoenergtic 192 MeV neutrons was determined. The increased uncertainties result from the application of the (0°-16°) difference method.

International Workshop on Fast Neutron Detectors
University of Cape Town, South Africa
April 3 – 6, 2006

1. Introduction

The spectral fluence of neutrons encountered behind the shielding of high-energy accelerators or at flight altitudes of civilian passenger aircraft exhibits distinct maxima at about 1 MeV and 100 MeV. To assess the radiation risk of personnel exposed to such radiation fields, data on the relative biological effectiveness at low dose (RBE_M) for high-energy neutrons are required. Among the various biological endpoints, the induction of dicentric chromsomes by ionising radiation is regarded as a representative measure for the radiation risk. A comprehensive set of RBE_M data for this endpoint is available for neutron energies below 14.8 MeV [1] which was recently extended to 60 MeV quasi-monoenergetic neutrons [2]. In the present contribution, another measurement of RBE_M for 192 MeV quasi-monoenergetic neutrons is reported. As for the earlier experiments, blood from the same donor was used for the present measurement to obtain a consistent data set for the full range of neutron energies.

2. Irradiation and analysis of the blood samples

2.1 General procedure

For the experiments, fresh peripheral blood was drawn from the same healthy male donor, as already used in our previous irradiation experiments with monoenergetic neutrons with energies between 0.036 MeV and 14.6 MeV [1], quasi-monoenergetic 60 MeV neutrons [2] and with ^{60}Co γ-rays [2,3] as reference radiation. The neutron irradiation was carried out at the neutron beam facility [4] of the iThemba Laboratory for Accelerator Based Sciences (iTL) in Somerset West, South Africa using a quasi-monoenergetic neutron beam with a peak neutron energy of 192 MeV. The blood samples were kept at room temperature (i.e. 20-22°C) during the exposure to neutrons. This irradiation temperature was determined by the experimental conditions in the neutron beam facility. For comparison, a control sample of blood was kept unirradiated at room temperature for the same time period.

2.2 Characterisation of the neutron beams

The quasi-monoenergetic neutron beam was produced by 200 MeV protons incident on a ^{9}Be target 13 mm in thickness. An iron collimator wall with quadratic $5\ \text{cm} \times 5\ \text{cm}$ openings was used to provide neutrons beams at emission angles of 0° and 16°. Fig. 1 shows a schematic layout of the experiment. The spectral fluence distribution of the 0° beam exhibits a prominent high-energy peak resulting from the direct reaction ^{9}Be(p,n)^{9}B and a continuum produced by breakup reactions. The high-energy peak is strongly suppressed in the spectral fluence of the 16° beam, while the phase space distribution of the breakup continuum is not very different from that at 0°. Hence this beam can be used to experimentally determine the biological effect of the continuum neutrons by a difference measurement [5] instead of carrying out a calculational correction as for the earlier 60 MeV experiment [2].

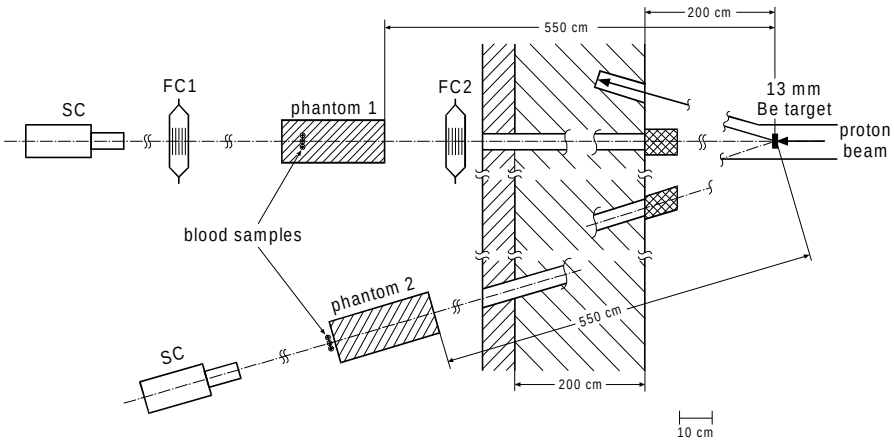

Fig. 1 Layout of the experimental set-up at the iTL neutron beam facility. The spectral fluence per monitor count (Φ_E/M) was measured with the scintillation detector and the ^{238}U fission ionisation chamber FC1 . The ^{238}U ionisation chamber FC2 served as a transmission monitor to relate the measurements for the beam specification and the irradiation of the blood samples. Graphite blocks 10 cm in thickness were used to prevent any stray protons from reaching the collimator openings. At 0°, the blood samples were positioned inside the lucite phantom behind a buildup layer, 24.5 cm in thickness. At 16°, the samples were positioned behind the phantom, 31.6 cm in thickness.

As in the experiment carried out at 60 MeV [2], the beams were characterised by time-of-flight (TOF) spectrometry using a fast liquid scintillation detector and a ^{238}U fission ionisation chamber. The combination of the measurements with these two detectors allows the spectral fluence Φ_E to be determined with high energy resolution and at the same proton beam focusing conditions as for the actual blood irradiations. The analysis showed that at the beam currents used for the blood irradiations, the 0° low-energy continuum had an additional contribution resulting from parasitic neutrons produced by protons hitting structures in front of the Be target. For the 16° beam, the collimator prevented these neutrons from reaching the measurement or irradiation position. The low-energy cutoff of the TOF measurements was 5 MeV. Below this energy a horizontal extrapolation of the spectral fluence was used.

The beam charge Q and the number of counts M in the ^{238}U transmission ionisation chamber positioned permanently in the 0° beam were used as monitors to relate the measurement of the spectral neutron fluence Φ_E to the irradiation of the blood samples. Fig. 2 shows the spectral fluences per monitor count (Φ_E/M) at a distance of 8 m from the Be target. The high-energy peak ($E_n \geq 182$ MeV) in the 0° spectral fluence comprises about 38% of the total fluence Φ while for the 16° beam only 5% of the total fluence are contained in this energy region. The kerma-weighted average energies of the 0° and the 16° spectral fluences are 162.1 MeV and 142.6 MeV, respectively. In addition to the 0° and 16° spectral fluences, the difference spectrum $(\Phi_E/M)^{(0°\text{-}16°)} = (\Phi_E/M)^{(0°)} - 0.908\cdot(\Phi_E/M)^{(16°)}$ is shown in Fig. 2. For this spectrum, the total fluence below $E_1 = 182$ MeV vanishes. The relative uncertainty of the total fluence per monitor count (Φ/M) is about 15%, including the estimated uncertainty of the ^{238}U(n,f) reference cross section [6].

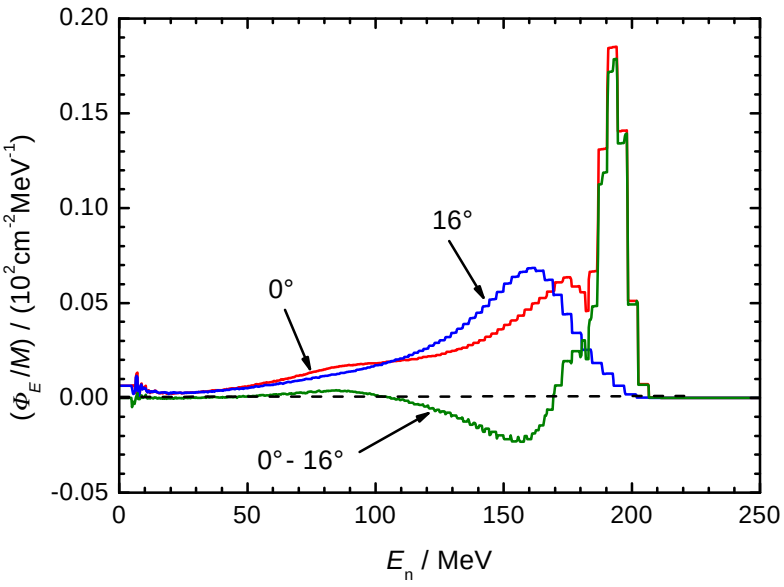

Fig. 2 Spectral fluence per unit monitor count (Φ_E/M) of the neutron beams used for the present experiment. The beams were produced by 200 MeV protons incident on a 13 mm Be target. The (0°-16°) spectral fluence is calculated from the spectral fluences of the beams produced under neutron emission angles of 0° and 16°: $(\Phi_E/M)^{(0°\text{-}16°)} = (\Phi_E/M)^{(0°)} - 0.908 \cdot (\Phi_E/M)^{(16°)}$.

2.3 Irradiation of the blood samples and determination of the energy dose

The irradiations of the blood samples were carried out in two lucite mini-phantoms with a cross sectional area of 13 cm × 13 cm. The phantoms were positioned at a distance of 5.5 m from the Be target under neutron emission angles of 0° and 16°. The shape of the neutron beam profile at the front face of the phantom was quadratic with a full width at half maximum (FWHM) of 6.7 cm. The buildup layer in front of the blood samples had a thickness of 24.5 cm and 31.6 cm for the 0° and 16° irradiations, respectively. The range of 200 MeV recoil protons in lucite is 21.8 cm, i.e. the irradiations were carried out under conditions close to charged particle equilibrium in forward direction. Three blood samples, 2 cm^3 in volume, were always irradiated simultaneously in each of the two phantoms. The absorbed dose rate (dD/dt) in the blood samples at 0° was about 10.3 mGy/h. The total time of 45 h available for the irradiation was divided in intervals chosen so that five samples received about 20 %, 40 %, 60 %, 80 % and 100 % of the total dose, respectively.

The absorbed dose to human blood [7] per unit neutron fluence (D/Φ) at the front face of the phantom was calculated using the Monte Carlo radiation transport code MCNPX, version 2.5.e [8] using the spectral fluences (Φ_E/M) shown in Fig. 2. For neutron energies up to 150 MeV, cross sections from the LA150N library were used. Above this energy, the default nuclear reaction models implemented in MCNPX were employed. All secondary charged particles included in the data tables and models were transported until their energy reached their

respective default cutoff energies. The remaining kinetic energy released in a nuclear reaction was deposited in kerma approximation. As for the 60 MeV experiment [2] double counting of recoil protons produced by neutrons with energies below 150 MeV had to be avoided. This was accomplished by separately calculating the kerma resulting from recoil protons using the calculated spectral fluence in the samples and the hydrogen kerma factor. This partial dose was then subtracted from the residual dose caused by recoil nuclei and heavier fragments ($A > 4$) which were not transported by MCNPX and which included also the contribution from recoil protons produced by neutrons with energies below 150 MeV. The absorbed dose in the central blood sample per neutron fluence at the phantom surface (D/Φ) was 65.4 pGy cm^2, 56.4 pGy cm^2 and 70.4 pGy cm^2 for the 0° spectrum, the 16° spectrum and the (0°-16°) difference spectrum, respectively. The relative uncertainty of the conversion coefficients (D/Φ) was determined from a comparison of results obtained at 150 MeV using the LA150N library and the default nuclear models and is estimated to be about 14%. The absorbed dose D_i received by sample i is given by $D_i = (D/\Phi)\cdot(\Phi/M)\cdot M_i$ where M_i denotes the number of monitor counts during the irradiation of sample i. The uncertainties of the dose values were correlated to almost 100% for all irradiated samples. The only uncorrelated contributions were the uncertainties of the numbers of monitor counts M_i which amounted to about 2% while the total relative uncertainties of the absorbed dose values D_i were about 20%

2.4 Blood culture conditions and chromosome analysis

Immediately after irradiation, cultures were established according to our standard procedure which was earlier described in detail [1,2]. Briefly, cultures contained 0.5 ml whole blood, 4.5 ml RPMI 1640 medium supplemented with 15% fetal calf serum, 1% glutamine, antibiotics and 2.5% phytohemagglutinin (PHA). Colcemid (0.03 μg/ml) was present during the entire incubation period of 48-52 hours. These culture conditions ensured that the chromosome analysis could be performed exclusively in complete metaphases of the first cell cycle in vitro. Chromosome preparation and Giemsa staining were carried out according to our earlier experiments [1-3]. All slides were coded. Although the frequencies of dicentrics, centric rings and supernumerary acentric fragments were determined, in the present study only the data for dicentric chromosomes were used in the quantitative analysis. During this particular experiment, however, it turned out that the PHA charge available at iTL to stimulate the division of the lymphocytes was of suboptimum quality. Hence, the total number of cells which could be inspected for chromosomal aberrations was significantly reduced compared to the earlier experiments which increased the statistical uncertainties.

3. Results

As for the earlier measurements, ^{60}Co γ-radiation was used as a reference. The data used to establish the reference yield curve $y = c + \alpha_{ref}\cdot D + \beta_{ref}\cdot D^2$ are reported in [2]. Here y denotes the number of dicentric chromosomes per cell and $c = (3.33\pm1.67)\cdot10^{-4}$ is the constant background observed in the absence of radiation. The linear yield coefficient α_{ref} was (0.0106±0.003) Gy^{-1}

[2]. The reference yield curve was determined at a dose rate of about 15 mGy min^{-1}. Table 1 shows the distribution of dicentric chromosomes on cells observed using the 0° and the 16° beams. In most samples a significant over-dispersion is noted. This is consistent with the high-LET nature of the radiation applied.

Table 1 Intercellular distribution of dicentric chromosomes in human lymphocytes induced by quasi-monoenergetic 192 MeV neutrons at the 0° and 16° irradiation positions. The asterix denotes the background frequency from the same donor examined earlier. The mean value and the variance of the distribution of dicentric chromosomes are deoted by y and σ^2, respectively. The u-value is the test quantity of the statistical test of Rao and Chakravati [9].

0° irradiation position							
Dose (Gy)	Analysed cells	Dicentrics per cell	Intercellular distribution of dicentrics				
			0	1	2	σ^2 / y	u-value
0*	15000	0.00027	14996	4	-	1.0	0
0	3000	0.00033	2999	1	-	1.0	0
0.115	1200	0.010	1188	11	1	1.147	3.75
0.176	1100	0.019	1081	17	2	1.172	4.12
0.265	1000	0.024	978	20	2	1.144	3.20
0.346	1000	0.038	966	30	4	1.173	3.92
0.439	1000	0.049	955	41	4	1.115	2.60

16° irradiation position							
Dose (Gy)	Analysed cells	Dicentrics per cell	Intercellular distribution of dicentrics				
			0	1	2	σ^2 / y	u-value
0	3000	0.00033	2999	1	-	1.0	0
0.0429	1550	0.0052	1542	8		0.99	-0.36
0.0659	1160	0.0095	1149	11		0.99	-0.25
0.0994	1080	0.0139	1066	13	1	1.12	2.89
0.1287	870	0.0195	855	13	2	1.22	4.73
0.1677	630	0.0222	616	14		0.98	-0.37

The 0° and 16° yield curves are shown in Fig. 3 together with the yield curve for the (0°-16°) difference spectrum. The yield curve for this spectral fluence was calculated according to $y^{(0°\text{-}16°)} = (y^{(0°)}\text{-}c)\text{-}0.908\cdot1.142\cdot(y^{(16°)}\text{-}c)$. The additional normalization factor of 1.142 was determined from the results of the MCNPX simulations and accounts for the larger distance of the blood samples to the Be target at 16° compared to that at 0°.

Linear functions $y = \alpha \cdot D$ were fitted to the yield data $y_i^{(0°)}$-c, $y_i^{(16°)}$-c and $y_i^{(0°\text{-}16°)}$. Reciprocals of the estimated variances were used as weights. From the linear yield coefficient α, the relative biological efficiency at low dose $\text{RBE}_M = \alpha/\alpha_{ref}$ was calculated.

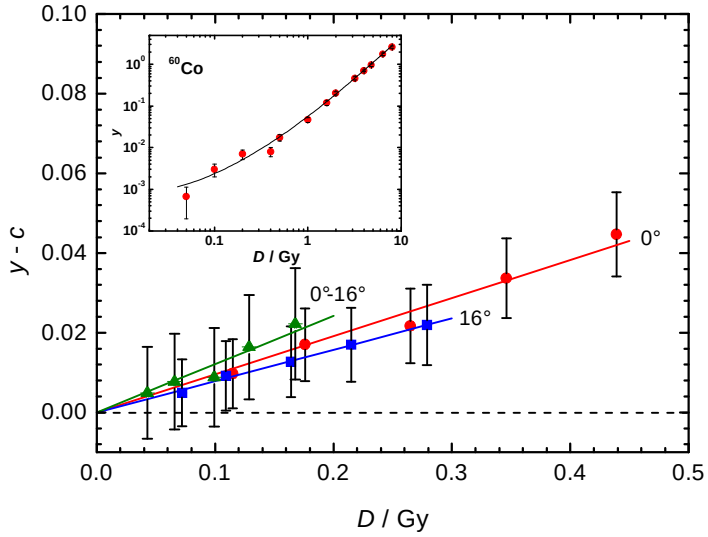

Fig. 3 Yield y of dicentric chromosomes observed for the 0° (circles) and 16° (squares) beams and the (0°-16°) difference spectrum (up triangles). Linear functions $y = \alpha \cdot D$ were fitted to the experimental data. The insert shows the yield curve for ^{60}Co γ-radiation which was used as reference.

Table 2 shows the linear yield coefficients and their type-A and type-B uncertainties as well as the resulting RBE_M values with their total uncertainties. The total uncertainties of the RBE_M values do not include the uncertainty of the linear yield coefficient α_{ref} for the reference radiation.

Table 2 Linear yield coefficients α for the irradiations at 0° and 16° as well as for the (0°-16°) difference spectrum. The type-A and type-B uncertainties $u_\alpha^{(A)}$ and $u_\alpha^{(B)}$ of α and the total uncertainties u_{RBE} of the RBE_M values are indicated for a coverage factor $k = 1$. The linear yield coefficient for ^{60}Co γ-radiation is $\alpha_{ref} = (0.0106 \pm 0.003)$ Gy^{-1}.

	α / Gy^{-1}	$u_\alpha^{(A)}$ / Gy^{-1}	$u_\alpha^{(B)}$ / Gy^{-1}	RBE_M	u_{RBE}
0°	0.096	0.004	0.020	9.0	2.0
16°	0.0787	0.0014	0.016		
0°-16°	0.121	0.008	0.025	11.4	2.5

Fig. 4 shows the present data together with the results of the earlier measurements carried out at the PTB accelerator facility [1] and in UCL cyclotron centre [2].

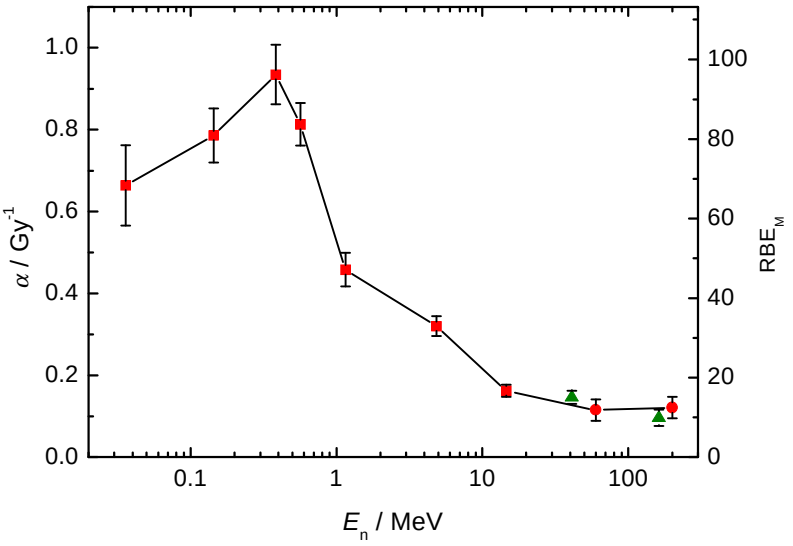

Fig. 4 Linear yield coefficient α and relative biological efficiency RBE_M at low dose for the induction of dicentric chromosomes. The squares show data measured at the PTB accelerator facility using monoenergetic neutrons [1] while the up-triangles indicate results obtained using quasi-monoeneregtic neutron beams. The quasi-monoenergetic data are shown at the dose-weighted mean energies. The circles show the quasi-monoenergetic data with a correction for the effect of the neutrons from the low-energy continua. The data points around 60 MeV were obtained at the UCL neutron beam facility [2]. The data points around 200 MeV show the present data.

4. Discussion

The result of the present measurement supports the observation from the earlier measurement at 60 MeV [2] that the RBE_M for the production of dicentric chromosomes by fast neutrons becomes almost energy independent above neutron energies of 20 MeV. This is already evident from Fig. 3 where the slope of the yield curve for the low-energy continuum is close to that of the full quasi-monoenergetic spectral fluence. In our recent publication [2], the results at 60 MeV were compared in detail with A. Heimers' results [10] who found RBE_M values between 47 and 113 for a radiation field which simulated the radiation environment at flight altitudes. These arguments are also applicable to the present measurements, since our results for 192 MeV quasi-monoenergetic neutrons show almost the same RBE_M value as found earlier for 60 MeV neutrons [2]. Here only one important difference between Heimers' work [10] and the present study should be emphasised once more. Due to the higher number and magnitude of different dose values employed in the present study, the statistical uncertainty of the number of observed dicentric chromosomes is sufficiently small that the slope of the dose-effect curve can be determined reliably while in Heimers' study the maximum number of detected chromosomes per sample was only 6. Therefore the ratio of the linear coefficients of the dose-effect curves for high-energy neutrons and ^{60}Co γ-radiation could be used in the present work to determine the maximum relative biological efficiency RBE_M while in Heimers' work

relative biological efficiencies could only be obtained for two individual dose values. Even with the increased uncertainty of the present data, the results for quasi-monoenergetic 192 MeV neutrons differ clearly from reported RBE_M values of up to 113 for simulated neutron spectra at flight altitudes [10].

Acknowledgment

The authors are grateful for the support received from the staff of PTB and iTL during preparation and carrying out of the experiment.

References

[1] E. Schmid, D. Schlegel, S. Guldbakke, R.P. Kapsch, D. Regulla, *RBE of nearly monoenergetic neutrons at energies of 36 keV-14.6 MeV for the induction of dicentrics in human lymphocytes*, Radiat. Environ. Biophys. 42 (2003) 87, and references cited therein

[2] R. Nolte, K.-H. Mühlbradt, J.P. Meulders, G. Stephan, M. Haney, E. Schmid, *RBE of quasi-monoenergetic 60 MeV neutron radiation for induction of dicentric chromosomes in human lymphocytes*, Radiat. Environ. Biophys. 44 (2005) 201

[3] M. Bauchinger, E. Schmid, S. Streng, J. Dresp, *Quantitative analysis of the chromosome damage at first division of human lymphocytes after ^{60}Co γ-irradiation*, Radiat. Environ. Biophys. 22 (1983) 225

[4] W.R. McMurray, D.G. Ashman, K. Baruth-Ram, R.W. Fearick, *The Faure cyclotron neutron source and a particle spectrometer for neutron induced emission of charged particles at energies between 60 MeV and 200 MeV*, Nucl. Instr. and Meth. A 329 (1993) 217

[5] R. Nolte, M.S. Allie, P.J. Binns, F. Brooks, A. Buffler, V. Dangendorf, J.P. Meulders, F. Roos, H. Schuhmacher, B. Wiegel, *High-energy neutron reference fields for the calibration of detectors used in neutron spectrometry*, Nucl. Instr. and Meth. A 476 (2002) 369

[6] A.D. Carlson, S. Chiba, F.J. Hambsch, N. Olsson, A.N. Smirnov, *Update to nuclear data standards for nuclear measurements*, Summary report of a consultants' meeting held in Vienna, Austria, 2 to 6 December 1996, ed. H. Wienke, Report INDC(NDS)-368, International Atomic Energy Agency (1997) Vienna

[7] ICRU, *Tissue substitutes in radiation dosimetry and measurement*, ICRU Report 44 (1989), Bethesda

[8] J.S. Hendricks, G.W. McKinney, L.S. Waters, T.L. Roberts, H.W. Egdorf, J.P. Finch, H.R. Trellue, E.J. Pitcher, D.R. Majo, M.T. Swinhoe, S.J. Tobin, F.X. Gallmeier, J.-C. David, W.B. Hamilton, J. Lebenhaft, *MCNPX, version 2.5.e*, Los Alamos National Laboratory report LA-UR-04-0569 (2004) Los Alamos

[9] C.R. Rao, I.M. Chakravati, *Some small sample test of significance for a Poisson distribution*, Biometrics 12 (1956) 264-282

[10] A. Heimers, *Cytogenetic analysis in human lymphocytes after exposure to simulated cosmic radiation which reflects the inflight radiation environment*, Int. J. Radiat. Biol. 75 (1999) 691