IAEA CRP E3.5008

RC17076 (R0)

STRENGTHENING OF "BIODOSIMETRY" IN DALAT NUCLEAR RESEARCH INSTITUTE

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Objectives of RC17076 (R0)

Improvement of dicentric assays, micronuclei assays,
development of FISH-translocation analysis and PCC technique
and intensification of collaboration and networking

THE FIRST MISSIONS OF RC17076
(RC17076 R0)

1. Upgrading of laboratory:

Building the new biodosimetry lab. In Nuclear Research Institute area: ~250 – 300 m^2 (biotechnology buiding ~700 m^2).

2. Upgrading of technique:

Developing of Micronuclei (MN) and Premature Chromosome Condensation (PCC) techniques.

3. Database on calibration curve:

Presentation of dose – effect calibration curve for dicentrics induced in lymphocytes exposed to thermal neutron at chanel No.3 of Dalat Nuclear Reactor.

Abstract:

The upgrading of biodosimetry lab., including foundation, equipment, technique and manpower are planted to satisfy the missions of RC 17076 "Strengthening of "Biological Dosimetry" at Dalat Nuclear Research Institute: Improvement of Dicentric Assays, Micronuclei Assays, Development of FISH-Translocation Analysis and PCC Technique and Intensification of Collaboration and Networking" with the missions of the first year RC 17076 (R0) were building the new biodosimetry lab., preparing the slides for MN analysis and PCC analysis, generating the calibrated curve dose-effect of dicentric aberration for thermal neutron plus gamma rays source at Dalat Nuclear Reactor.

The new biodosimetry lab. Were built in the Dalat Nuclear Reactor area, six rooms have been designed for developing Radio.Bio.Med.Pharmaceitical Section included biodosimetry.

The new protocol of Micronuclei slides were successful and have been applied for gamma rays dose 3 Gy. The result of PCC slides were not yet so good.

Abstract

Calibration of dose-effect was conducted with radiations of 3th chanel of Dalat Reactor: Thermal neutron flux ϕ_{th}=52.19x10^6n/cm^2/s, neutron resonant flux: ϕ_{epi}=1.43x10^6n/cm^2/s, and gamma rays dose rate: 0.02 mGy/s. Component of total dose in Gy was included 87.5% thermal neutron, 2.40% resonant neutron and 10.10% gamma rays.

Whole blood of each combination was separated and screened in 9 dose points in limited doses 0-6 Gy. 11 combinations were tested. Checking the dicentric distribution in each metaphase was presented that this fit a Poisson distribution, the evidence of irradiation in the uniformed dose fields. The relative biological efficiency (RBE) for creating dicentric was depended on exposed doses. The linear correlated coefficient was determined r (y,d) = 0.582 ± 0.084, along with the crude experimental curve have identified the general quadratic equation $y = \alpha D + \beta D^2 + C$. The experimental regression coefficients were determined with $\alpha = 4.291 \pm 2.125$, $\beta = 1.496 \pm 0.249$ and $C = 1.284 \pm 0.609$ ($\alpha = 10^{-2}$ x Gy^{-1}; $\beta = 10^{-2}$ Gy^{-2}).

The calibration equation of dose-effect was $y = 4.291D + 1.496D^2 + 1.284$.

UPGRADING OF LABORATORY

- A new house for radiobiological department have been put into use in December 2012. The Radio.Bio.Med.Pharmaceutical Section is responsible for the development of celline culture with applications in biodosimetry, testing and evaluating the targeted isotope carriers, the targeted pharmaceutical carriers. Six rooms have been designed for developing Radio.Bio.Med.Pharmaceitical Section.

- Biodosimetry laboratory are upgrading by investing of VINATOM with ~ 400 000 USD for equipments included Analysis Autosystem with M-Search sofware.

Front of house

Back of house

Radio.Bio.Med.Phar. Center

New house of Radiobiological Department and Radio.Bio.Med.Pharmaceutical Section.

Developing of Rdio.Bio.Med.Pharmaceutical field

1. Celline culture.

2. Biodosimetry.

Using multimarkers for biodosimetry:

- *Deal to accidents*
- *Medical management of radiation workers in the hospital, engineering.*

3. Isotope carriers: (monoantibodies and lyposome).

For diagnosistic and therapeutic of cancer

4. Primary cells:

Produce the biosuplies for isotope carriers

MICRONUCLEI (MN) TECHNIQUE

1. Test for hypotonic.

Test 1:

- Full steps of whole blood cuture for Micronuclei.

-Full steps of fixation with KCl 0.075 M and cacnoa 3/1 (methanol / acid acetic with ratio 3/1) for Micronuclei.

Test 2:

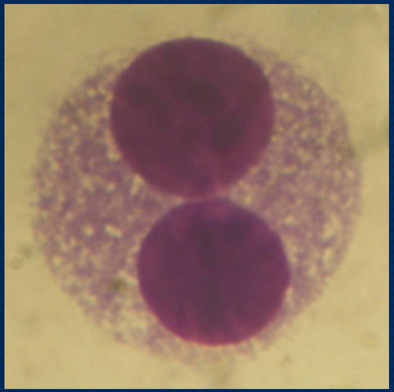
- Full steps of whole blood cuture for Micronuclei.

-Full steps of fixation with KCl 0.075 M; Cacnoa 10/1 (methanol / acid acetic with ratio 10/1); Ringer (9.0 g NaCl; 0.42 g KCl; 0.24 g $CaCl_2$ in 1000 ml DW); Solution cacnoa & ringer (cacnoa 10:1 / ringer with ratio 1/1).

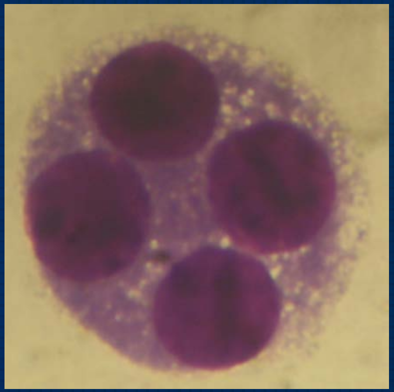
** Along with changing fixed solution and hypotonic treatment time, test 2 had given the best result on binuclei. Hypotonic way was important for micronuclei technique.*

2. Test for time of culture.

Mononuclei



Binuclei



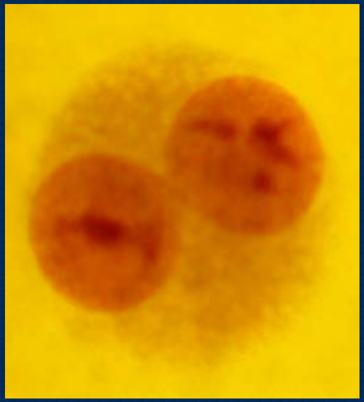
Tetranuclei

Gamma doses (Gy)	Cultural time	Number of cells	Mononuclei (%)	Binuclei (%)	Tetranuclei (%)
0	72h00	3225	2608 (80.87)	540 (16.74)	77 (2.39)
0	72h30'	2054	1372 (66.8)	618 (30.1)	64 (3.1)
3.0	72h00	1170	1060 (90.6)	88 (7.5)	22 (1.9)
3.0	72h30'	1373	1234 (89.9)	124 (9.0)	15 (1.1)

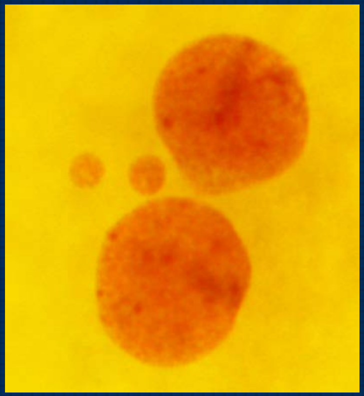
** Test 2 bester than test 1. Procedure of test 2 have been used for MN technique.*

** Binuclei in cultural time 72h30' higher than (near 2 folds) that in cultural time 72h,*

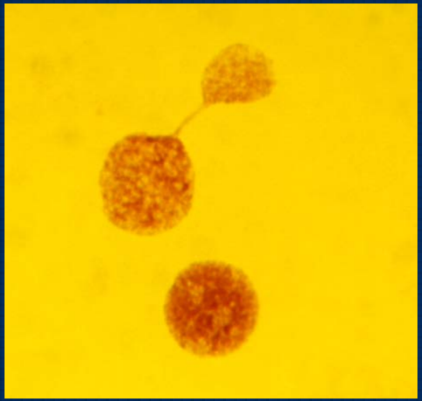
MN analysis and it distribution in lymphocytes exposed gamma rays 3.0 Gy



Binuclei



Micronucleis



Nuclei bridge

Gamma rays doses (Gy)	Cultural time	Binuclei	Nuclei bridge	Distribution of Micronuclei			
				0	1	2	3
0	72h00	565	-	564	1	-	-
0	72h30’	313	-	512	1	-	-
3.0	72h00	515	5	420	76	17	2
3.0	72h30’	711	3	615	75	17	4

- *Nuclei bridge was detected with frequency about 0.66%), the frequency was not correlation with dicentric frequency that analysed by CA analysis from gamma rays dose 3.0 Gy (~ 42%).*

It means that micronuclei included dicentrics and fragments (it is basic of analysis dicentric analysis from MN slides).

PREMATURE CHROMOSOME CONDENSATION (PCC) TECHNIQUE

Objectives: *Improve PCC technique in G1 phase of human lymphocytes with Caliculine A.*

Design experiment: Using 12 combinations (1-12) for testing:

1. Treated with Caliculine A 50 μM / 60' in whole blood cultured 0'
2. Treated with Caliculine A 50 μM / 60' in whole blood cultured 60'
3. Treated with Caliculine A 50 μM / 60' in whole blood cultured 180'

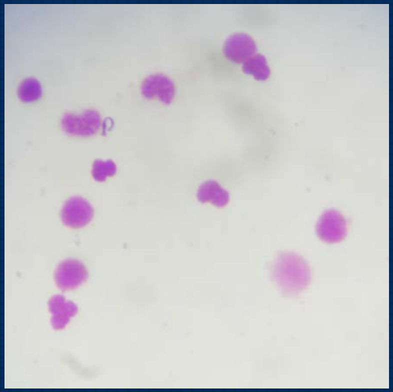
4. Treated with Caliculine A 50 μM / 180' in whole blood cultured 0'
5. Treated with Caliculine A 50 μM / 180' in whole blood cultured 60'
6. Treated with Caliculine A 50 μM / 180' in whole blood cultured 180'

7. Treated with Caliculine A 80 μM / 60' in whole blood cultured 0'
8. Treated with Caliculine A 80 μM / 60' in whole blood cultured 60'
9. Treated with Caliculine A 80 μM / 60' in whole blood cultured 180'

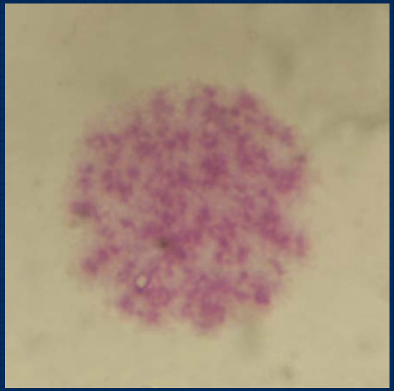
10. Treated with Caliculine A 80 μM / 180' in whole blood cultured 0'
11. Treated with Caliculine A 80 μM / 180' in whole blood cultured 60'
12. Treated with Caliculine A 80 μM / 180' in whole blood cultured 180'

Experimental results

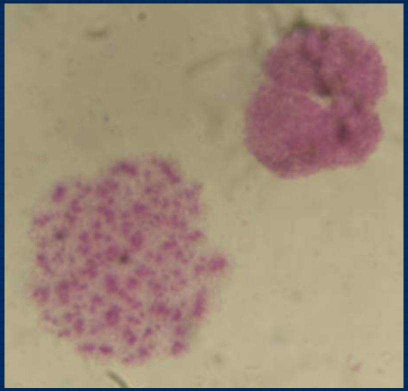
- The lymphocytes with PCC were detected in all 12 combinations.
- The questions on PCC effects have to answer: The difference between treatments caliculine A on and after cultured 60'; The difference between the caliculine A concentrations 50 and 80 μM; The difference between treatment times 60' and 180'.



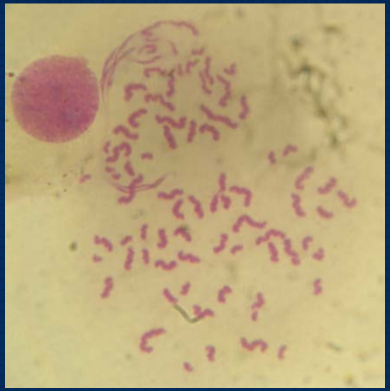
Only a few PCC



G1 PCC



G1 PCC



G2 PCC

The result was not yet give a good effect, We need an assistance from IAEA expert.

DATABASE ON CALIBRATION CURVE

Objectives:

Presentation of dose – effect calibration curve for dicentrics induced in lymphocytes exposed to thermal neutron at No.3 chanel of Dalat Nuclear Reactor such as a part of biodosimetry database to deal with radiation accident in future.

Objects and methods:

Objects:

- *Radiation source and dosimetry: Radiation source of the channel No.3 of Dalat Nuclear Reactor included thermal neutron plus gamma rays: Thermal neutron flux ϕ_{th}=52.19x10^{6}n/cm^2/s, neutron resonant flux: ϕ_{epi}=1.43x10^{6}n/cm^2/s, and gamma rays dose rate: 0.02 mGy/s. Component of total dose in Gy was included 87.5% thermal neutron, 2.40% resonant neutron and 10.10% gamma rays.*

- *Lymphocyte: Whole blood from 11 donors who were the healthy persons in year older from 25-40. All donors had tested dicentric per 1000 metaphases, the donors who had a dicentric / 1000 metaphases had to exclude.*

Methods:

1. Design of combination for exposing:

Blood of each healthy donor was sheared to 9 tubes (1 ml) for exposing by 8 supposing dose points in a range 0 to 6 Gy (combination). 11 combinations had been used for calibration of dose-effect. The absorbed doses were measured by nuclear physics and physical dose assessments.

2. Physical dosimetry:

The component of radiations and the component of radiation dose were counted by nuclear physicals of Nuclear Research Institute.

3. Lymphocytes culture:

All blood from each exposed tubes were cultured in plastic tubes with RPMI 16040 medium, PHA and FCS (sigma), incubated 48 hours/37^{o}C.

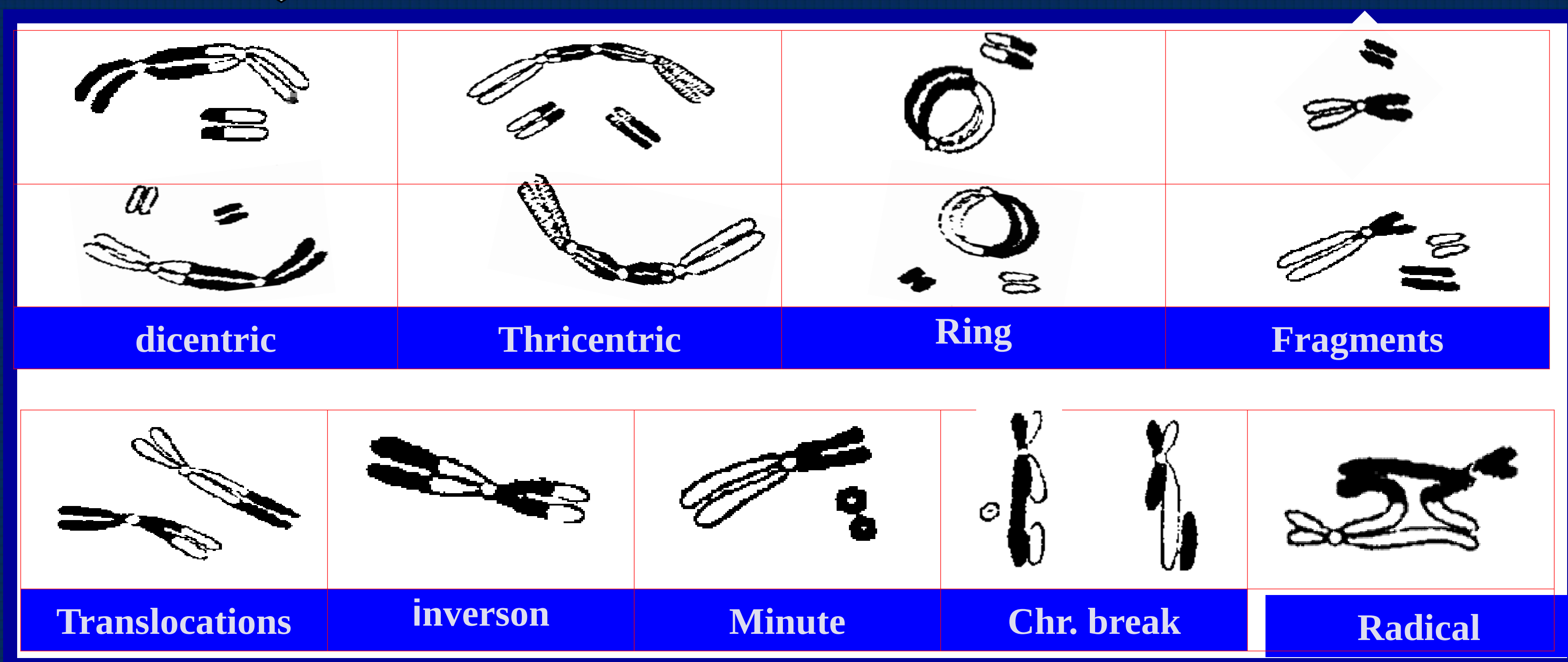
4. Chromosome aberration analysis:

Dicentric analysis relied on centromere, number of fragments and classification of Savage.

Dicentric analysis:

Normal chromosomes

Chromosome aberrations



5. Mathematic and statistic:

- Average, standard deviation, t-test.

- Test of Poisson distribution.

- Relative Biological Efficient (RBE) and RBEo.

- Test for distribution coefficients to find general equation.

Using linear relative coefficient $r(y,d) = [(\sum D_i Y_i - n.D.Y)] / [(\sum D_i^2 - D^2)^{1/2} . (\sum Y_i^2 - Y^2)^{1/2}]$*, and the experimental dose-effect curve.*

+ The general equation in linear form $y = kD + C$ *will be choose with* $r(y,d) \geq 0,8$.

+ The general equation in quadratic form $y = \alpha D + \beta D^2 + C$ *will be choose with* $r(,d) \leq 0.6$.

- Analysis of experimental regression equation: Finding the linear coefficient (α*) and square coefficient (*β*) and showed the dose-effect regression calibration equation:*

Solve the set of 3 equations:

$$cn + a\Sigma D + b\,\Sigma D^2 = \Sigma y \quad (1)$$
$$c\Sigma D + a\Sigma D^2 + b\Sigma D^3 = \Sigma yD \quad (2)$$
$$c\Sigma D^2 + a\Sigma D^3 + b\Sigma D^4 = \Sigma yD^2 \quad (3)$$

Or

$$cn + \alpha\Sigma D_i + \beta\Sigma D_i^2 = \Sigma y_i \quad (1')$$
$$c\Sigma D_i + \alpha\Sigma D_i^2 + \beta\Sigma D_i^3 = \Sigma y_i D_i \quad (2')$$
$$c\Sigma D_i^2 + \alpha\Sigma D_i^3 + \beta\Sigma D_i^4 = \Sigma y_i D_i^2 \quad (3')$$

Results and discussions

Table 1:

Com.	Parameters	Exposed dose points								
		1	2	3	4	5	6	7	8	9
I	Dose (Gy)	0	0.158	0.406	0.79	1.58	2.34	3.111	4.79	6.34
	Dicentric (%)	0	1.05	2.86	6.38	12.50	17.65	27.03	52.17	89.36
II	Dose (Gy)	0	0.139	0.367	0.78	1.50	2.25	3.00	4.69	6.15
	Dicentric (%)	0	0.86	3.00	8.00	12.70	19.61	28.57	56.60	87.88
III	Dose (Gy)	0	0.177	0.415	0.82	1.67	2.45	3.21	4.99	6.54
	Dicentric (%)	0	1.14	4.20	8.24	15.38	20.11	43.90	62.22	95.83
IV	Dose (Gy)	0	0.168	0.418	0.805	1.73	2.47	3.276	5.07	6.16
	Dicentric (%)	0	0.74	2.65	5.48	12.50	23.29	23.14	52.17	83.05
V	Dose (Gy)	0	0.135	0.363	0.727	1.40	2.17	2.946	4.51	6.17
	Dicentric (%)	0	0.80	3.52	5.06	15.09	19.69	27.27	47.06	89.36
VI	Dose (Gy)	0	0.176	0.424	0.821	1.72	2.58	3.23	5.00	6.34
	Dicentric (%)	0	0.70	5.17	8.94	21.67	22.95	36.73	61.90	100.0
VII	Dose (Gy)	0	0.168	0.408	0.805	1.66	2.44	3.22	4.87	6.46
	Dicentric (%)	0	0.87	3.26	7.32	20.50	19.44	34.05	59.64	90.38
VIII	Dose (Gy)	0	0.167	0.418	0.805	1.61	2.44	3.04	4.98	6.46
	Dicentric (%)	0	0.85	3.26	6.90	11.11	16.85	21.95	47.27	85.42
IX	Dose (Gy)	0	0.146	0.374	0.748	1.55	2.12	2.89	4.56	6.12
	Dicentric (%)	0	0.88	3.40	5.38	16.26	19.48	27.58	46.35	90.31
X	Dose (Gy)	0	0.136	0.362	0.771	1.49	2.12	2.94	4.57	6.01
	Dicentric (%)	0	0.91	3.26	7.75	11.54	18.76	26.67	57.51	89.63
XI	Ddose (Gy)	0	0.179	0.419	0.815	1.67	2.56	3.13	4.90	6.56
	Dicentric (%)	0	0.92	3.16	6.67	10.00	15.12	22.50	43.10	80.43
Aver.	Gy		0.159± 0.017	0.398± 0.025	0.790± 0.030	1.598± 0.105	2.358± 0.169	3.090± 0.135	4.812± 0.200	6.328± 0.181
	Di.%		0.884± 0.126	3.431± 0.700	6.92± 1.269	14.477± 3.786	19.359± 2.380	30.854± 10.061	53.272± 6.727	89.241± 5.424

Checking the uniform ability of exposed dose field

Using poisson test to check the distribution of dicentric among metaphases, 10 random samples screened in different exposed doses of 11 combinations were used for testing. Total of 1733 dicentrics distributed in 426 cells showed in table 2.

Table 2. Distribution of dicentric per cell .

Dicentrics / cell	0	1	2	3	4	5	6	7	8	9	10	Σ=1733
Number of cells	5	22	53	86	100	75	42	19	14	5	1	Σ=426

Hypothesis of Poisson distribution was done according to standard of Chi square (χ^2) with formula $\chi^2 = \sum_i^k \chi_i^2 = \sum_i^k [(mi - npi)^2/np_i]$ (i: i = 0, 1, 2, 3 … is natural number showed number dicentric in a cell; mi is the number of cells have i dicentrics; n is the total cells analyzed; pi is the theory numerical value of Poisson, $p_i = N'x/N = e^{-\lambda} . \lambda^x/x!$; λ: the average frequency of dicentrics per cell, $\lambda = \sum m_i/n$)

Table 3. Chi square (χ^2) counted from experimental data of table 2.

Dicentrics / cell	0	1	2	3	4	5	6	7	8	9	10
χ^2	0.71	1.97	0.88	0.23	3.43	0.81	0.32	2.18	0.02	0.21	0.87

The parameters of Chi square were $\lambda = \sum mi / \sum n = 1733 / 426 = 4.068$; $e^{-\lambda} = 0.0171$; $\sum \chi_i^2 = 0.71 + 1.97 + 0.88 + 0.23 + 3.43 + 0.81 + 0.32 + 2.18 + 0.02 + 0.21 + 0.87 = 11.63$. Consulting χ^2 table with k = 11, $\alpha = 0.01$ had $\chi^2_{k-1} (\alpha) = 16.9$. The data presented $\sum \chi_i^2 < \chi^2_{k-1} (\alpha)$, it means that induced dicentrics fited in Poisson distribution.

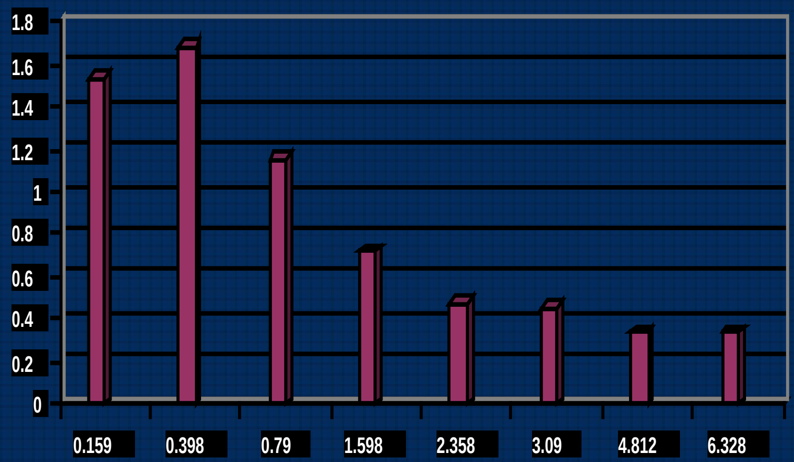
This result ensured the reliability of the samples exposed in the uniform radiation field.

Relative Biological Efficiency (RBE)

RBE = fi / f γ (fi: frequency of dicentric due to radiation i; fγ: frequency of dicentric due to the gamma rays in the same dose). The dose-effect calibration curve for gamma rays that reported by Masao Sasaki $y = 2.63D + 6.25D^2 + C$.

Table 4. RBE investigated from radiation source.

Dose (Gy)	0.159	0.398	0.790	1.598	2.358	3.090	4.812	6.328
fi (%)	0.884	3.431	6.92	14.477	19.359	30.854	53.272	89.241
fγ (%)	0.576	2.037	5.978	20.163	40.952	67.802	157.376	266.915
fi/ fγ	1.535	1.684	1.158	0.718	0.473	0.455	0.338	0.334



It showed RBE in the range of doses from 0.159 Gy to 6.328 Gy with top in 0.398 Gy.

The max relative biological efficiency (RBEo):

RBEo = αi / $\alpha\gamma$ (αi: linear regression coefficient of dose-effect calibration equation of experimental radiation source i; $\alpha\gamma$: linear regression coefficient of dose-effect calibration equation of gamma rays $y = 2.63D + 6.25D^2 + C$). The obtained result for the studied source was RBEo = 4.291 / 2.63 = 1.632. The new result from thermal neutron & gamma ray source proved the suitability with reports published by Fabry et al, Bender, Lloyd, Natarajan, Preston and Sasaki for neutron sources.

Result reflected exactly the radiation component nature of investigated source due to RBEo was higher than that for gamma rays but smaller than that for thermal neutron.

The dose–effect calibration equation and curve

The linear correlative coefficient and general equation of dose-effect response

The linear correlative coefficient

- $r(y,d) = [(\sum D_i Y_i - n.D.Y)] / [(\sum D_i^2 - D^2)^{1/2} . (\sum Y_i^2 - Y^2)^{1/2}]$ *or*

- $r(y,d) = [\sum D_i Y_i - (\sum D_i . \sum Y_i) / n] / [(\sum D_i^2 - (\sum D_i / n)^2)^{1/2} . (\sum Y_i^2 - (\sum Y_i / n)^2)^{1/2}]$.

Follow counting table:

No.	mi	Di	Yi	miDi	$miDi^2$	miYi	$miYi^2$	miYiDi
1								
2								
…								
∑	n = ∑mi			1*	2*	3*	4*	5*

With formular: $r(y,d) = [5^* - (1^* . 3^*) / n)] / [2^* - (1^* / n)^2]^{1/2} . [4^* - (3^* / n)^2]^{1/2}$.

Table 4: The results of linear correlative coefficients for 11 combinations.

Coefficient	Combinations						
	1	2	3	4	5	6	7
r(y,d)	0.549	0.548	0.537	0.532	0.553	0.553	0.535
	8	9	10	11			
r(y,d)	0.548	0.746	0.754	0.544			

The linear correlative coefficient was r(y.d) = 0.582 ± 0.084,

r(y,d) value showed that there was a average linear correlation between doses and dicentric frequencies, but diagram (picture 1) of correlation between dose and frequencies of dicentric showed the parabola form for all of 11 combinations. It fited to the low LET radiation for the radiation of No.3 channel of Dalat Nuclear Reactor with component of total dose included 87.5% thermal neutron, 2.40% resonant neutron and 10.10% gamma rays and thermal neutron have energy level ≥0.5 eV. The r (y,d) reflected exactly the effect of low LET radiation.

The general experimental equation had the form of $y = \alpha D + \beta D^2 + C$.

The experimental recurrent coefficients of equation $y = \alpha D + \beta D^2 + C$.

Finding the experimental recurrent coefficients α, β and c of equation $Y = \alpha D + \beta D^2 + C$ was conducted by solving of the set of 3 equations:

$$cn + \alpha \Sigma D + \beta \Sigma D^2 = \Sigma y \quad (1)$$
$$c\Sigma D + \alpha \Sigma D^2 + \beta \Sigma D^3 = \Sigma yD \quad (2)$$
$$c\Sigma D^2 + \alpha \Sigma D^3 + \beta \Sigma D^4 = \Sigma yD^2 \quad (3)$$

Finding the experimental recurrent coefficients α, β and c of equation $Y = \alpha D + \beta D^2 + C$ by following table:

	m_i	$m_i x_i$	$m_i x_i^2$	$m_i x_i^3$	$m_i x_i^4$	$m_i y_i$	$m_i y_i x_i$	$m_i y_i x_i^2$
	-							
	-							
$\sum$	$n = \sum mi$	1*	2*	3*	4*	5*	6*	7*

With the set of three new equations:

$$cn + \alpha.1^* + \beta.2^* = 5^* \quad (1)$$
$$c.1^* + \alpha.2^* + \beta.3^* = 6^* \quad (2)$$
$$c.2^* + \alpha.3^* + \beta.4^* = 7^* \quad (3)$$

Using experimental data of table 1 and using replaced method for solving of the set of 3 equations above showed the values of α, β and C in table 5:

Table 5: The results of the experimental recurrent coefficients of general equation $y = \alpha D + \beta D^2 + C$.

Coefficients	Combinations						
	1	**2**	**3**	**4**	**5**	**6**	**7**
α	3.177	5.169	8.149	2.568	4.254	5.624	6.950
β	1.658	1.448	0.979	1.648	1.553	1.483	1.069
c	1.302	0.926	0.006	1.379	1.543	1.798	0.552
	8	**9**	**10**	**11**			
α	1.489	4.11	4.346	1.365			
β	1.708	1.605	1.726	1.58			
c	2.056	1.603	1.102	1.863			

The experimental recurrent coefficients were: $\alpha = 4.291 \pm 2.125$, $\beta = 1.496 \pm 0.249$ and $C = 1.284 \pm 0.609$ ($\alpha = 10^{-2} \times Gy^{-1}$; $\beta = 10^{-2}\ Gy^{-2}$).

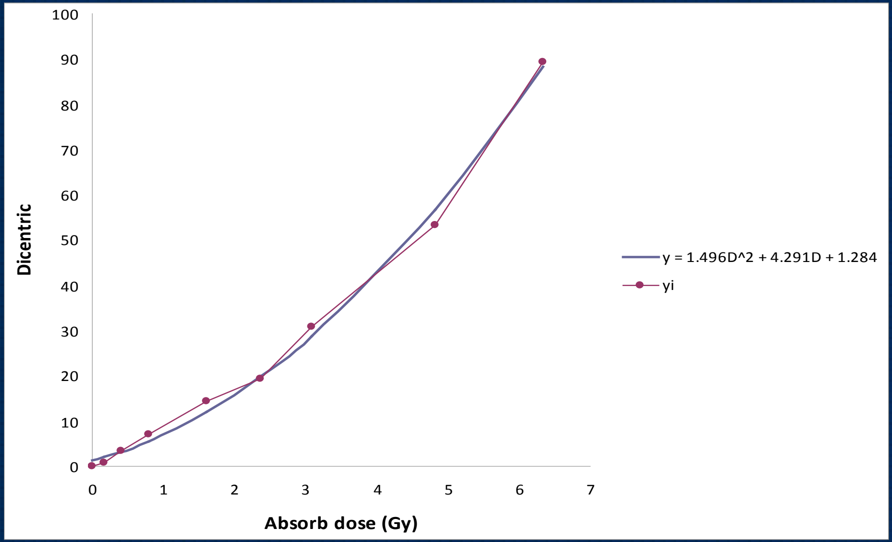
The experimental recurrent equation $Y = 4.291D + 1.496D^2 + 1.284$.

The dose-effect calibration curve

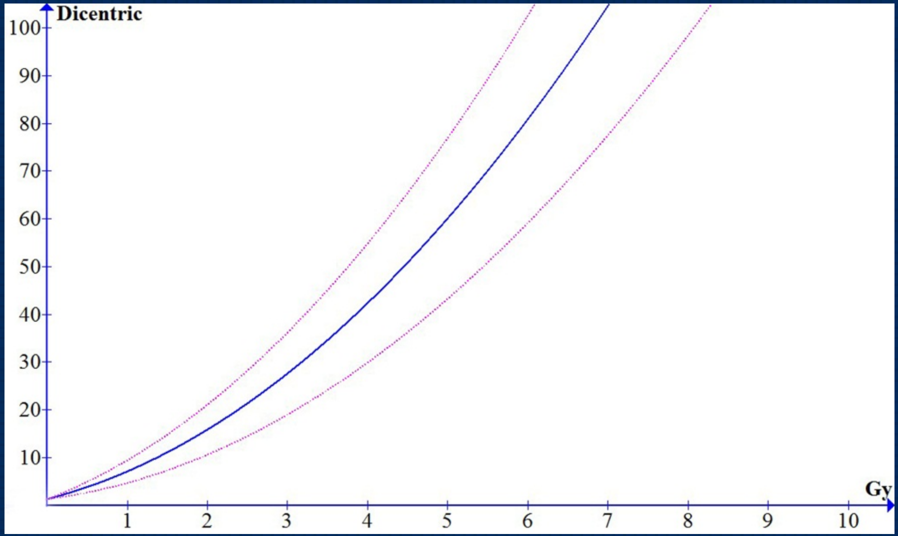
The dose-effect calibration curve was presented by dose-effect correlation data from experimental result of table 1 (average) and experimental recurrent equation.

Dose (Gy)	0	0.159	0.398	0.790	1.598	2.358	3.090	4.812	6.328
Aver.(di.%	0	0.884	3.431	6.92	14.477	19.359	30.854	53.272	89.241
Cali.(di.%	1.284	2.004	3.229	5.608	11.961	19.720	28.827	56.573	88.343

The dose-effect calibration curve was represented in the picture 1 (red: experimental data, blue: graph of calibration equation):



Graph of genaral distribution



and dose-effect calibration curve

The results of the linear relative coefficient, general equation and experimental regression coefficients reflected the radiation nature of investigated source. Radiation source of the channel No.3 of Dalat Nuclear Reactor included thermal neutron plus gamma rays: Thermal neutron flux ϕ_{th}=52.19x10^6n/cm^2/s, neutron resonant flux: ϕ_{epi}=1.43x10^6n/cm^2/s, and gamma rays dose rate: 0.02 mGy/s. Component of total dose in Gy was included 87.5% thermal neutron, 2.40% resonant neutron and 10.10% gamma rays:

** The investigated source had the mix energy of thermal neutron and gamma rays and difference with other reported radiation sources. The data showed the significant difference with the calibration equations for gamma rays or X-rays had been reported and thermal neutron in the radiation component created a difference in the dose-effect calibration equation. However, the result also showed the suitability of the basic principles of radiation effects, such as dependence on LET, dose rate, radiation nature which had investigated before.*

** Considering the radiation component, it showed that energy level of thermal neutron was not high, further neutron dose component accounted only 87.5%, it can be said that the general equation represented by low LET radiation $y = \alpha D + \beta D^2 + C$ was reflected exactly. The ratio $\alpha/\beta = 4.291 / 1.496 = 2.868$ was higher than it in the gamma rays results reported by Bauchinger, Brewen, Fabry, Littlefield, Lloyd. Considering the dose rate, the observed results of α, β and α/β had a difference with the reported results for gamma rays of dose rate 12.5 mGy/s ($\alpha = 1.82$; $\beta = 4.10$; $\alpha / \beta = 0.44$) and 0.036 mGy/s ($\alpha = 0.61$, $\beta = 4.10$ and $\alpha/\beta = 0.12$). This data was explained by the involvement of thermal neutron.*

** The relationship between dicentric frequencies and absorbed doses of thermal neutron & gamma rays showed the mathematical relationship between dicentric frequencies and square of absorbed doses and the quantitative relationship between the values α, β, α/β with LET, nature and dose rate of radiation source. The result was explained satisfactorily when compared with data that reported by Barjaktarovic, Bauchinger, Bender, Biola, Brewen, Fabry, Lloyd, Natarajan, Obe, Prosser, Preston, Sasaki, Schmid, Takahashi.*

Conclusion.

** Calibration of dose-effect was conducted for radiation source of the channel No.3 of Dalat Nuclear Reactor included thermal neutron plus gamma rays: Therma neutron flux ϕ_{th}=52.19x10^{6}n/cm^2/s, neutron resonant flux: ϕ_{epi}=1.43x10^{6}n/cm^2/s, and gamma rays dose rate: 0.02 mGy/s. Component of total dose in Gy was included 87.5% thermal neutron, 2.40% resonant neutron and 10.10% gamma rays.*

** The investigated data of 11 combinations, each combination was exposed to 9 dose points in limited dose 0 to 6 Gy. The dicentric distribution among metaphases of exposed cells was fited a Poisson distribution with p = 99%, the evidence of irradiation in the uniformed dose field.*

** The relative biological efficiency (RBE) for creating dicentric was depended on exposed doses and the nature of radiation. The linear related coefficient r (y,d) = 0.582 ± 0.084.*

** The general equation was quadratic equation $y = \alpha D + \beta D^2 + C$. The experimental regression coefficients were determined $\alpha = 4.291 \pm 2.125$, $\beta = 1.496 \pm 0.249$ and $C = 1.284 \pm 0.609$ ($\alpha = 10^{-2} \times Gy^{-1}$; $\beta = 10^{-2}\ Gy^{-2}$) and the dose-effect calibration equation was presented $Y = 4.291D + 1.496D^2 + 1.284$.*

Strengthning of biodosimetry in future

1. The role of dicentric marker is very important and deciding development of biodosimetry at Nuclear Research Institute. Upgrading of dicentric analysis in CA, MN and PCC slides will be the main mission of biodosimetry laboratory.

2. The missions of biodosimetry laboratory are following:

- The database of the calibration dose-effect for the radiation sources with the specific radiations of nuclear reactor and the specific dose rate of medical radiation.

- The database of chromosome aberration managements for radiation workers .

3. Training: Manpower scholaship is necessary.

Proposal to IAEA

1. Aplication of CA marker needs to recomment in 2 levels:

- Level 1: Enough amount for dose assessment (whole body and organ).

- Level 2: The marker for controling residents

2. IAEA need to recoment on the standards to apply biodosimetry in acut irradiation also cronic irradiation.

3. IAEA need to recoment on using the calibration curve for the other range of dose rate.

3. The relation of Asia biodosimetry laboratories need to organize. We think that Japan is compotable for a centrum of this relation.

1. The plan of RC 17076 (2012 – 2016).

- *Upgrading of laboratories to response the tasks of biodosimetry before the actual and plans to develop nuclear power electric, development so fast of radiation sources for medical, scientific and industrial uses and before the actual effects of specific environmental contamination (a new IAEA TC project will be propose to IAEA).*
- *Implementation of the recommended scientific objectives of the Project:*

** Implement adequate technical information and expertise in LMI-countries to perform biodosimetry and human risk assessment (i.e. in scenarios of environmental, occupational, clinical and accidental exposures to radiation of different qualities at low and high dose levels).*

** Update conventional biological assays and to implement state of the art technology in research centers having expertise on applying different types of cytogenetic assays for biodosimetry immediately and/or retrospectively following (controlled and uncontrolled) exposure to radiation of different qualities.*

** Unify/harmonize technically cytogenetic assays that are applicable for human biodosimetry among different laboratories in the IAEA MSs, and by initiating different networks (national and international collaborations).*

** Initiate and give advice on different relevant research programs in order to enhance the current and future research programs to assess precisely the effect of ionizing radiations and human risk.*

** Monitor individuals exposed to radiation environmentally, occupationally, clinically as well as accidentally.*

** Complement and add to already existing IAEA and WHO activities in this field; RANET (IAEA-Incidence and Emergency Unit, Nuclear safety); BiodoseNet (WHO).)*

2. The missions of RC 17076 (R1, 2013).

- *Install the new devices for Center of Radiation Medical Pharmaceutical Biology at Nuclear Research Institute, Dalat. The total of devices about 400 000 USD will be installing for celline culture, biodosimetry, isotope carrier. The automatic analysis system (metafer) is including in this plan.*

- *Training and using FISH techniques for CA, MN and PCC slides at the laboratory, these technique will be learned by arranging a visit to an IAEA-expert (such as Dr. Darroudi from LUMC, Leiden, The Netherlands).*

- *Generation of calibration curve for low LET radiation (60Co) that is essential for biological dose estimation: for this study lymphocytes from 5-6 donors will be irradiated with different doses in the range of 0.25 up to 6 Gy. This investigation will be performed by using gamma rays (60Co) source at Nuclear Research Institute, Dalat.*

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