IL NUOVO CIMENTO **43 C** (2020) 153
DOI 10.1393/ncc/i2020-20153-9

Colloquia: SIRR 2020

NATO Science for Peace and Security (SPS) project "BioPhyMeTRE": "Novel biological and physical methods for triage in radiological and nuclear (R/N) emergencies"

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received 25 January 2021

Summary. — In case of Radiological and Nuclear (R/N) emergencies, the early knowledge on the individual radiation absorbed dose is of paramount importance for sorting out unaffected subjects from those requiring medical intervention. Retrospective dosimetry by using biological and physical methods aims to prioritize the selection of over-exposed individuals for a rapid triage-dose assessment. In this context, the *BioPhyMeTRE* project focuses on the validation of innovative biological and physical methods allowing a rapid screening/triage of potential victims, by using inexpensive and user-friendly analytical procedures and devices. The biological method combines the two most standardized biodosimetry methods into a single one and the physical technique concerns the use of a low-cost, portable mini photo-luminescence reader for the individual dose assessment by using personal objects that civilians wear or carry every day. Next to the experimental work, the project includes training for the transfer of knowledge and skills among the partners, inter-laboratory exercises for the validation of the analytical procedures and disseminations of the results. The preliminary results of these activities are here reported.

1. – Introduction

Nowadays, the importance of the retrospective dosimetry has been increased, due to constant possibility of accidental over-exposure of the population, either because of a possible terrorist attack using radiological material, an accident in a nuclear power plant or in the case of small-scale accidents in medicine or industry.

At this time, efficient and reliable triage of casualties, using biological, physical and clinical endpoints, able to rapidly identify individuals suspected of over-exposure to life-threatening doses, is urgently needed, as the immediate assessment of the absorbed dose allows to identify individuals who need urgent medical intervention [1, 2].

The retrospective dosimetry (by biological and physical methods) is an essential tool for estimating radiation doses received by individuals in case of lack of personal dosimeters (in particular for civilians) and accuracy concerning the circumstances of the accident.

The range of biological and physical dosimetry techniques now available has led to proposals for a multi-parametric approach to investigating an over-exposed person and having a variety of assays available may be particularly useful if a laboratory has to deal with an event involving many casualties.

It should be noted that most of the retrospective biological and physical techniques available until now are generally time-consuming, expensive and expertise-demanding.

To overlap these limits the *BioPhyMeTRE* project ("Novel biological and physical methods for triage in radiological and nuclear (R/N) emergencies"), funded by the NATO Science for Peace and Security (SPS) programme and started in April 2020, proposes the development of innovative biological and physical methods allowing a rapid triage of potential victims, by using inexpensive and user-friendly analytical procedures and devices.

The biological method here proposed combines the two most standardized cytogenetic methods used in biodosimetry (dicentric and micronucleus assays) into a single one, in order to have a more exhaustive "two in one" assay representing a time-saving, inexpensive and potentially automatable tool for triage purposes in case of mass casualty radiological events.

This protocol offers the advantage of simultaneous scoring of chromosome aberrations (dicentrics) and micronuclei on the same slide (fig. 1), leading to a considerable reduction of: a) manual work (a general critical point in large-scale emergencies), b) costs for chemical reagents and c) quantity of blood per subject required, while maintaining the same accuracy in the dose assessment.

The proposed protocol, recently designed and published by the ENEA researchers [3], has showed its technical feasibility, but requires further methodological improvements and validation in order to verify its applicability in case of a triage dose assessment on realistic radiological/nuclear scenarios. Once validated for dose assessment, this protocol may provide an easier, time-saving and low-cost alternative to the two separate standard methods.

Concerning the physical method, the project focuses on the use of a low-cost, portable mini photo-luminescence reader for the individual dose assessment by using personal objects that civilians wear or carry every day. This system, designed and commercialized for irradiated food analysis, allows extremely rapid measurements (about 60 s), is transportable and usable *in situ* even by not skilled operators. It is here proposed as a novel method for the individual dose estimation by using materials of no considerable value as cigarettes, snacks, paper towels, etc., which people usually bring with them and would easily hand in in case of a radiological emergency.

The objective of the *BioPhyMeTRE* project is the improvement and validation of the biological and physical methods through the set-up of calibration curves for dose assessment. To this purpose, inter-laboratory comparisons will be performed to verify the

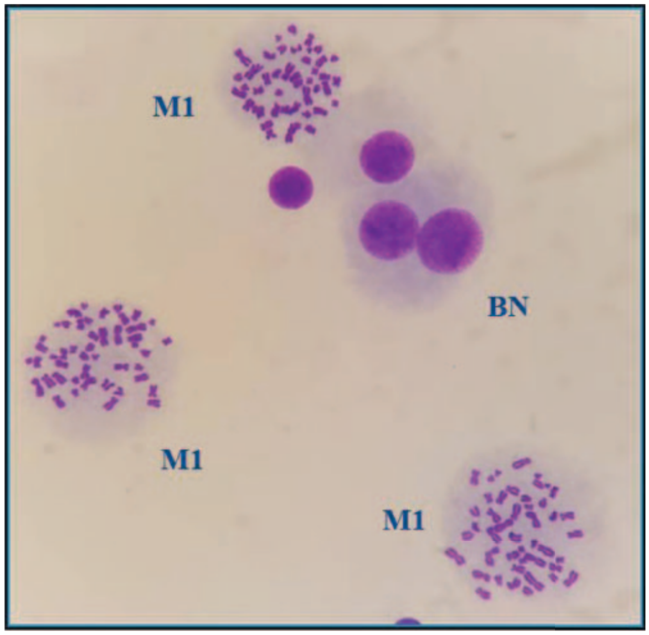

Fig. 1. – Simultaneous visualisation of metaphases (M1) for analysis of dicentrics and binucleated cells (BN) for the analysis of micronuclei on the same slide (400× magnitude).

reliability of both methods for triage in R/N emergencies. Moreover, automation systems for the novel biological dosimetry protocol will be evaluated. Inter-comparison between the biological and physical method will be performed in order to evaluate the accuracy and sensitivity of the two techniques. Next to the experimental work, the project includes training for the transfer of knowledge and skills among the partners, inter-laboratory exercises for the validation of the analytical procedures and disseminations of the results. In this paper, the status of art of the work is described.

2. – Status quo of the *BioPhyMeTRE* project

2˙1. *Biological method*. – Compared to the published novel biological dosimetry protocol [3], the experimental procedures concerning cell culture conditions and timing, fixation and staining were appropriately optimised in order to obtain the best possible quality of metaphases as well as binucleated cells in the same slide.

In particular, different combinations of experimental conditions were tested, regarding the main critical steps of the protocol: colcemid concentration, incubation time with colcemid, duration of hypotonic shock, fixation conditions and staining time of slides.

The slides obtained with the different experimental conditions were observed under the microscope and the optimum cell culture, fixation and staining conditions were selected on the basis of the quality of the metaphases and binucleated cells achieved. The following modifications of the protocol have been adopted: first, 4 h incubation of cell culture with colcemid was selected, maintaining a concentration of 0.1 μg/ml. The longer incubation time with colcemid resulted in an increased number of harvested metaphases in the first cell division (M1), reaching an average of 500 M1 per slide. Moreover, the incubation time with hypotonic solution was increased to 30 min and, as regards the fixation procedure, a pre-fixing step was introduced after the incubation with hypotonic solution, adding 1 ml of cold fixative (3:1 methanol/acetic acid). These two modifications resulted in improved quality of metaphases, without affecting the quality of binucleated cells. Finally, the staining of slides with 5% Giemsa solution was performed for a maximum of 8 min, in order to optimise the intensity of staining of metaphases and binucleated cells.

2·2. *Web scoring inter-comparison exercise*. – An inter-comparison exercise was performed between the two institutions working on the biological method within the *BioPhyMeTRE* project.

The web-based scoring of digital images of metaphases or binucleated cells is a recent approach recommended by the International Organization for Standardization [4, 5] and already utilised in the RENEB project [6] representing an opportunity to compare the scoring criteria of the involved laboratories which is one source of uncertainty in the assessed dose.

Within the *BioPhyMeTRE* project, a gallery of 500 images of Giemsa staining metaphases at high resolution mode, captured automatically with the autocapture software module Metasystems (Germany) in 63× magnification, was created and shared to be analyzed in the two biological laboratories. The results were recorded with a standardized scoring sheet.

Some aspects were investigated: which cells have to be rejected and the scoring criteria for dicentrics, centric rings, acentric fragments and acentric rings.

The two laboratories provided a good agreement in the scoring of as far as the 90% of the images, in particular the observed yield of dicentrics was very homogeneous, while the discrepancies in the scoring concerned almost exclusively the centric rings. This aspect was deepened by analyzing collectively image by image and dwelling on the controversial points in order to harmonize the scoring criteria.

2·3. *Physical method*. – A revision of the literature [7-12] about the use of the stimulated luminescence techniques for emergency dosimetry allowed a first selection of the matrices to be tested: cigarette tobacco, salty snacks, paper handkerchiefs and banknotes. In particular, in tobacco and salty snacks, responsible for the luminescence signals are the contaminants present in the materials: silicates in tobacco, salt in the snacks. Those give a PLS response even when present in low amount and to doses in the range of emergency dosimetry. These materials, purchased from local markets and vending machines for drinks and snacks, were investigated by using the Pulsed PSL system for irradiated food screening (SUERC, Scotland) with infrared stimulation (890 nm). Samples of each material, unirradiated and irradiated, were analysed. Two packets of cigarettes of the same brand and two packets of salty snacks of the same brand were purchased. Irradiation was performed with cesium-137 (Gammacell 40 available at ISS); the samples were irradiated inside their original packaging to simulate a real situation. The analysis did not require any pre-treatment of the sample which was put directly (or after breaking it into small pieces if necessary) in a Petri dish for the measurement. For each dose, four aliquots of sample were analysed; about 1 g of tobacco (the content of two cigarettes) and 2 g of crackers were used for each aliquot. As expected, both tobacco and snacks showed a PSL radiation sensitive response as shown in table I and II and fig. 2 and 3. However, the radiation sensitivity of irradiated samples differs depending on the sample type. Salty crackers show better PSL responses with respect to tobacco, which is in correlation with the concentration of minerals. In particular, the infrared stimulated luminescence

TABLE I. – *PSL response/mass recorded with infrared stimulation (890 nm) at different absorbed doses for tobacco. The values in the table are the average values for four aliquots.*

Dose/Gy	0	1	2	3	LOD
Total counts/g Mean ± SD	401 ± 100	755 ± 524	534 ± 7	1295 ± 194	702

TABLE II. – *PSL total counts/mass recorded with infrared (890 nm) stimulation with salty crackers taken from two packets. For each packet, the PSL response was the mean of four aliquots and the data shown in the table are the mean values and the standard deviation over the two groups.*

Dose/mGy	0	100	300	500	LOD
Total counts/g Mean ± SD	152 ± 108	3998 ± 74	13798 ± 1719	21581 ± 415	478

of tobacco in the dose range investigated for emergency dosimetry appears weak and differs significantly from the detection limit (LOD) (zero dose response increased by 3 standard deviations) only above 3 Gy, as shown in table I and fig. 2.

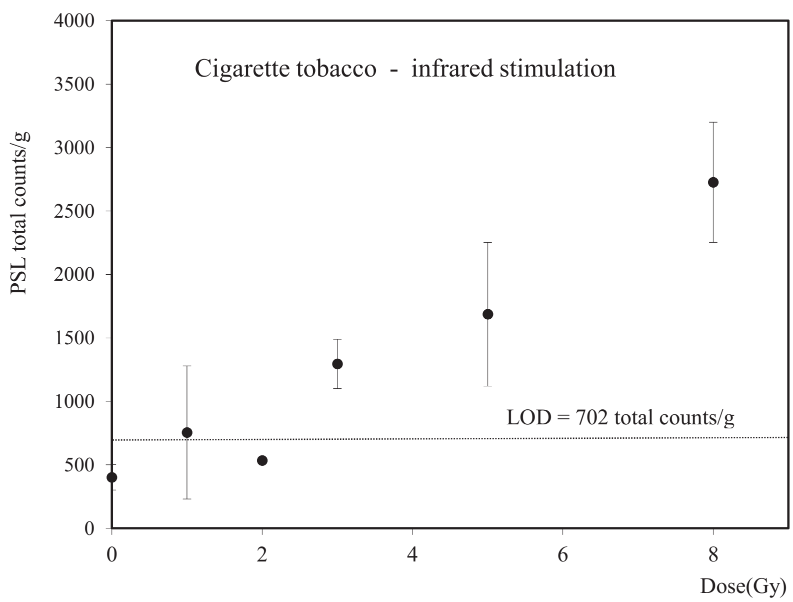

Fig. 2. – PSL dose response of cigarette tobacco obtained with infrared stimulation. The values in the graph are the average total counts/mass for four aliquots and the errors are the standard deviations.

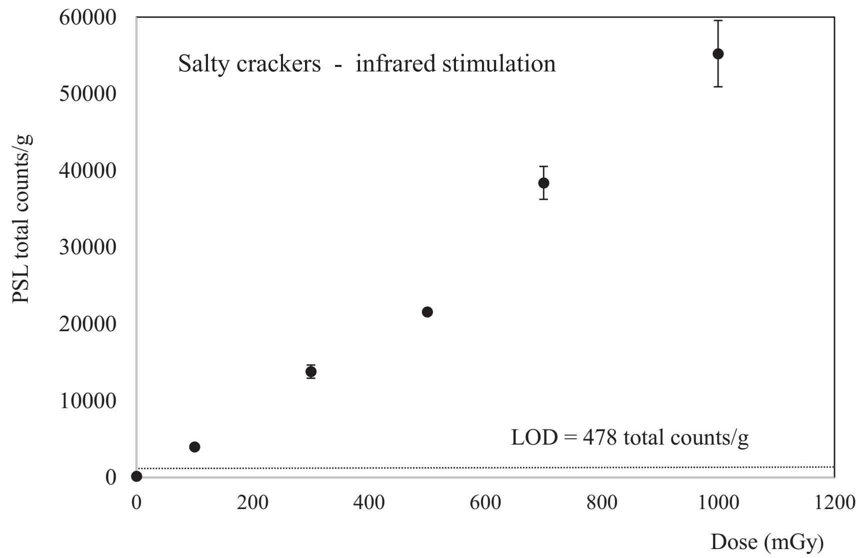

Fig. 3. – PSL dose response of salty snacks taken from two packets. For each packet and each dose, the PSL response was the mean of PSL total counts/mass of four aliquots; the values shown in the graph are the mean values and the standard deviations over the two groups from the two packets.

The high variability of PSL response (standard deviation) is a consequence of the non-homogeneous distribution in the tobacco of the luminescent contaminants (silicates) responsible for the PSL signal. It can be reduced adopting a protocol of analysis which requires examining more aliquots.

Anyway, the method is proposed for a rough evaluation of the dose to be used for a rapid triage of civilians soon after the radiological incident. More accurate dose assessment can be carried out later for those civilians more seriously exposed to radiation.

Salty crackers, unlike tobacco, due to the high radiation sensitivity of the salt, allow to detect absorbed doses as low as 100 mGy. As represented in table II, the LOD (478 counts/g) is well below the PSL response at 100 mGy.

Due to the inhomogeneity of the sample, also for salty crackers the uncertainty of the measurement is variable; however, it appears reduced with respect to tobacco due to their higher sensitivity to radiation as well as to the major number of aliquots analysed for each dose point.

These results were obtained within 20 minutes after the irradiation process. Preliminary data on the signal stability indicated that the PSL signal decreases by about 50% during the first 22 hours. Considering the high radiation sensitivity of the salt, it may be expected that this decrease cannot substantially change the result, but an experimental validation will be required.

Figure 3 shows the dose response of PSL response of salty crackers. The experiment was carried out in parallel on two packets of crackers. The data indicated a linear increase from 100 to 500 mGy; above 500 mGy the behaviour seems to become supralinear. A similar behaviour was observed also with salt by using blue light stimulation [10-12]. Further investigations are in progress to clarify this point.

2·4. *Education and training.* – The training courses planned to teach the biological and the physical method, respectively, to the IRSE team by the ENEA staff and to the RBI team by the ISS staff, could not be held due to the COVID19 pandemic. Alternatively, online training courses were organized. Theoretical lessons on retrospective dosimetry topics were given by the co-directors of the *BioPhyMeTRE* project during sessions of training online. In addition, the IRSE young researchers contributed to these sessions conducting online seminars on biological and physical dosimetry.

Regarding the physical techniques two lessons were proposed. The first one presented, in general, the definition, scope and objectives of retrospective dosimetry focusing on Optically Stimulated Luminescence (OSL), Termo-Luminescence (TL), Electron Paramagnetic Resonance (EPR) whose main advantages and disadvantages were presented. Moreover, the need for a complementary approach regarding biological and physical methods was emphasized.

The second lesson focused on luminescence emergency dosimeters and reported the results obtained by applying stimulated luminescence techniques to common-place materials which can be localized in the vicinity of individuals involved in a nuclear/radiological incident. Particular attention was dedicated to electronic components and glass from mobile phones, widely studied also in European projects, and household salt, salty snacks and tobacco that are being investigated in the project.

All the online theoretical lessons were held for all the participants in the project on the Zoom platform and are now online on the project website at the following link: `https://biophymetre.com/activities/`.

2·5. *Dissemination activities.* – As requested by the NATO SPS Programme, the official website of the *BioPhyMeTRE* project was created and launched: the URL of the home page of the website is `https://biophymetre.com/`.

The home page of the website contains a description of the project, essential information about the partners, a description of the NATO SPS Programme (with the link to the NATO science website) and a photo gallery. The other pages of the website contain more detailed information about the partners and their institutions (with the links to the institutional home pages), information about the project activities and important events and conferences that will take place in the field of radiation research.

The objectives of the project as well as some preliminary results were presented in several national and international conferences.

3. – Conclusions

In general, after a R/N emergency, the multi-parametric approach, by both biological and physical methods, is recommended, as the "ideal dosimeter" does not exist, since each single method has limits and advantages related to specific parameters (sensitivity to radiation, signal stability of the induced damage, etc.). Moreover, most of the methods now available and validated for individual dose assessment, are generally time-consuming, expensive and expertise-demanding.

In relation to this, the *BioPhyMeTRE* project aims at developing innovating biological and physical dosimetry methods for the prompt identification of possible overexposed individuals, using simplified and cost-effective procedures: a two-in-one biological method for the scoring of chromosomal aberrations and micronuclei and a physical technique that utilises a portable mini photo-luminescence reader for the individual dose assessment on no-value personal objects.

As regards the biological combined method, the experimental activity conducted so far focused on the optimization of the main steps of the protocol allowed to obtain a high quality of metaphases and binucleated cells to be scored.

In addition, the web-based scoring performed on the analysis of chromosomal aberrations and micronuclei established univocal scoring criteria between the two laboratories involved in the development of the biological method.

These preliminary results are very important in view of the subsequent phases of the project, including the set-up of calibration curves for dose assessment, the automation of the technique and further inter-comparison exercises.

The preliminary results obtained with the physical method were encouraging. Salty snacks, in particular, seem to be very promising as fortuitous dosimeters as they can detect dose as low as 100 mGy; however, a further analysis of increased statistics of dose response linearity, dose assessment and fading is needed before this method can be recommended.

The biological and physical dosimetry methods proposed in the "*BioPhyMeTRE*" project, once they have been fully developed and validated, could represent useful tools for the categorization of subjects overexposed to ionising radiation in R/N emergencies. The use of both methods could be extended to the National and International Retrospective Dosimetry Networks (*e.g.*, NATO platforms, WHO BioDoseNet, WHO REMPAN, IAEA RANET, EURADOS, RENEB) activated in case of mass casualty radiological events.

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The *BioPhyMeTRE* Project is fully funded by NATO Science for Peace and Security Programme (Grant G5684). We are grateful to the personnel of the Medicine Service of ENEA Casaccia for their collaboration in collecting blood samples from healthy donors.

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