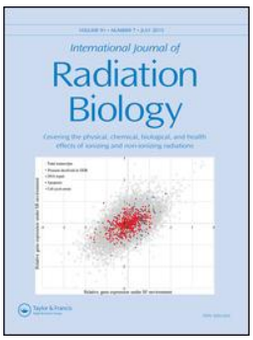

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RESEARCH ARTICLE

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Capabilities of the RENEB network for research and large scale radiological and nuclear emergency situations

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ABSTRACT

Purpose: To identify and assess, among the participants in the RENEB (Realizing the European Network of Biodosimetry) project, the emergency preparedness, response capabilities and resources that can be deployed in the event of a radiological or nuclear accident/incident affecting a large number of individuals. These capabilities include available biodosimetry techniques, infrastructure, human resources (existing trained staff), financial and organizational resources (including the role of national contact points and their articulation with other stakeholders in emergency response) as well as robust quality control/assurance systems.

Materials and methods: A survey was prepared and sent to the RENEB partners in order to acquire information about the existing, operational techniques and infrastructure in the laboratories of the different RENEB countries and to assess the capacity of response in the event of radiological or nuclear accident involving mass casualties. The survey focused on several main areas: laboratory's general information, country and staff involved in biological and physical dosimetry; retrospective assays used, the number of assays available per laboratory and other information related to biodosimetry and emergency preparedness. Following technical intercomparisons amongst RENEB members, an update of the survey was performed one year later concerning the staff and the available assays.

Conclusions: The analysis of RENEB questionnaires allowed a detailed assessment of existing capacity of the RENEB network to respond to nuclear and radiological emergencies. This highlighted the key importance of international cooperation in order to guarantee an effective and timely response in the event of radiological or nuclear accidents involving a considerable number of casualties. The deployment of the scientific and technical capabilities existing within the RENEB network members seems mandatory, to help other countries with less or no capacity for biological or physical dosimetry, or countries overwhelmed in case of a radiological or nuclear accident involving a large number of individuals.

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Biodosimetry; capacity of laboratory response; emergency preparedness; radiological accident/emergency; questionnaire/survey

Introduction

Ionizing Radiation (IR) is widely used for medical, industrial, environmental, energy generation and security applications. Therefore, accidents/incidents involving IR can happen and may involve a large number of potential casualties that need to be categorized according to the degree of injury. Furthermore, many countries use nuclear power as a source of energy and an uncontrolled nuclear accident can have

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immediate (e.g. irradiation, injury and deterministic effects) as well as long-term consequences that can lead to an increased risk of developing radiation-induced diseases (stochastic effects, e.g. cancer). Accidents like Chernobyl in April 1986 involved large numbers of individuals (amongst the population, the staff working in the nuclear power plant, first responders and liquidators) that were exposed to the radiation released. More recently, an earthquake and tsunami resulted in the accident at the Fukushima Daiichi power plant with over 1000,000 people affected. Malicious acts such as a terrorist attack using radioactive sources, for example, a Radiological Dispersal Device (RDD, dirty bomb) in a crowded place or a concealed Radiological Exposure Device (RED) in the public transportation system are also possible. Not to mention the detonation of an Improvised Nuclear Device (IND) over a populated area.

During the initial steps of emergency management in the aftermath of such events, where triage and evaluation prioritize individuals according to their degree of injury and exposure is fundamental, biodosimetry can be used to provide timely assessments of radiation exposure since physical dosimetry is often not available or reliable (Kulka et al. 2012; Maznyk et al. 2012).

Also the identification and reassurance of the large number of 'worried well' individuals is of paramount importance in order to prevent the health care infrastructure from being overwhelmed. Following a large-scale radiological incident, fast medical and radiological triage of patients according to the degree of radiation exposure will be required (Kulka et al. 2015), with the number of people who may need to be screened easily exceeding the capacity of a single or even some laboratories. Networking has been recognized as a sensible and important emergency response strategy after a radiological accident in several regions of the world (Roy et al. 2007). The already existing network Radiation Emergency Medical Preparedness and Assistance *Network* (REMPAN) from the World Health Organization (WHO) is one good example. One of the critical factors related to the study of biological effects of IR and widely distributed across the body is the estimation of dose (Rodrigues et al. 2005) and this is essential in an accident scenario. The majority of biodosimetric studies use human lymphocytes, which besides their availability are known to be very sensitive to IR. Monitoring humans exposed to IR through biodosimetry has relied heavily on the evaluation of cytogenetic indicators such as unstable chromosomal aberrations, especially dicentric chromosomes (considered the gold standard assay), stable chromosomal aberrations, namely reciprocal translocations (using fluorescence *in situ* hybridization (FISH)/chromosome painting), and other cytogenetic biomarkers such as micronuclei (MN), premature chromosome condensation (PCC), or the γ-H2AX assay for radiation-induced double-strand breaks. With the exception of the γ-H2AX assay, all of these techniques are very time-consuming. Doses to individuals can also be estimated from dosimetric assays using electron paramagnetic resonance (EPR) and thermally – or optically – stimulated luminescence (TSL, OSL) measurements (Trompier et al. 2016). When the number of individuals exposed is very high and/or a timely response is needed, the capacity of a single laboratory can be overwhelmed. These may be applied to biological materials such as teeth (bio-physical assay), or to inorganic materials carried by the individual such as components of their mobile phone (physical assay). Such assays provide orientation to specific dose estimates to complement whole body dose estimates from biodosimetric assays.

The RENEB project (Realizing the European Network of Biodosimetry) aimed to establish and develop a sustainable European network in biological and physical dosimetry that can be activated in case of an IR accident or incident. The project included 23 institutions from 16 European countries.

This paper identifies the available equipment, techniques and infrastructure in addition to the number of staff and existing experience in each laboratory and the different assays that are carried out. The capacities and capabilities of these laboratories to respond to a radiological or nuclear accident are summarized.

Materials and methods

A survey designed to evaluate capacity of response in the event of a radiological accident was sent in February 2013 to 23 institutions of the RENEB consortium. The questionnaire was designed to investigate several areas of information, starting with questions about general information and the kind of institution (hospital-based institute, military, national institute of health, national research institute, radiation protection authority, or university-based institute). Also, questions about the research activities in biological and physical dosimetry and/or emergency preparedness developed in each laboratory and the available response capacity to an accident were asked. The questionnaire also inquired about the type of existing cooperation, if any, between the different laboratories in the consortium. Questions about the research activities developed by the members were included.

Specific questions about six biological assays (dicentric, micronucleus, γ-H2AX foci, FISH, M-FISH and PCC) and two physical assays (EPR and OSL/TL) included the number of persons involved in each assay, the equipment available and existing experience in each laboratory which could be offered and made available in the case of an accident involving exposure to IR. Responders to the survey could also specify other assays performed in the laboratory. The questionnaire also collected data on the existence of other laboratories in each country that could also perform the same biological and/or physical techniques. In December 2014 a second questionnaire was sent to the RENEB members in order to update the information on the capacity of the network, in terms of human resources, assays and techniques, for response to radiological emergencies. Data from both surveys were compiled, analyzed and are presented here.

Results

The results were obtained from the compilation of all the information provided by the 17 institutions that answered the questionnaire sent to the RENEB consortium. Table 1

Table 1. Types of institutions involved in the RENEB project.

Type of institution	Number of institutions
Civilian research institute	1 (5.9%)
Hospital based institute	2 (11.8%)
Military	1 (5.9%)
National institute of health	3 (17.6%)
National research institute	5 (29.4%)
Radiation protection authority	2 (11.8%)
University based institute	3 (17.6%)

Table 2. Main areas of research activities of RENEB members.

Research area	Number of institutions
Control group	16 (94%)
Radiation qualities	9 (53%)
Relative biological effectiveness	6 (35%)
Radiation sensitivity	15 (88%)
Low dose effect	11 (65%)
Radiotherapy patients	7 (41%)
Radiation accidents	13 (75%)
Environmental exposure	7 (41%)
Radiation protection	10 (59%)
Emergency preparedness	11 (65%)
Validation methods	13 (41%)
Biomarkers	14 (82%)
DNA repair	9 (53%)
Automation of assays	10 (59%)

summarizes the types of institutions that answered the questionnaire.

With regard to cooperation, some institutions already had collaborations with other partners either through existing projects/platforms (EURADOS, MULTIBIODOSE, WHO BioDoseNet, WHO REMPAN, etc.) or through regular cooperation as the 2004 established tripartite network between BfS, PHE and IRSN. Also, biological dosimetry laboratories of International Atomic Energy Agency (IAEA) member states are improving the preparedness to react to radiation/nuclear accidents at a national level and supporting if necessary the neighbouring countries.

The analysis of the answers concerning the main areas of research of the RENEB institutions in the field of biological or physical dosimetry and/or emergency preparedness has shown that the majority of laboratories are also involved in research activities such as studies of control groups, radiation qualities, radiation sensitivity, low dose effect, radiation accidents, radiation protection, emergency preparedness, biomarkers, DNA repair and automation of assays as shown in Table 2. Other areas of interest are *relative biological effectiveness (*RBE), radiotherapy patients, environmental exposure and validation of methods and are also shown in Table 2.

Table 3 presents the number of laboratories using biological and physical dosimetry assays obtained in the first questionnaire (2013). The second questionnaire (2014) was created to evaluate the upgrade of capabilities of some RENEB members following laboratory staff training and courses in methodology, statistics or quality maintenance carried out within the RENEB consortium.

By the analysis of Table 3, the dicentric assay 'gold standard' is the one that is most used, followed by the micronuclei assay, whole chromosome painting and γ-H2AX. It is also clear that a difference exists between biological and physical methods, with physical methods implemented less frequently.

Table 3. Number of institutions in the RENEB project performing biological and physical dosimetry assays.

	Number of involved laboratories	
Biological assay	2013	2014
Dicentric	17	17
Micronucleus	11	13
γ-H2AX	12	12
M-FISH	4	5
WCP	11	12
PCC	4	5
Physical assay		
EPR	1	2
OSL/TL	3	4

M-FISH: multiplex fluorescence *in situ* hybridization; WCP: whole chromosome painting; PCC: premature condensed chromosomes; EPR: electron paramagnetic resonance; OSL: optically stimulated luminescence.

Table 4. Number of available assays/laboratory taking into account both biological and physical assays.

Number of assays per laboratory	Number of laboratories
Biological assays	
1	1
2	3
3	2
4	8
5	1
6	1
Physical assays	
1	2
2	1

Table 5. Number of trained permanent and non-permanent staff, the number of samples that can be analyzed per week and the number of automatic systems available inside the network.

Biological assay	Permanent staff	Non-permanent staff	Samples/ week	Automated systems
Dicentric	43	18	2049	20
Micronucleus	25	11	1420	8
γ-H2AX	20	13	3845	14
M-FISH	9	4	26	7
WCP	26	4	150	19
PCC	6	2	15	1
Bio-physical assay				
EPR	5	1.5	850*	–
OSL/TL	6	2.3	1200	5

M-FISH: multiplex fluorescence *in situ* hybridization; WCP: whole chromosome painting; PCC: premature condensed chromosomes; EPR: electron paramagnetic resonance; OSL/TL: optically stimulated luminescence. *800 sample in triage mode.

The survey showed that some laboratories have implemented and have available more than one assay. Table 4 shows the number of available assays and the number of laboratories using the biological (dicentric, MN, γ-H2AX, PCC, FISH, etc.) and the physical (EPR, OSL/TL) assays.

Table 5 shows the number of permanent and non-permanent staff, with the capability to conduct all the assays and data analysis in each laboratory, along with the number of samples that can be processed per week. Also shown in Table 5 is the distribution of automatic equipment systems among the laboratories involved in the RENEB network.

Table 6 presents the capacity of each country concerning the available assays and the possible sample throughput per week of each assay.

The survey also included questions about the number of dose-response calibration curves implemented per assay taking into account different radiation qualities among the RENEB laboratories (Figure 1). This is an important consideration, as the interpretation of dose using a calibration curve produced in another laboratory may introduce additional uncertainty, and therefore any laboratory intending to carry out biological dosimetry should establish its own dose-response curve (IAEA 2011).

Most of laboratories (15) that perform the dicentric assay have calibration curves for γ radiation and 10 have calibration curves for X-rays, four for neutrons and just one for α-particles. For the micronuclei assay, nine and four laboratories have calibration curves for γ and X-rays, respectively. For γ-H2AX only five laboratories have calibration curves for γ radiation and three for X-rays. For the FISH translocation assay, based on whole chromosome painting, seven laboratories have dose-response curves for γ radiation and two for X-rays; based on M-FISH just one out of four laboratories have a dose-response curve for γ radiation. One laboratory is just establishing a calibration curve. In the case of the PCC assay, two laboratories have calibration curves for γ radiation and one for X-rays.

Table 6. RENEB capacity per week concerning the different assays available per country.

	DIC	MN	γ-H2AX	M-FISH	WCP	PCC	EPR	OSL
Belgium	50	300	250	–	–	–	–	–
Bulgaria	70	200	0	–	49	–	–	–
Finland	50	–	60	–	15	–	–	–
France	1150	–	1400	10	30	–	800*	250
Germany	140	550	110	1	6	–	–	–
Greece	20	–	–	10	10	10	–	–
Italy	55	50	5	5	15	5	50	300
Poland	40	120	10	–	–	–	–	–
Portugal	30	20	10	–	5	–	–	650
Romania	4	20	0	–	–	–	–	–
Spain	90	–	–	–	10	–	–	–
UK	350	160	2000	–	10	–	–	–

Dic: dicentric; MN: micronuclei; M-FISH: multiplex fluorescence *in situ* hybridization; WCP: whole chromosome painting; PCC: premature condensed chromosomes; EPR: electron paramagnetic resonance; OSL: optically stimulated luminescence. *in triage mode.

There are four laboratories that also perform dose assessment for new methods (gene expression, gene and protein expression RT-QPCR, apoptosis and telomere length), and two of them have dose-response curves for γ radiation and one for X-rays.

With regard to the physical assays, the laboratory that performs EPR has a calibration curve for γ/X-rays and laboratories that perform OSL/TL all have calibration curves for γ/X-rays. In addition, one of them also has a calibration curve for α-particles.

The questions related to the statistical method(s) and software programmes used for dose assessment of the biological assays, show that CABAS and Dose Estimate are the most common software programmes for biological dosimetry: 11 laboratories use Dose Estimate, five use CABAS and five laboratories use both programmes. Software packages such as R, SPSS and ORIGIN are used by laboratories performing EPR and OSL/TL.

Discussion

In the event of a large scale radiological/nuclear accident or malevolent act using an RDD, RED or an IND, after the first clinical triage of the casualties, it is very important to estimate, with reasonable accuracy, the radiation dose to which individuals have been exposed in order to anticipate the development of stochastic or deterministic effects associated with the radiation exposures. Biological and physical dosimetry can be used in a triage mode to help the initial clinical evaluation as it allows the categorization of potentially exposed individuals according to dose. Furthermore, it can identify people exposed to a high dose, but also any 'false positives', i.e. people that have not been exposed, but have clinical symptoms that can be confused with those caused by radiation exposure.

The RENEB project paved the way to establish a sustainable network involving European laboratories with experience, knowledge, skills and competence in biological and physical assays, which can be used to perform triage dose assessment for a high number of individuals in the event of a large scale radiological emergency (as shown in Table 5). In addition to the accident simulation exercise that was also performed in the framework of the RENEB project

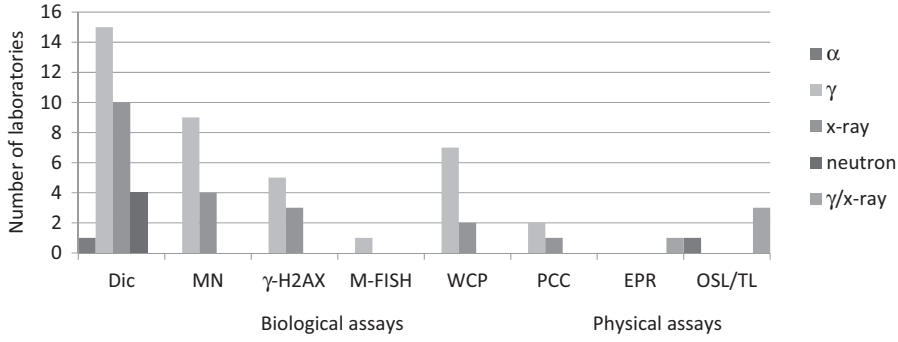

Figure 1. Number of calibration curves implemented per assay, taking into account the radiation quality, in the RENEB laboratories. Dic: dicentric; MN: micronuclei; M-FISH: multiplex fluorescence *in situ* hybridization; WCP: whole chromosome painting; PCC: premature condensed chromosomes; EPR: electron paramagnetic resonance; OSL/TL: optically stimulated luminescence.

(Brzozowska et al. 2016), the maximum capacity of each particular assay established in a laboratory, was identified. The results collected and the analysis of the survey showed the existence of a network featuring competence and skills covering a wide range of biological and physical dosimetry techniques. The analysis of the results of the questionnaires pinpoint that there are a larger number of laboratories performing biological assays, relative to the corresponding number of those performing physical techniques.

In order to ensure the accuracy and quality of the results of individual laboratories and the comparability of results between laboratories it is very important to establish a common methodology. This would need to encompass robust quality assurance and quality management procedures, as well as established, validated and documented protocols (Gregoire et al. 2016). These procedures and protocols are essential in order to have good laboratory practice throughout the biological and physical dose assessment process (Voisin 2015). For instance, the construction of calibration curves, established in vitro, is very important as they allow the conversion of a specific endpoint (e.g. dicentrics, micronuclei, γ-H2AX foci, etc.) into absorbed dose. In the group of RENEB members almost all the laboratories involved have these calibrations curves for at least one radiation quality and for different assays. Concerning the physical assays, in general these do not use predefined calibration curves: instead at least one calibration point, or ideally an extended calibration curve, is measured for each sample analysed. The types of calibration curve that the laboratory uses therefore depend on the availability of different irradiators in calibrated geometries.

By compiling all of the survey data, it has been shown that many of the laboratories involved in the RENEB network have more than one assay operational and available. This is ideal for a timely and fast dose assessment because the response time of each test is rather variable. Indeed, for example for γ-H2AX and gene expression, results can be obtained in the same day, while for dicentrics and micronuclei several days are required. The variability in the number of available techniques, from 1–6 per laboratory, cover different dosimetric aspects, as some assays are good after acute exposure (dicentric, MN and γ-H2AX), after chronical exposure or long time ago (FISH translocations) or very high dose exposures (PCC). Further aspects may be factors such as the costs involved to improve and validate the assays, the influence of the costs and of the number of existing technicians on the responsiveness to that assay, as well as the costs associated to the need to get quick but good quality results. It is also clear that for the more expensive assay, there are fewer laboratories able to perform them or keep them operational.

After the training courses performed in other RENEB laboratories the response capability has been improved (Gregoire et al. 2016, Wojcik et al. 2016). Moreover, it is important to mention that the capacity of the assays increased drastically with the network and that these assays can be used, e.g. for clinical investigations in silent periods, which gives further benefit to the community.

Conclusion

The sustainability of an international network of institutions deploying technical and scientific skills and competence in biodosimetry is of the utmost importance in the event of a radiological or nuclear accident or malevolent act involving mass casualties. In such scenarios, a single institution will be overwhelmed and not be able to cope with the high number of samples in time. An international infrastructure would therefore be required and has been accomplished by the RENEB project. However, this network still needs official recognition of the national and international organizations holding responsibilities for the management of the emergency response to radiological and/or nuclear emergencies.

Disclosure statement

The authors report no conflicts of interest. The authors alone are response for the content and writing of the paper.

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