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Emergency preparedness exercise for biological dosimetry - BIOPEX (2008)

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Abstract

As a continuation to the NKS-funded BIODOS project, the BIOPEX project has aimed at testing and validation of the newly established dose calibration curve for PCC rings, a specific chromosome aberration for use in biodosimetry in large casualty emergency preparedness. The testing of the PCC ring technique was performed by direct comparison to the conventional dicentric assay, both conducted with a triage approach that gives a crude dose estimate through analysis of a relatively small number of cells. Altogether 62 blood samples were analysed, each irradiated with an individual dose using γ-rays, and representing casualties in a simulated radiation accident resulting in a broad spectrum of whole body and partial body doses, ranging from zero dose up to a lethal whole body dose of 13 Gy. The results indicated that both triage assays were capable of discerning non-exposed cases and that in the uniform irradiations, the dose estimates based on data from both assays were fairly consistent with the given dose. However, differences were observed depending on the dose level. At doses about 5 Gy and below, dicentric scoring resulted in more accurate whole-body dose estimates than PCC rings. At very high doses, PCC rings appeared to give more accurate dose estimates than dicentrics. The discrepancies are mainly caused by shortcomings in the respective dose calibration curves. In non-uniform irradiations, the PCC ring assay was slightly better in the approximation of the partial body dose than dicentrics, but neither assay enabled accurate estimation of either dose or fraction of cells irradiated. The irradiated fraction of cells for the casualties in this scenario was apparently too small (10-40%) to be distinguished with the triage approach applied in the current study. With respect to the technical aspects, scoring of the PCC rings is easier and therefore somewhat faster but may be more sensitive to quality aspects. In conclusion, the study demonstrated that the PCC ring assay is suitable for use as a biodosimeter, especially for estimation of very high doses.

Key words

Biological dosimetry, PCC assay, dicentrics, triage, mass casualty radiological accident

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Final report

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1. Introduction

In the event of radiological accidents, a number of dose assessment techniques are available. Biological dosimetry provides an approach to determine the quantity of radiation exposure on personal level. When the number of casualties is large, there is a need for a method that allows for a fast and reliable dose evaluation that may be crucial for early decision of medical care. Biodosimetry also provides information on inhomogeneous exposure thus helping to recognize patients to develop severe local reactions. The classical dicentric method is generally considered the best of the biodosimetry methods, but it is relatively time-consuming and requires adequate training for successful determination of dose. Also, very high doses cannot be accurately assessed. Several biological techniques have challenged the dicentric method, one of them the PCC method, which is based on the analysis of cells where chromatin has been prematurely condensed with the help of either chemicals or fusion with mitotic cells from another species. Premature chromosome condensation is closely regulated by the maturation/mitosis promoting factor (MPF), originating either from an external source (mitotic cells) in the fusion techniques, or from the cell itself in methods applying chemical induction (Gotoh and Durante 2006).

The PCC technique was proposed for biological dosimetry purposes by Pantelias and Maillie (1984). In the original version, the technique was based on fusion of interphase lymphocytes with mitotic Chinese hamster ovary cells resulting in condensation of chromatin into chromosomes in the resting cells. The analysis is based on the counting of excess chromosome fragments from Giemsa-stained preparations. This procedure includes a number of technical drawbacks, e.g. a decrease in sensitivity over time due to rejoining of radiation-induced breaks and the more demanding preparation and analysis of PCC cells than in the classical chromosome analysis. However, the PCC assay has important potential applications (IAEA Technical report 2001). In high-dose exposures, lymphopenia reduces the number of cells available for classical chromosome analysis. High doses also induce low mitotic index due to cell cycle delay. In the PCC assay, these difficulties are reduced by scoring of radiation-induced damage in cell samples where cell division is not required. In the recently developed chemical induction of PCCs, Okadaic acid- and Calyculin A-induced chromosome condensation of stimulated, proliferating cells has enabled the scoring of condensed chromosomes (Durante et al. 1998; Kanda et al. 1999) up to a dose of 20 Gy. The combination of FISH chromosome painting and the PCC assay has enabled the recognition of chromosome exchanges (Durante et al. 1996). To reduce the assay time, further development of the assay

for the induction of PCC in unstimulated cells was facilitated by cyclin B kinase (Prasanna et al. 2000).

The assay of chemically induced PCC cells is technically relatively easy and thus fulfils the requirements for an emergency method. In our previous NKS-project, BIODOS, the quality and quantity of PCC cells and the evaluation of different aberration types were assessed. The results demonstrated that okadaic acid induction and ring chromosome analysis provide the most promising PCC assay for the purpose of fast dose estimation of a large number of casualties encompassing a wide range of doses. Evaluation of rings that have clearly defined open spaces and those large spherical rings without open spaces provided the most reproducible results. The dose response data for rings were fitted into a linear model, although other more complicated models may also have been valid. Dose values at low dose range (1 Gy and to some extent 2.5 Gy) and above 15 Gy weakened the model considerably.

In the work performed during the BIOPEX project, the aim was to compare and validate the PCC ring assay with respect to the routinely applied dicentric assay. This was accomplished by simulating an accident scenario involving a large number of casualties each with an individual uniform or non-uniform irradiation. The scoring of PCC rings and dicentrics on the slides prepared from the individual blood samples was conducted in a triage mode, i.e. a small number of cells were analysed in order to obtain fast and crude dose estimation by both assays The purpose was also to evaluate the usefulness of obtained dose estimates for supporting the later radiological triage of the subjects and medical decisions about the level of hospital care needed. Further, the study was expected to provide valuable information on transportation, culturing and data processing of large number of samples assayed.

2. Material and methods

2.1. Planning of the accident scenario

For the purpose of testing the biodosimetry assays a scenario of malevolent use of radiation was simulated by in vitro irradiation of blood samples. The simulation of doses was planned independently, the only information given to the designer was the number of samples that can be managed, the dose range of the PCC dose calibration curve and that both whole body and partial body exposures can be included. The scenario was based on the assumption that a very strong gamma source had been hidden in a public transport vehicle, giving rise to a dose rate of 20 Gy/h close to the source and 2 Gy/h at 5 m distance. Close to the source, because of partial shielding it would be possible to get a high dose to only part of the body. Before dis-

closure of the source, hundreds of persons could potentially have been exposed. All persons who might have been exposed and who present with gastrointestinal symptoms indicating possible exposure need to be evaluated. Rapid decisions have to be made concerning which of the victims need hospital care and at which level. In this scenario, 62 persons with clinical symptoms and potential exposure were evaluated. According to the scenario, the actual exposures spanned a broad spectrum of doses. Seven persons were supposed not to have been exposed at all, despite presenting with symptoms, while in the simulation, 37 persons were exposed to whole body doses between 0.4 Gy and 13 Gy. 18 cases received partial body doses of between 7 Gy and 13 Gy to between 10% and 40% of the body. These exposures were simulated by exposure of fresh blood to gamma radiation with a dose rate of 0.3 Gy/min, as described below. To simulate partial body exposure, exposed blood was mixed with unexposed blood from the same person in corresponding proportions. The given dose and exposed fraction for each case appears form tables 3 - 6.

2.2 Irradiation of blood and shipment of samples

In all, 14 volunteers (6 males and 8 female) from among STUK employees were enrolled for donating blood. Blood samples, each containing 10 ml Li-heparin blood in a vacuum tube, were exposed to specific doses using Co-60 γ-rays with a dose rate of 0.3 Gy / min making use of the equipment and dosimetry expertise of the Radiation Metrology laboratory at STUK. Irradiations were performed in a water bath with a fixed temperature of 37 ºC. The dosimetry was calculated as dose in water and the total error of the dose as 4 %. After irradiation and blood mixing, the samples were kept for 2 h in 37 ºC to allow for repair of DNA damage. The irradiations and blood handling were performed by persons not involved in the analyses and samples were blind coded before culturing took place. Blood handling also included the processing of samples with "partial body" exposures, i.e. mixture of irradiated and non-irradiated blood in different proportions. Out of 62 irradiated samples, 36 were cultured at STUK, 26 samples were sent to FOI for culturing and both laboratories made slide preparations. NRPA received preparations of 11 samples (5 from STUK; 6 from FOI) for analysis and the remaining 31 and 20 at STUK and FOI, respectively.

For the shipment of blood samples, several courier companies were contacted to determine the best alternative for sending a large number of blood samples from STUK to FOI. Factors affecting the success of shipping blood samples were considered: speed of delivery, controlled temperature conditions and required documentation for smooth passage through cus-

toms. Since the courier companies could not provide guaranties for temperatures above 0ºC during transport, blood samples were packed in Styrofoam boxes provided with proper insulation and heated gel packs. The irradiation experiments were initiated after the transport issues were clarified. The irradiations were performed during three different occasions in March - April 2008, with a pace of approximately 20 samples / week. Due to unforeseen problems in the culture procedures at FOI, another two irradiation experiments, encompassing 25 different doses, were performed in May - June 2008 in order to repeat the FOI samples. Parallel backup cultures were processed at STUK from the 25 repeat samples.

2.3 Culture of samples and analysis

Procedures established during the BIODOS project were applied in the culturing and analysis of the current samples. Similar protocols were used at both FOI and STUK. Samples arrived at FOI on the following day after irradiation and shipment. To establish as similar conditions as possible for all samples, the samples to be processed at STUK were kept overnight at room temperature before further processing. Lymphocytes were isolated using either Histopaque or Lymphoprep. Cultures were established using RPMI 1640 medium with 20% FCS, 1% PHA, 1% L-glutamine and 1% penicillin-streptomycin. A lymphocyte density of at least 0.5 x 10^6 cells / ml in 5 ml cultures was required for successful cell growth. Parallel cultures for both dicentrics and PCC ring assays were established for all irradiated samples. For dicentric assay, colcemid (final concentration 0.2 μg/ml) was added for the last 2.5 h of the 48 h cultures. The PCC assay cultures obtained Okadaic acid (final concentration 500 nM) during the last 1 h of the 48 h cultures.

At FOI, STUK and NRPA, the analysis of dicentric samples were performed according the laboratory routine in use in the respective laboratories and the PCC ring analysis as established in the BIODOS project. From PCC preparations, the starting point was to analyse 50 rings or 500 PCC cells per sample, whereas 30 dicentrics or 50 metaphases were to be scored for the dicentric assay. The criteria for scoring dicentrics were considered applicable for triage analysis for estimating doses in samples receiving either uniform or non-uniform irradiation (Lloyd et al. 2000). The number of PCC rings or cells to be scored was approximated based on the proportion of rings formed in proportion to dicentrics and to achieve similar accuracy as for dicentrics. At STUK, the dicentric and PCC assays were facilitated by a metaphase finder. PCC cells in any cell cycle phase were scored and rings were analysed irrespective of the presence of a centromere.

2.4 Statistical analyses and dose estimation

The distribution of dicentrics and PCC rings were tested with Poisson test and the dose estimates performed using specific software (DoseEstimate, Health Protection Agency). Dose estimation was based on the PCC dose calibration curve established in the BIODOS project as well as the routinely used dicentric curves in the respective laboratories. For PCC rings, a linear relationship, $D= C + \alpha D$, was used for dose estimations whereas for dicentrics, the dose estimation was based on linear quadratic curve, $D= C + \alpha D + \beta D^2$. The values for the coefficients used for different assays and laboratories are given in Table 1.

For cases showing non-Poisson distribution of dicentrics or PCC rings, defined as a deviation in the variance / mean relationship and characterized as u-value > ± 1,96 (Papworth 1970), an attempt was made to estimate the partial body doses and the fraction of body exposed.

Table 1. Dose coefficients used for dose estimations

	control (± S.E.)	linear coefficient	quadratic coefficient
PCC rings	0,0018 ± 0,0001	0,0489 ± 0,001	
Dicentrics, STUK	0,0005 ± 0,00024	0,0135 ± 0,0043	0,054 ± 0,003
Dicentrics, FOI and NRPA	0,0016 ± 0,001	0,014 ± 0,006	0,065 ± 0,003

2.5 Dose categories

One of the original tasks of this project was to evaluate the effectiveness of dose estimation for both assays by placing each case into crude dose categories. In addition to the non-exposed category, the biological dose estimation was evaluated with respect to the following categories which are based on early symptoms after acute whole body exposure (see table 1): no exposure (0 Gy), >0 and <2 Gy, 2-4 Gy, 4-6 Gy and $\geq$ 8 Gy. . This categorisation is of relevance when there is a need to decide upon the adequate level of hospital care for a great number of victims, while hospital resources are limited (Table 1).

Table 2 Level of hospital care needed, based on early estimation of acute whole body dose[1]

Estimated dose	Decision
Less than 2 Gy	Follow up in general hospital or on outpatient basis
2 – 4 Gy	Transfer to hematological department
4 – 6 Gy	Transfer to well equipped hematological department within two days. Early initiation of cytokine therapy
6 – 8 Gy	Early transfer to hematological department with capacity for reverse isolation and allogenic stem cell transplantation
More than 8 Gy	Palliative care if resources are strained. Otherwise as above. Prognosis for long term survival is bad.

1- Taken from *Radiation accidents. Examination and treatment of persons exposed to radiation. Helsinki 2008. 47pp. (Publications of the Ministry of Social Affairs and Health,Finland, ISSN 1236-2050, 2008:15), ISBN 978-952-00-2606-6 (PDF).* In Finnish and Swedish;
http://www.stuk.fi/julkaisut_maaraykset/muut_julkaisut/sv_FI/muut_julkaisut/

3. Results and Discussion

The results of scoring 62 samples, each presenting a "casualty" in a simulated mass casualty exercise, are given in Tables 3 and 4 for dicentrics and Tables 5 and 6 for PCC rings. In these tables, the detailed analysis data are given with statistics and estimated whole body dose with lower and upper 95% confidence limits as well as partial body dose. The tables reveal that for dicentrics, the triage scoring criteria of 50 cells or 30 dicentrics was fulfilled in all but two samples. For the PCC assay, successful triage scoring defined as 300 PCC cells or 50 rings, was not obtained in 12 samples out of the 62. However, it can be noted that the insufficient number of scored PCC cells had substantial impact on the dose estimate only in a few cases.

Table 7 demonstrates how well whole body dose estimates based on triage scoring of an average of 50 cells for dicentrics and 300 cells for PCC rings are placed into correct dose categories as defined in Table1. Both assays are equally efficient at control level and in the < 2 Gy category. In the following two categories (i.e. 2-4 Gy, 4-6 Gy), however, the dicentric assay is far more efficient in placing the cases into correct category; PCC ring estimates were correct in only 2 cases out of 10, whereas the dicentrics scoring was able to find right dose at a rate of 9 /10. In the two highest categories, 6-8 Gy and above 8 Gy, the assays showed reverse efficiency; PCC ring scoring was superior in placing dose estimates in correct catego-

ries. As a whole, data from the dicentric assay were slightly better in placing doses into the right category (77% vs. 59%).

Further, Table 7 also illustrates how often the given dose coincides with the calculated 95% confidence limits for the whole body estimates. From 7 control level samples, i.e. those with no given irradiation, the correct value of 0 Gy was obtained in 6 cases for dicentrics and 5 cases for PCC rings. The two incorrect estimates for PCC rings and the one for dicentrics were low and their lower confidence limits included or were very near to zero, indicating that the dose in these cases in fact was negligible. In the following category with doses above zero but less than 2 Gy, the given dose fell within the estimated 95% limits in 8/10 for dicentrics and 6/10 for PCC rings. In the 2-4 Gy category, the data demonstrate 7 and 5 correct estimates out of 10 cases for dicentrics and PCC rings, respectively. Based on the criteria of confidence limits, out of 17 samples in the 4-6 Gy, 6-8 Gy and above 8 Gy classes, the scoring of dicentrics found 12 correct doses, whereas the PCC ring dose estimate were within the limits 11 times. At really high doses, the PCC ring assay is more efficient in picking out correct estimates. Taking into account all data, the given doses were within the 95% confidence limits more often based on dicentrics than on PCC ring data (73% vs. 64%).

Results of the whole body dose estimates with respect to the given dose are also illustrated in Figure 1. In general, it can be observed that all laboratories performed relatively well in the comparison between assays and given dose. Poor quality of PCC preparations sent to NRPA influenced the scoring performed there. Figure 1 indicates that the dicentric assay produces valid dose estimates at doses of 5 Gy and below, whereas the PCC assay appears to recognize higher doses more efficiently. This can be explained by the the PCC ring dose calibration curve that displayed dose effect for doses up to 20 Gy. The dicentrics curve, however, is established for doses up to only 5 Gy due to saturation of dicentrics frequency at higher doses.

There may be a number of reasons for the poorer estimates at low doses using PCC rings. The most plausible explanation is the use of linear calibration curve also at low doses although the two data points below 5 Gy gave indications of this not being the case. In the data obtained for the dose points in order to establish calibration curve during the BIODOS activity (2006-2007), the frequency of rings at 1 Gy was very low and this data point was omitted from the curve data due to very poor fit in the linear model. The ring frequency at 2.5 Gy showed a better fit but was still somewhat low. In this situation, applying linear dose relationship entails underestimation of doses in the low dose range. Further statistics will be performed to obtain more accurate dose estimates at lower doses. In the literature, studies with

PCC ring dose-response have not included doses below 5 Gy (Lamadrid et al. 2007; Kanda et al. 1999).

As indicated in Table 4 and 6, non-uniform exposures, ie. over-dispersion of aberrations assuming a non-Poisson distribution and characterized by a u-value of greater than ± 1,96, were similarly observed with both assays. From the total 18 cases of partial-body irradiations, no over-dispersion of aberrations was seen in 7 and 8 cases in dicentric and PCC ring scoring, respectively. It is apparent that the low number of scored cells affected the efficiency of finding non-uniform exposures. Furthermore, the low percentage of exposed cell fraction (10-40%) also affected the success rate of discovering non-uniform irradiations. In addition, the same factors affected the poor estimation of irradiated fraction, ie. percentage of cells irradiated (data not shown). It has been shown for dicentrics that the scoring criteria of 50 metaphases / 30 dicentrics are appropriate for cases that involve partial body irradiation when the fraction of irradiated cells is 50% or higher (Lloyd et al. 2000). Figure 2 implies that there is large variation in the dose estimation of partial-body exposures. In the dicentric assay the doses are underestimated, whereas the PCC rings appear to overestimate the dose. The low dicentric dose may be caused by the fact that the doses in the non-uniform irradiations were generally much higher than is possible to estimate from the calibration curve established for dicentrics; the dicentric curve go up to 5 Gy. Concerning the overestimation of partial body doses from PCC rings, the cause may be overdispersion of aberrations at uniform exposure. This is because the methods for estimating partial-body exposures assume Poisson-distributed aberrations at whole-body exposures. The uncommon distribution of PCC rings became evident also in some cases of uniform exposure; seven of the samples displayed overdispersion of aberrations, whereas none of the uniform irradiations were overdispersed regarding dicentrics. The time required for microscopic analysis is of importance when comparing the efficiency of the two assays since the culture and slide preparation times are essentially the same. For dicentric assay, a skilled scorer, with the help of a metaphase finder, is able to evaluate about 50 metaphases / hour. For the PCC ring analysis, we approximated that, with the assistance of scanning and relocation of PCC cells, the average number of good quality PCC cells scored is 350 / hour. Therefore, using the applied scoring numbers for triage cases, the PCC assay is somewhat faster than the dicentric assay, but the difference is not significant. The scoring speed in both assays is sensitive for preparation quality, so large variations may occur. In the current study, the PCC assay demonstrated large variability in the success of producing good quality chromosome condensation.

4. Conclusions

The work performed in the BIOPEX project has aimed at comparison of two biodosimetry assays for triage purposes in a large accident involving exposure to low-LET ionizing radiation. The classic dicentric method was used as the reference technique to which the newly established PCC ring assay was compared. In general, the PCC ring assay was, depending on the dose, equally or somewhat less efficient than the dicentric assay in estimating correct dose in cases of uniform irradiations. Both triage assays were capable of discerning non-exposed cases. At doses about 5 Gy and below, dicentrics scoring resulted in more accurate whole-body dose estimates than PCC rings. This may be caused by the incorrect assumption of linear dose response for PCC rings at lower doses, when in reality it may be sublinear. For a more appropriate comparison, PCC ring data at small doses require more appropriate calibration. At very high doses, PCC rings appeared to give more accurate dose estimates than dicentrics, the natural explanation being that in contrast to dicentrics, the yield of PCC rings increase at constant rate up to very high doses. Applying the limited triage scoring, the dicentric assay was in 27 cases of 37 (with more than zero dose) capable of correctly placing cases in dose categories based on level of hospital care needed. For estimates from PCC ring scoring, the corresponding number was 21 out of 37.

In non-uniform irradiations, the PCC ring assay was slightly better in the approximation of the partial body dose than dicentrics, but neither was satisfactory. In addition, the fraction of cells irradiated could not be calculated with data from either of the assays. The difficulties in identifying non-uniform irradiation with the set analysis numbers in this study originates mainly from the irradiated fraction of cells being too small with respect to the triage approach. It should be noted that the study was not designed to test the general efficiency of estimating partial body doses, it was based on a possible scenario involving a number of cases with inhomogeneous exposure. In other words, the aim was not to investigate the smallest detectable fraction of non-irradiated cells with either assays, an issue of medical interest when the question of the fraction of non-irradiated bone marrow emerges.

As a general conclusion based on the results obtained in this study, the assay of analysing PCC rings has proven to be a suitable method for estimating doses in an accident involving a large number of exposed casualties and is especially applicable of estimation of high doses.

Table 3. Results from dicentric scoring for samples with uniform irradiation showing distribution statistics, given irradiations and whole body dose estimates. The different dose categories, ie. 0 Gy, 0-2 Gy, 2-4 Gy, 4-6 Gy, 6-8 Gy and above 8 Gy, in relation to the given dose are indicated in alternating shades of grey.

				Distribution of dicentrics												Calculated		
Code	**Cells analysed**	**Dic:s**	**Dic:s / cell**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Variance /mean**	**U**	**Irradiation given (%)**	**Given WB dose (Gy)**	**Whole body dose (Gy)**	**Lower Confidence Limit**	**Upper Confidence Limit**
BP09	50	0	0	50	0	0	0	0	0	0	0	0			-	0	0,00	0,00	0,00
BP32	50	0	0	50	0	0	0	0	0	0	0	0			-	0	0,00	0,00	0,00
BP61N	50	0	0	50	0	0	0	0	0	0	0	0			-	0	1,55	1,01	2,18
BP62	50	0	0	50	0	0	0	0	0	0	0	0			-	0	0,00	0,00	0,00
BP66	50	0	0	50	0	0	0	0	0	0	0	0			-	0	0,00	0,00	0,00
BP84N	50	1	0,020	49	1	0	0	0	0	0	0	0	1	0	-	0	1,45	0,92	2,09
BP91	50	0	0	50	0	0	0	0	0	0	0	0			-	0	0,00	0,00	0,00
BP29	50	1	0,020	49	1	0	0	0	0	0	0	0	1,00	0,00	100 %	0,4	0,46	0,00	1,31
BP41	50	2	0,040	48	2	0	0	0	0	0	0	0	0,98	-0,14	100 %	0,4	0,73	0,18	1,51
BP49N	50	4	0,080	46	4	0	0	0	0	0	0	0	0,94	-0,35	100 %	0,4	0,99	0,46	1,66
BP42	50	2	0,040	48	2	0	0	0	0	0	0	0	0,98	-0,14	100 %	0,8	0,73	0,18	1,51
BP80	50	6	0,120	44	6	0	0	0	0	0	0	0	0,90	-0,55	100 %	0,8	1,25	0,71	1,89
BP19	50	4	0,080	46	4	0	0	0	0	0	0	0	0,94	-0,35	100 %	1,2	1,09	0,52	1,82
BP88N	57	30	0,526	32	21	3	1	0	0	0	0	0	0,89	-0,60	100 %	1,2	2,73	2,23	3,30
BP 100	50	8	0,160	42	8	0	0	0	0	0	0	0	0,86	-0,76	100 %	1,2	1,46	0,92	2,09
BP03	50	11	0,220	39	11	0	0	0	0	0	0	0	0,80	-1,06	100 %	1,6	1,89	1,30	2,58
BP67	50	4	0,080	46	4	0	0	0	0	0	0	0	0,94	-0,35	100 %	1,6	1,00	0,46	1,66
BP04	50	17	0,340	37	9	4	0	0	0	0	0	0	1,15	0,78	100 %	2,1	2,38	1,79	3,05
BP20N	41	30	0,732	17	18	6	0	0	0	0	0	0	0,69	-1,43	100 %	2,1	3,24	2,65	3,9
BP34	50	7	0,140	43	7	0	0	0	0	0	0	0	0,88	-0,66	100 %	2,1	1,48	0,90	2,19
BP07	50	28	0,560	28	17	4	1	0	0	0	0	0	0,96	-0,21	100 %	2,7	3,09	2,50	3,75
BP10	50	27	0,540	27	20	2	1	0	0	0	0	0	0,85	-0,77	100 %	2,7	3,03	2,44	3,69
BP24	23	18	0,783	7	11	2	1	0	0	0	0	0	0,73	-0,87	100 %	3,2	3,68	2,81	4,66
BP90	47	31	0,660	25	14	7	1	0	0	0	0	0	1,01	0,03	100 %	3,2	3,08	2,52	3,69
BP106N	24	30	1,250	5	9	9	1	0	0	0	0	0	0,57	-1,47	100 %	3,2	4,27	3,49	5,13
BP01	44	31	0,705	23	14	5	1	1	0	0	0	0	1,23	1,07	100 %	3,6	3,48	2,85	4,18
BP85	61	32	0,525	38	15	7	1	0	0	0	0	0	1,12	0,66	100 %	3,6	2,73	2,24	3,27
BP16	21	32	1,524	4	6	9	0	2	0	0	0	0	0,83	-0,55	100 %	4,4	5,18	4,27	6,19
BP57	20	31	1,550	4	5	7	4	0	0	0	0	0	0,71	-0,90	100 %	4,4	5,23	4,29	6,26

Table 3. Continued

				Distribution of dicentrics												Calculated		
Code	**Cells analysed**	**Dic:s**	**Dic:s / cell**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Variance /mean**	**U**	**Irradiation given (%)**	**Given WB dose (Gy)**	**Whole body dose (Gy)**	**Lower Confidence Limit**	**Upper Confidence Limit**
BP14	17	32	1,882	1	4	8	4	0	0	0	0	0	0,39	-1,75	100 %	4,9	5,78	4,76	6,89
BP70	15	48	3,200	0	1	5	3	3	2	1	0	0	0,63	-0,98	100 %	4,9	6,91	5,92	7,97
BP82	26	34	1,308	10	6	3	6	1	0	0	0	0	1,33	1,19	100 %	4,9	4,38	3,62	5,19
BP50	23	30	1,304	9	3	6	5	0	0	0	0	0	1,15	0,49	100 %	5,7	4,79	3,91	5,75
BP75N	15	30	2,000	0	4	7	4	0	0	0	0	0	0,29	-1,92	100 %	5,7	5,44	4,45	6,52
BP86	17	34	2,000	2	3	8	2	1	1	0	0	0	0,81	-0,54	100 %	5,7	5,44	4,51	6,45
BP36	19	31	1,632	7	3	4	1	3	1	0	0	0	1,72	2,19	100 %	6,2	5,37	4,41	6,42
BP87	22	44	2,000	4	4	6	6	0	2	0	0	0	1,05	0,16	100 %	6,2	5,44	4,62	6,32
BP21	12	35	2,917	1	0	4	3	2	2	0	0	0	0,71	-0,68	100 %	7	7,22	5,96	8,54
BP65	21	30	1,429	5	8	3	4	1	0	0	0	0	1,02	0,06	100 %	7	4,58	3,74	5,49
BP59	8	26	3,250	0	0	2	3	2	1	0	0	0	0,33	-1,28	100 %	8	7,63	6,15	9,27
BP12N	6	30	5,000	0	0	1	1	0	1	2	0	1	0,96	-0,06	100 %	9	8,66	7,1	10,37
BP52	10	31	3,100	0	0	4	3	1	2	0	0	0	0,46	-1,16	100 %	10	7,45	6,12	8,90
BP99	16	33	2,063	1	7	2	2	4	0	0	0	0	0,94	-0,18	100 %	10	5,52	4,56	6,57
BP77	39	161	4,128	1	0	2	13	11	3	5	3	1	0,65	-1,52	100 %	13	7,86	7,25	8,50

Table 4. Results from dicentric scoring for samples with non-uniform irradiation showing distribution statistics, given irradiations and partial-body dose estimates.

				Distribution of dicentrics													Calculated			
Code	**Cells analysed**	**Dic:s**	**Dic:s / cell**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Variance /mean**	**U**	**Irradiation given (%)**	**PB dose given (Gy)**	**Partial body dose (Gy)**	**Whole body dose (Gy)**	**Lower Confidence Limit**	**Upper Confidence Limit**
BP06	50	1	0,020	49	1	0	0	0	0	0	0	0	1,00	0,00	10 %	9	No o.d.	0,46	0	1,31
BP47	50	1	0,020	49	1	0	0	0	0	0	0	0	1,00	0,00	10 %	8	No o.d.	0,46	0	1,31
BP08	50	0	0,000	50	0	0	0	0	0	0	0	0			10 %	13	No o.d.	0,00	0,00	
BP101	50	3	0,060	48	1	1	0	0	0	0	0	0	1,64	3,88	15 %	7	3,56	0,85	0,31	1,53
BP13N	50	10	0,200	44	5	0	0	0	1	0	0	0	2,86	9,69	15 %	8	4,05	1,64	1,1	2,27
BP23	50	4	0,080	48	0	2	0	0	0	0	0	0	1,96	5,48	15 %	8	5,31	1,09	0,52	1,82
BP110	51	3	0,059	50	0	0	1	0	0	0	0	0	3,00	12,20	15 %	10	6,49	0,84	0,31	1,51
BP22	50	5	0,100	49	0	0	0	0	1	0	0	0	5,00	22,10	20 %	9	9,46	1,23	0,65	1,95
BP26	50	4	0,080	49	0	0	0	1	0	0	0	0	4,00	17,10	20 %	9	8,4	1,09	0,52	1,82
BP44N	50	0	0,000	50	0	0	0	0	0	0	0	0			20 %	9	No o.d.	0	0	0
BP79N	50	11	0,220	42	6	1	1	0	0	0	0	0	1,54	2,79	20 %	10	3,12	1,72	1,19	2,35
BP30	50	2	0,040	49	0	1	0	0	0	0	0	0	2,00	7,00	20 %	13	5,31	0,73	0,18	1,51
BP89	50	1	0,020	49	1	0	0	0	0	0	0	0	1,00	0,00	20 %	13	No o.d.	0,44	0,00	1,20
BP31	50	8	0,160	47	1	0	1	1	0	0	0	0	3,15	11,40	25 %	7	6,59	1,59	1,01	2,29
BP97	50	0	0,000	50	0	0	0	0	0	0	0	0			25 %	7	No o.d.	0,00	0,00	0,00
BP72	51	16	0,314	45	1	3	0	1	1	0	0	0	3,12	11,00	30 %	10	6,01	2,09	1,55	2,69
BP105	52	1	0,019	51	1	0	0	0	0	0	0	0	1,00	0,00	30 %	13	No o.d.	0,42	0,00	1,17
BP45	50	9	0,180	46	0	3	1	0	0	0	0	0	2,20	6,29	40 %	7	5,84	0,46	0	1,31

No o.d. = no overdispersion of aberrations

Table 5. Results from PCC ring scoring for samples with uniform irradiation showing distribution statistics, given irradiations and whole body dose estimates. The different dose categories, ie. 0 Gy, 0-2 Gy, 2-4 Gy, 4-6 Gy, 6-8 Gy and above 8 Gy, in relation to the given dose are indicated in alternating shades of grey.

				Distribution of rings										Calculated		
Code	**Cells analysed**	**Rings**	**Rings / cell**	**0**	**1**	**2**	**3**	**4**	**5**	**Variance /mean**	**U**	**Irradiation given (%)**	**Given WB dose(Gy)**	**Whole body dose (Gy)**	**Lower Confidence Limit**	**Upper Confidence Limit**
BP09	300	0	0	300	0	0	0	0	0			-	0	0,00	0,00	0,00
BP32	300	0	0	300	0	0	0	0	0			-	0	0,00	0,00	0,00
BP61N	260	3	0,012	257	3	0	0	0	0	0,99	-0,11	-	0	0,15	0,01	0,65
BP62	300	0	0	300	0	0	0	0	0			-	0	0,00	0,00	0,00
BP66	300	5	0,017	297	2	0	1	0	0	2,19	16,30	-	0	0,26	0,07	0,76
BP84N	300	1	0,003	299	1	0	0	0	0	1,00	0,00	-	0	0,00	0,00	0,00
BP91	300	0	0	300	0	0	0	0	0			-	0	0,00	0,00	0,00
BP29	300	2	0,007	298	2	0	0	0	0	1,00	-0,06	100 %	0,4	0,06	0,00	0,45
BP41	300	0	0	300	0	0	0	0	0			100 %	0,4	0,00	0,00	0,00
BP49N	170	2	0,012	168	2	0	0	0	0	0,99	-0,08	100 %	0,4	0,16	0,00	0,83
BP42	300	4	0,013	296	4	0	0	0	0	0,99	-0,14	100 %	0,8	0,19	0,03	0,66
BP80	302	6	0,020	299	1	1	1	0	0	2,32	17,80	100 %	0,8	0,33	0,11	0,85
BP19	300	4	0,013	298	4	0	0	0	0	0,99	-0,14	100 %	1,2	0,19	0,03	0,66
BP88N	300	8	0,027	292	8	0	0	0	0	0,98	-0,31	100 %	1,2	0,46	0,20	1,04
BP100	301	18	0,060	283	18	0	0	0	0	0,94	-0,71	100 %	1,2	1,14	0,69	1,90
BP03	300	18	0,060	284	14	2	0	0	0	1,17	2,09	100 %	1,6	1,15	0,69	1,90
BP67	312	17	0,054	298	11	3	0	0	0	1,30	3,89	100 %	1,6	1,04	0,61	1,75
BP04	300	26	0,087	276	22	2	0	0	0	1,07	0,88	100 %	2,1	1,69	1,12	2,56
BP20N	189	9	0,048	180	9	0	0	0	0	0,96	-0,44	100 %	2,1	0,89	0,41	1,81
BP34	300	24	0,080	276	24	0	0	0	0	0,92	-0,96	100 %	2,1	1,56	1,01	2,40
BP07	271	24	0,089	249	20	2	0	0	0	1,08	0,97	100 %	2,7	1,73	1,12	2,66
BP10	300	24	0,080	279	18	3	0	0	0	1,17	2,17	100 %	2,7	1,56	1,01	2,40
BP24	300	38	0,127	276	30	4	0	0	0	1,09	1,08	100 %	3,2	2,51	1,80	3,52
BP90	300	40	0,133	264	33	2	1	0	0	1,12	1,49	100 %	3,2	2,65	1,91	3,68
BP106N	300	26	0,087	275	24	1	0	0	0	0,99	-0,08	100 %	3,2	1,69	1,12	2,56
BP01	218	22	0,101	196	22	0	0	0	0	0,90	-1,03	100 %	3,6	1,99	1,26	3,09
BP85	262	55	0,210	219	34	7	1	1	0	1,38	4,35	100 %	3,6	4,22	3,20	5,56
BP16	300	50	0,167	255	40	5	0	0	0	1,04	0,45	100 %	4,4	3,33	2,49	4,46
BP57	210	50	0,238	166	38	6	0	0	0	1,01	0,07	100 %	4,4	4,80	3,58	6,39

Table 5. Continued

				Distribution of rings										Calculated		
Code	**Cells analysed**	**Rings**	**Rings / cell**	**0**	**1**	**2**	**3**	**4**	**5**	**Variance /mean**	**U**	**Irradiation given (%)**	**Given WB dose(Gy)**	**Whole body dose (Gy)**	**Lower Confidence Limit**	**Upper Confidence Limit**
BP14	300	53	0,177	250	47	3	0	0	0	0,94	-0,75	100 %	4,9	3,54	2,67	4,69
BP70	290	76	0,262	223	58	9	0	0	0	0,98	-0,26	100 %	4,9	5,29	4,19	6,68
BP82	174	50	0,287	131	37	5	1	0	0	1,04	0,36	100 %	4,9	5,81	4,33	7,72
BP50	300	54	0,180	253	41	5	1	0	0	1,12	1,48	100 %	5,7	3,61	2,73	4,77
BP75N	72	31	0,431	49	18	2	3	0	0	1,30	1,80	100 %	5,7	8,62	5,89	12,19
BP86	151	50	0,331	111	31	8	1	0	0	1,12	1,02	100 %	5,7	6,70	5,00	8,91
BP36	140	41	0,293	107	26	6	1	0	0	1,15	1,30	100 %	6,2	5,92	4,27	8,10
BP87	90	50	0,556	52	27	10	1	0	0	0,98	-0,17	100 %	6,2	11,30	8,41	14,97
BP21	84	32	0,381	58	22	2	2	0	0	1,13	0,87	100 %	7	7,72	5,30	10,98
BP65	183	72	0,393	127	43	10	3	0	0	1,14	1,35	100 %	7	7,98	6,27	10,11
BP59	117	52	0,444	82	26	6	0	1	2	1,80	6,17	100 %	8	9,03	6,76	11,90
BP12N	15	7	0,467	8	7	0	0	0	0	0,57	-1,22	100 %	9	9,48	3,80	19,66
BP52	115	65	0,565	66	36	10	3	0	0	1,03	0,22	100 %	10	11,50	8,90	14,72
BP99	42	51	1,214	12	14	11	5	0	0	0,83	-0,80	100 %	10	24,80	18,49	32,68
BP77	125	57	0,456	80	35	8	2	0	0	1,04	0,35	100 %	13	9,26	7,04	12,07

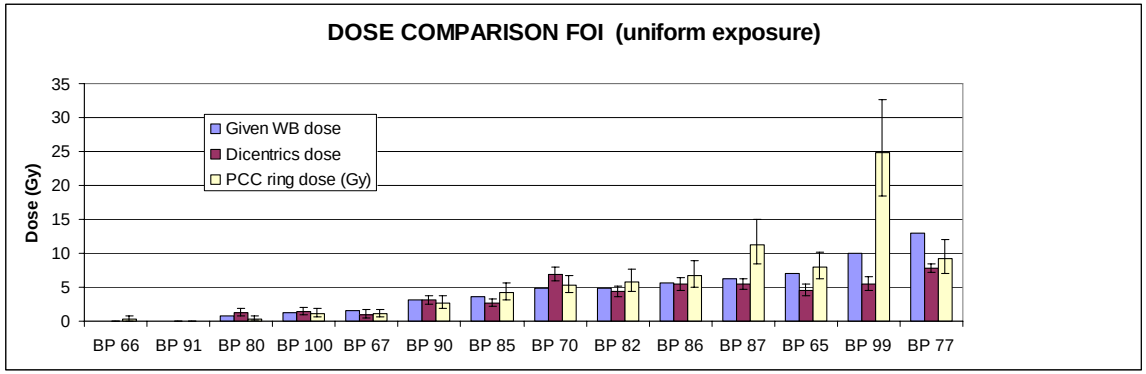
Table 6. Results from PCC ring scoring for samples with non-uniform irradiation showing distribution statistics, given irradiations and partial-body dose estimates.

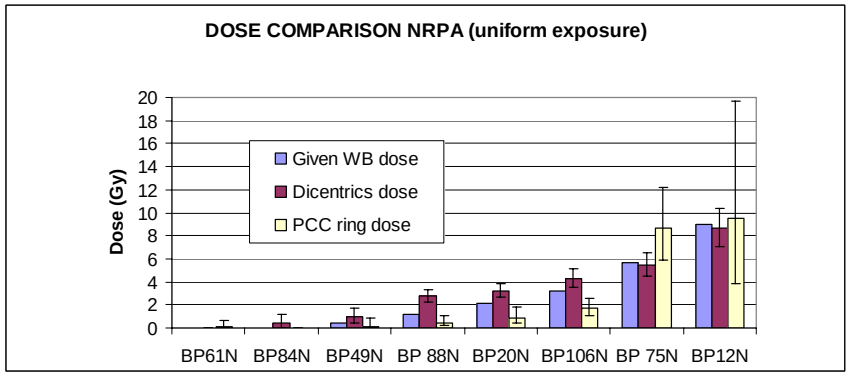
				Distribution of rings										Calculated			
Code	**Cells analysed**	**Dic:s**	**Dic:s / cell**	**0**	**1**	**2**	**3**	**4**	**5**	**Variance /mean**	**U**	**Irradiation given (%)**	**PB dose given (Gy)**	**Partial body dose (Gy)**	**Whole body dose (Gy)**	**Lower Confidence Limit**	**Upper Confidence Limit**
BP06	300	5	0,017	297	2	0	1	0	0	2,19	16,30	10 %	9	23	0,26	0,07	0,76
BP47	245	0	0	245	0	0	0	0	0			10 %	8	No o.d.	0		
BP08	300	10	0,033	293	3	0	1	1	0	2,78	22,90	10 %	13	15,6	0,60	0,29	1,22
BP101	300	4	0,013	296	4	0	0	0	0	0,99	-0,14	15 %	7	No o.d.	0,19	0,03	0,66
BP13N	220	10	0,045	212	7	0	1	0	0	1,56	6,20	15 %	8	9,5	0,85	0,41	1,67
BP23	300	2	0,007	298	2	0	0	0	0	1,00	-0,06	15 %	8	No o.d.	0,06	0,00	0,45
BP110	300	8	0,027	294	4	2	0	0	0	1,48	6,25	15 %	10	12,4	0,46	0,20	1,04
BP22	300	2	0,007	298	2	0	0	0	0	1,00	-0,06	20 %	9	No o.d.	0,06	0,00	0,45
BP26	300	5	0,017	297	1	2	0	0	0	1,79	10,80	20 %	9	23	0,26	0,07	0,76
BP44N	68	2	0,029	66	2	0	0	0	0	0,99	-0,12	20 %	9	No o.d.	0,52	0,03	2,14
BP79N	300	20	0,067	286	10	3	0	1	0	1,84	10,50	20 %	10	15,6	1,28	0,79	2,07
BP30	300	10	0,033	291	8	1	0	0	0	1,17	2,20	20 %	13	4,4	0,60	0,29	1,22
BP89	310	6	0,019	304	6	0	0	0	0	0,98	-0,22	20 %	13	No o.d.	0,32	0,11	0,82
BP31	300	13	0,043	288	11	1	0	0	0	1,11	1,45	25 %	7	No o.d.	0,81	0,43	1,48
BP97	300	20	0,067	283	14	3	0	0	0	1,24	2,98	25 %	7	6,9	1,28	0,79	2,07
BP72	242	50	0,207	205	29	5	2	0	1	1,64	7,10	30 %	10	13	4,15	3,10	5,54
BP105	300	5	0,017	297	2	0	1	0	0	2,19	16,30	30 %	13	23	0,26	0,07	0,76
BP45	300	18	0,060	283	16	1	0	0	0	1,05	0,69	40 %	7	No o.d.	1,15	0,69	1,90

No o.d. = no overdispersion of aberrations

Table 7. Number of cases with estimates in correct dose categories.

	Estimated dose in correct dose category		Given dose within estimated 95% confidence limits	
Dose category	Dicentrics	PCC rings	Dicentrics	PCC rings
Non-exposed	6 / 7	5 / 7	6 / 7	5 / 7
>0 to < 2 Gy	9/10	9/10	8/10	6/10
2 to < 4 Gy	9 /10	2/10	7/10	5/10
4 to < 6 Gy	7 / 8	3 / 8	7 / 8	5 / 8
6 to 8 Gy	1 / 5	3 / 5	4 / 5	4 / 5
> 8 Gy	1 / 4	4 / 4	1 / 4	3 / 4
All	34 /44 (77%)	26 /44 (59%)	32 /44 (73%)	28 / 44 (64%)





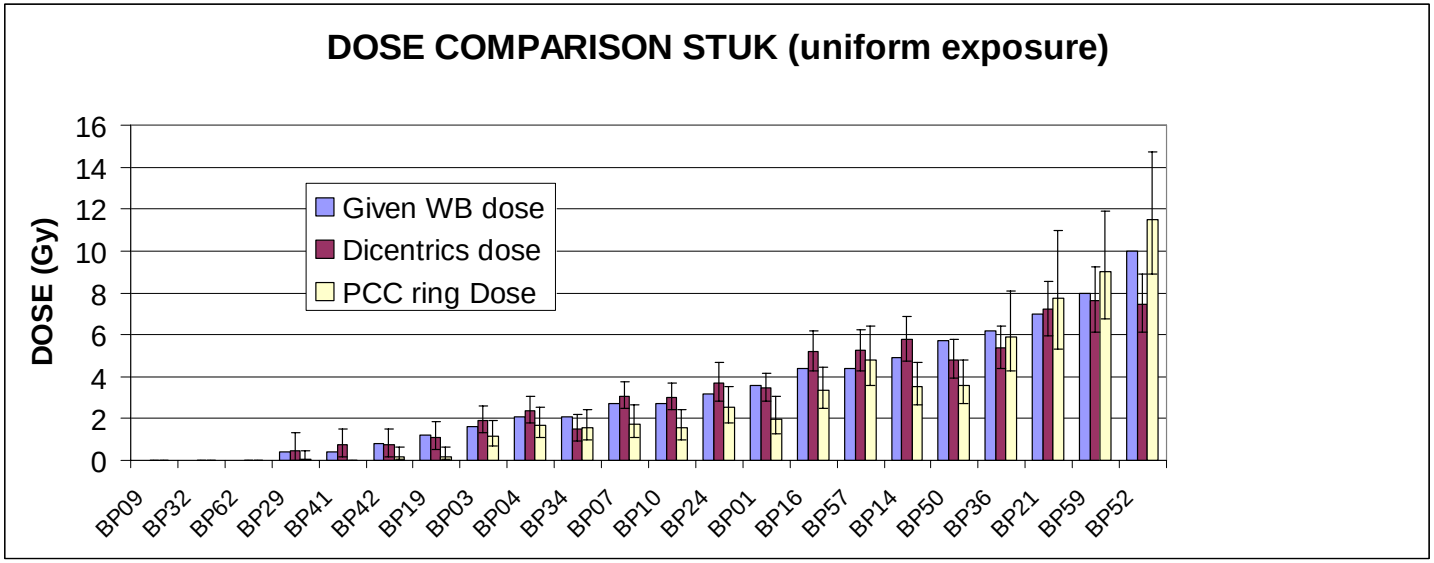


Figure 1. Dose comparison between dicentric and PCC ring assays in triage scored samples with uniform irradiation. Error bars indicate 95% confidence intervals for calculated doses.

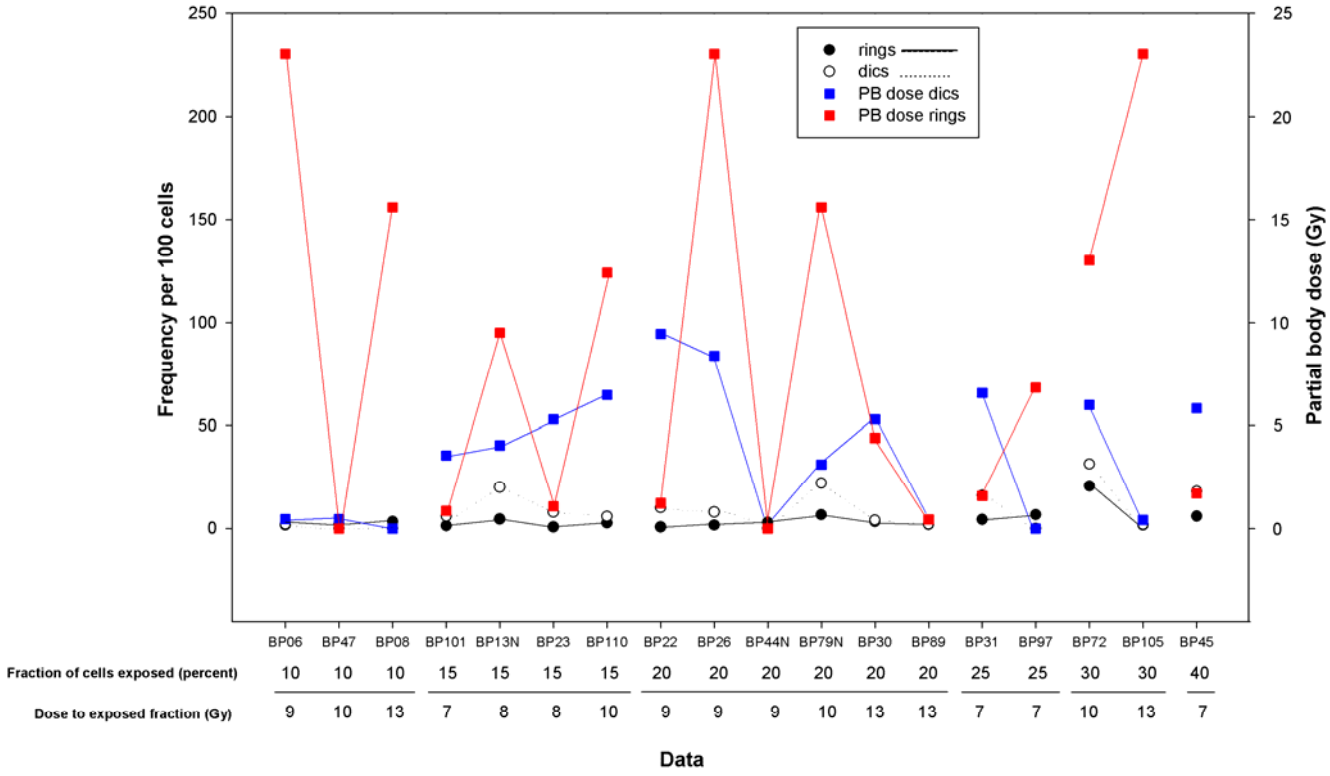


Figure 2. Frequency of dicentrics and PCC rings scored in 18 samples with partial body exposure and the calculated dose estimates.

In the dicentric assay the estimates of partial body dose are generally below the given dose, whereas the PCC rings appear to overestimate the dose.

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Abstract

As a continuation to the NKS-funded BIODOS project, the BIOPEX project has aimed at testing and validation of the newly established dose calibration curve for PCC rings, a specific chromosome aberration for use in biodosimetry in large casualty emergency preparedness. The testing of the PCC ring technique was performed by direct comparison to the conventional dicentric assay, both conducted with a triage approach that gives a crude dose estimate through analysis of a relatively small number of cells. Altogether 62 blood samples were analysed, each irradiated with an individual dose using γ-rays, and representing casualties in a simulated radiation accident resulting in a broad spectrum of whole body and partial body doses, ranging from zero dose up to a lethal whole body dose of 13 Gy. The results indicated that both triage assays were capable of discerning non-exposed cases and that in the uniform irradiations, the dose estimates based on data from both assays were fairly consistent with the given dose. However, differences were observed depending on the dose level. At doses about 5 Gy and below, dicentric scoring resulted in more accurate whole-body dose estimates than PCC rings. At very high doses, PCC rings appeared to give more accurate dose estimates than dicentrics. The discrepancies are mainly caused by shortcomings in the respective dose calibration curves. In non-uniform irradiations, the PCC ring assay was slightly better in the approximation of the partial body dose than dicentrics, but neither assay enabled accurate estimation of either dose or fraction of cells irradiated. The irradiated fraction of cells for the casualties in this scenario was apparently too small (10-40%) to be distinguished with the triage approach applied in the current study. With respect to the technical aspects, scoring of the PCC rings is easier and therefore somewhat faster but may be more sensitive to quality aspects. In conclusion, the study demonstrated that the PCC ring assay is suitable for use as a biodosimeter, especially for estimation of very high doses.

Key words

Biological dosimetry, PCC assay, dicentrics, triage, mass casualty radiological accident

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