Cytogenetic Biological Dosimetry: Past, Present and Future Perspectives

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Annual Permissible Dose to Public = 1 mSv (mGy) andOccupationally is 20 mGy

Acute & Chronic exposure at Low and High Dose Rates
Ionizing radiation: Dose ranges for natural and occupational exposure

**Dose Ranges (Sievert)**

- **Life Span Study (A-bomb survivor epidemiology)**
- **Solar flare dose on moon, no shielding**
- **Typical mission doses on Int'l. Space Station (ISS)**
- **EPA radiological emergency guideline for public relocation**
- **OEJ Low Dose Program**
- **Medical Diagnostics (A-J)**

**Regulations & Guidelines**

- **Max releases**
- **DOE facilities**
- **Round-trip NY to London**
- **EPA dose limit from releases in air:** 0.10 mSv/yr
- **ANSI Standard N43.17 Limit Security Personnel Scanners:** 0.25 mSv/yr/person
- **DOE, NRC Dose Limit for Public:** 1 mSv/yr

**Cancer Radiotherapy**

- **Total Body Irradiation (TBI) Therapy**
- **Acute Radiation Syndromes**
- **Cancer Epidemiology**

**Evidence for small increases in human cancer above 0.1 Sv acute exposure, 0.2 Sv chronic exposure**

**Medical Diagnostics, mSv**

- A. Chest x-ray (1 film)
- B. Dental oral exam
- C. Mammogram
- D. Lumbosacral spine
- E. PET
- F. Bone (Tc-99m)
- G. Cardiac (Tc-99m)
- H. Cranial CT (MSAD) (multiple scan average dose)
- I. Barium contrast G-I
- J. Spiral CT - full body

**LD_{50} = L_{thai} Dose to 50%**

- Absorbed dose: 1 Gray = 100 rad
- Dose equivalent: 1 Sievert = 100 rem
- 1 Sv = 100 mrem

*Note: Whole body acute prognoses assume no medical intervention.*

**Chart compiled by NF Metting, Office of Science, DOE/ER**

“Orders of Magnitude” revised March 2006
Responses of the cell upon induction of DNA damage

Cell cycle arrest

DNA Repair → Complete

DNA Repair → Incomplete

*Genomic instability
*Mutations *Chromosomal aberrations

G1/S checkpoint (ATM, ATR, p53)
S-phase checkpoint (ATM, p53, Chk2)
G2/M checkpoint (Cdc2 / CyclinB)

Cell death (Apoptosis, necrosis)

Damaged DNA

SSB = Single Strand Breaks
DSB = Double Strand breaks
BD = Base Damage

Cancer and Genetic Diseases
Consequences of exposure to physical and chemical agents

Cellulare Damages
(DNA, Protein, Membrane, Cellular organization)

Clinical Trials
(Cell killing, Cancer)

Genetic Differences

Difference in sensitivity
I. Hagmar et al., 2004 reported on the association between level of chromosomal aberrations (in human peripheral blood lymphocytes) and cancer risk and could provide novel information on the type of aberrations more strongly predictive of cancer risk and on the types of cancer more strongly predicted by chromosomal aberrations.

II. The cancer-predictive ability of chromosomal aberrations received further support from a recently published study (Boffetta et al., 2007), based on data from 6,430 individuals from 5 regions in Central and Eastern Europe. Chromosomal aberrations and cancer risk: results of a cohort study from central Europe (Am J Epidemiol.; 65(1):36-43).

III. The ability of another type of genetic damage, micronuclei, to predict future cancer risk has been under investigation. Using data obtained from over 6,700 subjects in 10 countries, it was found that persons with medium or high levels of micronuclei in peripheral blood lymphocytes had a higher probability of developing cancer, especially gastrointestinal or urogenital cancer (Bonassi et al., 2007) [An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans, Carcinogenesis; 28(3):625-631.]
Biological Assays for Dose Assessment Following Exposure to Ionizing Radiation

1. Dicentrics

2. Micronuclei

3. Premature Chromosome Condensation (PCC)

4. Translocations using Fluorescence *in situ* hybridization (FISH)

(International Atomic Energy Agency, Technical Reports Series No. 405, 2001)
Background level in unexposed individuals

<table>
<thead>
<tr>
<th>Biological end-points</th>
<th>Spontaneous frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dicentrics</td>
<td>1-3 / 1000 cells</td>
</tr>
<tr>
<td>2. Micronuclei in binucleated cells (BNC)</td>
<td>2-30 / 1000 BNC*</td>
</tr>
<tr>
<td>3. Premature Condensed Chromosome</td>
<td>0-2 / 1000 cells</td>
</tr>
<tr>
<td>4. Translocations using Fluorescence in situ hybridization (FISH)</td>
<td>0-18 / 000 cells*</td>
</tr>
</tbody>
</table>

*In both micronucleus and FISH based translocation assays, spontaneous frequency was found to be age dependent.
The pattern of dose response curve following low and high LET radiation (acute and chronic exposure)

Dose estimation of acute vs chronic exposure for low and high LET radiation

\[
Y = c + \alpha D + \beta D^2
\]

\[
Y = c + \alpha D
\]
Development and validation of biological assays for rapid dose assessment in radiation accidents (mass casualties)

1. Assessment of low <1 Gy, as well as high >3 Gy doses of Low LET radiation

2. Rapid assessment (short culturing time, low number of cells to be analyzed)

3. Discriminating between whole and partial body exposure
Experimental designs:

Irradiation: X-rays at doses of 5 and 8 Gy (2 Gy/min)

Irradiated lymphocytes mixed with different proportions with unirradiated lymphocytes, as follows: 0 (i.e. 100% irradiated fraction), 3%, 5%, 10, 20%, 30%, 50%, 80% and 90% (i.e. 10% irradiated fraction).
A. Interphase nuclei fused with B. Mitotic cells in 1 hr (PEG) lead to C. PCC of A

Panthelias and Maillie, 1986; Darroudi and Natarajan, 1989; Vyas et al., 1991; Darroudi et al., 1998a,b, 2001; 2005, 2008.
Assessment of whole and partial body exposure

Metaphase

MN in Binucleated cells

Premature Chromosome Condensation

(A) A metaphase; arrow indicates a tricentric chromosome. (B) A binucleated cell; arrows indicate position of two micronuclei. PCC of normal (C) and X-irradiated (D) monkey G_0 lymphocytes.

F. Darroudi et al., Int. J. Radiation Biology, 1998, 74(2) 207-215; F. Darroudi et al., 2008 (IJRB, in press).
A comparative study between, Dicentric, MN and PCC assays for detecting accurately whole and partial body irradiation (*in vitro*, at 8 Gy X-rays)

<table>
<thead>
<tr>
<th>True Irradiated fraction</th>
<th>Estimated fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCC /Dicentrics / MN</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>30</td>
<td>28 7 8</td>
</tr>
<tr>
<td>50</td>
<td>47 15 15</td>
</tr>
<tr>
<td>70</td>
<td>72 35 30</td>
</tr>
<tr>
<td>90</td>
<td>87 66 48</td>
</tr>
<tr>
<td>97</td>
<td>97 100 78</td>
</tr>
</tbody>
</table>

**Conclusions:** PCC could accurately estimate the fraction of irradiated cells (independent of fractions irradiated). Unlikely dicentric and MN analyses consistently underestimate, namely due to cell killing and mitotic delay effects that are operating at high exposure levels.

Application of PCC assay for dose assessment in cases of mass casualties

To simulate a radiation accident, human lymphocytes irradiated \textit{(in vitro)} with different doses of X-rays and PCC performed at different intervals 0, 1 and 7 day(s).

1 Gy X-rays:

- \textbf{PCC}: 3.5 Br./Cell
- \textbf{Dic.}: 0.25 Br./Cell
- \textbf{MN}: 0.15 Br./Cell

Three calibration curves were established.
The "problem" at low doses

Epidemiological models use human population exposures and outcomes.

Models confirmed to an extent by animal research.

Molecular and cellular data are not fully utilized.

(LNT: a testable hypothesis)

0.05 & 1 mGy/day for 400 days - no effect

20 mGy/day for 400 days – Neoplasms (Tanaka et al., 2007)

The "Gold Standard" A-bomb Survivors

Supra-Linear

Linear

Low Dose Extrapolations

5-10% Cancer Risk

Dose (Sv)
Frequency and shape of dose response curve at low dose levels of X-rays, in human lymphocytes: Using PCC technique

X-rays-induced Premature Condensed Chromosomes in G0 lymphocytes:
Frequency of PCC at low doses, immediately following exposure

Supra-linear!
A Linear dose effect relationship is found for low doses of X-rays in G2 assay, correlated with impairment of DSB repair.

G2-assay to assess low doses radiosensitivity in normal VH10 and Artemis-1 fibroblasts.

Origin of chromosome aberration formation
(Simple Chromosome Exchanges)

Stable (Ai, Aiii, and Bi) and unstable aberrations (Aii and Bii)
Application of Fluorescence *in situ* hybridization-based translocation assay for Biological Dosimetry (acute and chronic exposure)
Human cell with an apparently reciprocal chromosome translocation (arrows) detected by fluorescence *in situ* hybridization (FISH) using whole chromosome paints. Chromosome pairs 1, 2, and 4 are painted red, and 3, 5, and 6 are painted green.

A.J. Sigurdson et al., Mutat Res. 2008 Apr 30;652(2):112-121
Detection of translocations: Application of multi-color FISH plus a pan-centromeric probe

*Chromosomes labeled, 1, 4, 8 plus CP*

F. Darroudi et al., Int. J. Radiat. Biol. (in press)
Age-specific mean translocation frequencies were slightly lower when data were limited to 300 GEs, however a model with a slope and curvature term continued to fit the data best.

A.J. Sigurdson et al., Mutat Res. 2008 Apr 30;652(2):112-121
Race significantly modified the age and translocation frequency relationship

Age-specific translocation frequencies by race for those with 300 or more cell equivalents scored.

- Asian (n = 41)
- Black (n = 256)
- White (n = 1064)
- Others (n = 39)
FISH-based translocation is a suitable assay for retrospective Biological Dosimetry

Application of FISH chromosome painting for quantification of past radiation exposures/ persistence of translocations

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Radiation exposure</th>
<th>Time since exposure or accumulated (years)</th>
<th>The basis for comparison</th>
<th>Long-term stability of translocations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>accident, tritium</td>
<td>6</td>
<td>urine</td>
<td>yes</td>
<td>Lucas et al., 1992</td>
</tr>
<tr>
<td>4</td>
<td>occupational</td>
<td>36 (acc.)</td>
<td>Film dosimetry</td>
<td>yes</td>
<td>Staume et al., 1992, 1995</td>
</tr>
<tr>
<td>1</td>
<td>accident, tritiated water</td>
<td>11</td>
<td>Dicentrics, initial yield, urine</td>
<td>yes</td>
<td>Lloyd et al., 1998</td>
</tr>
<tr>
<td>4</td>
<td>Accident, Y-12</td>
<td>30</td>
<td>in vitro curve</td>
<td>no</td>
<td>Lucas et al., 1992</td>
</tr>
<tr>
<td>2</td>
<td>Goiania accident</td>
<td>1</td>
<td>Dicentrics, initial yield</td>
<td>no</td>
<td>Straume et al., 1991</td>
</tr>
<tr>
<td>15</td>
<td>Goiania accident</td>
<td>5</td>
<td>Dicentrics, initial yield</td>
<td>no (&gt;1 Gy) yes (&lt;0.8 Gy)</td>
<td>Natarajan et al., 1994</td>
</tr>
<tr>
<td>24</td>
<td>Goiania accident</td>
<td>8</td>
<td>Dicentrics, initial yield</td>
<td>no (&gt;1 Gy) yes (&lt;0.8 Gy)</td>
<td>Natarajan et al., 1998, Darroudi, 1999, Darroudi &amp; Natarajan, 1999</td>
</tr>
<tr>
<td>61</td>
<td>Hiroshima workers</td>
<td>50</td>
<td>DS 86</td>
<td>yes</td>
<td>Salassidis et al., 1995</td>
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<tr>
<td>75</td>
<td>Mayak nuclear-workers</td>
<td>35</td>
<td>Film dosimetry</td>
<td>no</td>
<td>Lindholm et al., 1998</td>
</tr>
<tr>
<td>12</td>
<td>Chernobyl accident</td>
<td>5-8</td>
<td></td>
<td>yes</td>
<td>Darroudi, 1999, Darroudi &amp; Natarajan, 1999</td>
</tr>
<tr>
<td>4</td>
<td>Estonian accident</td>
<td>0-2</td>
<td>Film dosimetry</td>
<td>yes</td>
<td>Darroudi, 1999, Darroudi &amp; Natarajan, 1999, Granath, Darroudi et al., 1996, Lloyd et al., 1996, Lindholm et al., 1998</td>
</tr>
<tr>
<td>26</td>
<td>Estonian cleanup workers</td>
<td>8</td>
<td>Film dosimetry</td>
<td>no</td>
<td>Darroudi, 1999, Darroudi, and Natarajan, 1996,1999, Granath, Darroudi et al., 1996, Lloyd et al., 1996</td>
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<tr>
<td>60</td>
<td>Chernobyl cleanup workers</td>
<td>8</td>
<td>Film dosimetry</td>
<td>no</td>
<td>Darroudi, 1999, Darroudi, and Natarajan, 1996,1999, Granath, Darroudi et al., 1996, Lloyd et al., 1996</td>
</tr>
<tr>
<td>118</td>
<td>Estonian cleanup workers</td>
<td>10</td>
<td>Film dosimetry</td>
<td>no</td>
<td>Littlefield et al., 1998</td>
</tr>
</tbody>
</table>

*F. Darroudi Guest Editor (IJRB, 2009 in press.): Special Issue on Biological Dosimetry
*C. Whitehouse et al. (2005) Int. J. Radiation Biology 81, 139-145.
Development of multi-color FISH, COmbined Binary RAtio labeling technique (COBRA)

J. Wiegant et al., Genome Res., 10, 861-865, 2000;
Detection of X-ray-induced chromosome aberrations in human lymphocytes, using COBRA-FISH - For retrospective dosimetry (acute and chronic exposure)

Radiation accident ($^{60}$Co) in Istanbul (Turkey)

December 2002

Blood samples collected in January 2003 from 10 individuals

Dicentric, Micronucleus and FISH-based translocation assays were employed to obtain a dose estimate immediately, and retrospectively

A comparative study
Immediate dose assessment using Dicentrics, but has no potential for retrospective dosimetry

Istanbul follow up studies

Cobalt-60 exposure

Data indicate that irrespective of estimated dose of exposure, dicentric frequency declined quickly with post-exposure time.

The half-life is estimated to be around 120 days.
Immediate dose assessment using Micronuclei but has no potential for retrospective dosimetry.
FISH based translocation assay is suitable for dose assessment immediately as well as retrospectively.
Using FISH assay for retrospective Biological dosimetry at low and high doses of low LET radiation

*Istanbul follow up studies
Persistance of Translocations

*Tralocations persisted at different post-exposure times. Slight reduction in the frequency at first 4 months could be due to the existence of unstable aberrations in cells having stable ones.

*The frequency of stable translocations remained constant in stable cells at different post-exposure times.
Mayak cohorts: 24,500 registered, 7,500 dead at the end of 2004.

Mayak workers employed at the main plants (reactor, plutonium, radiochemical) on 1948-1958 (average age at the time of hiring was 20 years old). Most of them worked for a long period (25-30 years), up to 1982.

Consequently, they were exposed to chronic radiation at very low dose rate in a wide range of recorded doses (accumulated dose range of 0.03 – 4.5 Gy).
Mayak cohorts: Comparison between FISH and physical dose estimates

Data generated so far are novel, and for the first time revealed that FISH assay is a useful biological system to be applied for retrospective individual dosimetry following exposure to ionizing radiation (occupationally) at low doses as well as at very low dose rates.

**Cytogenetic dose:**
The measured translocation yield (FISH) was converted to dose to the bone marrow using a standard calibration curve for chronic exposure and age-dependent control yield was considered.

**RBM dose:**
Physical estimates of bone marrow dose based on film badge measurements.

**Conclusion:**
The agreement between the two estimates of bone marrow dose was confirmed in 75% of cases.

<table>
<thead>
<tr>
<th>ID No.</th>
<th>Genome</th>
<th>Translocations</th>
<th>Cytogenetic dose (Gy)</th>
<th>SE (Gy)</th>
<th>RBM dose (Gy)</th>
</tr>
</thead>
</table>
| 8677   | 1395    | 65             | 0.047                 | 2.1     | 0.5          | 1.92 (
| 8678   | 1047    | 37             | 0.035                 | 1.4     | 0.6          | 1.63 (
| 8679   | 1047    | 54             | 0.052                 | 2.6     | 0.5          | 2.32 (
| 8680   | 837     | 56             | 0.067                 | 3.5     | 0.6          | 3.02 |
| 8681   | 2091    | 100            | 0.048                 | 2.1     | 0.6          | 1.82 |
| 8682   | 2094    | 55             | 0.026                 | 0.7     | 0.4          | 2.81 |
| 8683   | 1032    | 34             | 0.033                 | 1.4     | 0.4          | 2.06 |
| 8684   | 1375    | 30             | 0.022                 | 0.6     | 0.3          | 0.82 |
| 8685   | 1032    | 30             | 0.029                 | 1.1     | 0.4          | 1.41 |
| 8688   | 1395    | 32             | 0.023                 | 0.6     | 0.3          | 0.93 |
| 8689   | 1395    | 67             | 0.048                 | 2.2     | 0.6          | 2.28 |
| 8690   | 1264    | 70             | 0.055                 | 2.7     | 0.6          | 2.45 |
| 8708   | 1047    | 53             | 0.051                 | 2.5     | 0.5          | 1.83 |
| 8710   | 1047    | 31             | 0.030                 | 1.0     | 0.4          | 2.22 |
| 8748   | 1280    | 52             | 0.041                 | 2.0     | 0.6          | 1.75 |
| 8749   | 1534    | 43             | 0.028                 | 1.0     | 0.4          | 2.74 |
| 8750   | 1718    | 34             | 0.020                 | 0.4     | 0.4          | 0.78 |
| 8751   | 1047    | 34             | 0.032                 | 1.4     | 0.4          | 1.90 |
| 8752   | 1032    | 27             | 0.026                 | 0.7     | 0.3          | 0.54 |

**Explanation of the discrepancies between biological- and physical- dose estimates:**
High-energy β-rays (that hardly reach the target cells) were recorded by the badges before 1960 and thus might have give rise to lower-than expected ChA frequency.
Mayak cohorts: Induction of numerical aberrations was evident

Extra copy of #8

Acentric fragment of chromosome # 8, 46 centromeres, acentric – 47th.

2 Extra copies of #4

Truncated # 4 (46 centromeres)
Conclusions:

*The measurement of translocation using FISH is able to estimate the average doses to the bone marrow of an individual. *Studies performed thus far validating the FISH translocation assessment protocols give reasons to be optimistic that it is a reliable system for retrospective biological dosimetry even at low doses (<1 Gy).

• However, a number of parameters requires further investigations with regard to the persistence of stable translocations with time after exposure, and to shorten time necessary to score adequate number of cells:

* Influence of internal contamination.
* The occurrence of expanding clones of aberrant cells \textit{in vivo}.
* Influence of partial-body exposure
* Development and validation of automatic analyzer system
Correlation between exposure to low dose of radiation, and higher incidences of chromosomal aberrations and cancer


The Techa River cohort provides strong evidence that low-dose, low-dose rate exposures lead to significant increases in solid cancer risks that appear to be linear in dose. The results do not suggest that risks associated with low-dose rate exposures are less than those seen following acute exposures such as were received by atomic bomb survivors.
Hall mark(s) of high LET radiation?
Occurrence of three or more breaks in two or more chromosomes will lead to formation of complex translocations.

Detection of Complex chromosome exchanges, using COBRA-MFISH:

Arrow indicates the position of a centromere of a specific chromosome.

(Darroudi et al., IJRB, 2008, in Press)
Complex chromosome exchanges and insertions are hall-marks of high LET Neutron radiation.

Darroudi et al., Rad. Prot. Dos., 2002; Darroudi et al., IJRB (in press)

Hallmark of high LET (Neutron) radiation?
1. Complex exchanges
2. Insertions
Heavy ions, neon-induced chromosomal aberrations in human lymphocytes: Frequencies and Spectra

Spectra of Neon induced translocations

F. Darroudi et al., IJRB, 2008 (in press)
Development and Application of PCC and M-FISH to analyze chromosomal aberrations in normal cells and primary tumors

Bezrockove ...Darroudi, Genes, Chromosomes & Cancer 38, 177-186 , 2003; Darroudi et al., Cancer Letters, 2008.
Detection of C-Myc Oncogen in a normal Metaphase

Cobra MFISH combined with the detection of the C-Myc oncogen visualized with indirect Cy7
FISH-based translocation assay on Germ Cells:
Sequential G-banding FISH on human sperm chromosomes

Fig. 1: (a) Shows the occurrence of two breaks in two different chromosomes, this leads to the formation of either one reciprocal translocation (b) or one dicentric with one acentric fragment (c). Two breaks in one chromosome (d) may lead to ring formation (e). However, if three or more breaks occur in two or more chromosomes (f and h) this will lead to the formation of complex-type translocations (i.e. g, j and k).
Detection of Pericentric inversion using arm specific probe

Bezrookove et al., Genes, Chromosomes & Cancer 38, 177-186, 2003; F. Darroudi et al., Cancer Letters, 2008 (in press)
Detection of Para-centric inversion using bands specific probe

Chromosome 5
INVERSION (a marker for Leukemia)
Leiden University Medical Centre:

Janna Fomina
Matty Meijers
V. Bezrookove
Y. Xiao
H. Tanke

A.T. Natarajan (The Netherlands)
G. Koksal (Turkey)
A. T. Ramalho (Brazil)
H.J. Oh; M.S. Kang (South Korea)
A. Mahmoudzadeh (Iran)
All members of EU COD consortium
T. Azizova, A. Vozilova, A.A. Edwards (SOUL consortium)

Financial Support from European Union: COD, RISCRAD and SOUL Projects
Thank You For Your Attention

Tulips banding patterns

Noordwijkerhout - The Netherlands

Chromosomes banding patterns

#2 #6 #16

#5 #5 #5