



Final publishable summary report

Level of dissemination: public

TENEb: Towards a European Network of Excellence in Biological Dosimetry

Web page address: <http://www.teneb.eu>

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Type of funding scheme: Coordination and Support Actions: Supporting (CSA-SA)

Work programme topics addressed: Euratom Programme for Nuclear Research and Training

Fission 2008-6.0.3: Actions supporting programme implementation and other activities

Stockholm, 24.09.2009

Executive Summary

The viability study of a network of excellence (NoE) for biological dosimetry to assess radiation casualties

The aim of TENEB was to assess the capacity of laboratories in the EU to perform biological dosimetry in a large scale radiological event. A letter was sent to the radiation protection authorities of the 27 member states of the European Community plus Switzerland and Norway. The letter included a technical questionnaire to be passed on to the appropriate laboratories. In addition the same questionnaire was independently sent to 23 laboratories known personally to the TENEB participants.

Outcome of the questionnaire

We obtained no information from seven countries. From personal knowledge of the TENEB consortium, there is no biological dosimetry undertaken in those countries. However some of them do have arrangement for having analyses performed in other countries. Four countries informed us that they had no interest in establishing biological dosimetry. It was nevertheless mentioned that they would like to have access to such services if needed in the future. Three countries currently have no capacity but are at various stages in commissioning laboratories.

The remaining discussion concerns the 15 countries that do have biological dosimetry of varying proficiencies. They can be divided into two subgroups: those with a routine service as part of the laboratory statutory tasks and those with experience but no routine service provided. The majority of the 15 countries only have one laboratory however five countries have two to five laboratories capable of undertaking biological dosimetry. The total number of operational laboratories is 24. These can be summarized as 7 university based, 16 located in governmental bodies (including research institutions and hospitals), and one military.

The dicentric assay is established in 21 laboratories. The overall capacity of those 21 laboratories to perform biological dosimetry in a triage mode is around 1500 victims per week. This calculation was based on the trained staff at the time of the questionnaire, asking the laboratory head to assess the capacity based on the scoring 50 cells per victim. The total capacity of these laboratories to perform biological dosimetry in the full mode (500 cells) is approaching 200 victims per week.

The micronucleus assay is established in 15 laboratories. The overall capacity of those 15 laboratories to perform biological dosimetry in a triage mode is around 900 victims per week (500 binucleated cells per case).

The dicentric and micronucleus assay are the most widely applicable. However, we also asked for other assays that were available: 11 laboratories have the γ -H2AX assay, 9 have PCC and 17 have FISH. In general, most laboratories expressed their intention to extend the range of assays that they can undertake.

At any given moment, the combined consumables stockpile of the 15 laboratories would enable about 1000 blood samples to be processed. However in many countries, restocking can be achieved within 48 hours.

The TENEB consortium considers that a gamma calibration curve is the most important for dealing with a large scale accident or terrorist event. Seventeen laboratories are calibrated with such a curve for dicentrics. Of these 17 ten are also calibrated for X-rays, whilst four laboratories have only X-rays calibration. In addition, a small number have dose response curves for other radiation qualities (alpha, neutrons). The respective numbers for the 15

laboratories with the micronucleus assay are 9 calibrated for gamma and 9 for X-rays with 6 for both. Three laboratories, although having the capacity to perform the micronucleus assay, have no calibration curves.

Assessment of viability

Out of the 27 member states 18 countries recognise the necessity to perform biological dosimetry and 15 have established laboratories operating. Most are integrated in national emergency response planning and are regularly performing dose assessment on individual cases that arise from time to time in industry and medicine. Over many years, often with EC research funding, a good level of collaboration has developed among many of these laboratories on biological dosimetry related research. However, there has been no substantial funding for biological dosimetry *per se*. Individually, a few laboratories have recognised the need for networking and set up formal agreements for mutual assistance for serious radiological events. This forms an ideal basis for expansion to a European NoE. Networking at the European level has the potential to enhance and improve the existing resources to form an efficient and prepared network across Europe.

Previous experience in EURATOM and other EC-programmes has shown that the main difficulty when establishing a NoE is in moving towards a sustainable integration. Reaching this objective will be dependent on the willingness of the partners to sustain biological dosimetry beyond the duration of a NoE. Therefore, we asked the directors of authorities/laboratories in the 18 countries which recognise the necessity to perform biological dosimetry to confirm that biological dosimetry will remain or become a continuing activity in the addressed institution. We received positive statements from 18 institutions.

These statements and the long term expertise and the integration of the laboratories in their national governmental structures makes it highly probable that a network will be sustained beyond the period when EC funding has ceased.

Conclusion

The European Community has a large number of biological dosimetry laboratories but nevertheless, each laboratory has a limited capacity and can not handle a mass casualty event. It is only possible for a surge to be handled by European cooperation. There is a strong willingness among the European laboratories to come together as a network to prepare for a mass casualty event somewhere in Europe. Funding of most laboratories seems to be assured. This is an essential prerequisite for a sustained interacting network across Europe.

Full report

The viability study of a network of excellence (NoE) for biological dosimetry to assess radiation casualties

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1. Rational for creating a NoE in biological dosimetry

Following a radiological accident, biological dosimetry based on the analysis of cytogenetic damage in peripheral blood lymphocytes provides an approach to estimate individuals' absorbed doses (1). The dicentric assay is generally considered the gold standard of biodosimetric methods and it has been successfully applied on numerous occasions to estimate the absorbed doses in a number of small-scale radiation accidents, where the number of casualties was low (2). When the number of accident casualties is large, the precision of individual dose estimates is of lesser importance in the early stage of accident management. A fast approximate estimate of dose should provide confirmation of each patient's triage position and in that way support the physician in providing help to the accident victims (3). It should confirm or reject the doses suggested by the clinical triage and, in addition, help to identify false positive and false negative cases. False positive are people exhibiting the rather non-specific early prodromal reactions that are in fact due to causes other than radiation. False negative are those people who were exposed, but did not show any initial clinical symptoms. This latter possibility is especially important to calm the worried well amongst the public and to assist in the counselling of those people who were exposed and carry a risk of late stochastic disease.

In recent years a number of events have occurred that highlight the necessity of being prepared for a possible large-scale radiological accident or a terrorist attack, where the casualties will most likely be members of the public (4). These include the Tokaimura criticality accident in 1999, the September 11th attacks in USA in 2001, the Madrid and London train bombings in 2004 and 2005 or the polonium-210 poisoning of Alexander Litvinenko in 2006. Irrespective of whether the spreading of radioactive isotopes is malevolent or accidental, the number of affected people can vary from a few to a number of hundreds or thousands. Furthermore, as with the polonium event, future incidents may have an international/transnational scale. Following the discovery of possible exposure, there will be an uncertainty about the extent of exposure that will likely lead to great public concern, as was demonstrated during the 2006 event. The radiation exposure can range from very low to substantial, possibly combined with conventional injuries.

An important question is how well are European Union (EU) member states prepared to cope with mass radiological casualties. The present report addresses one aspect of this; the capacity of EU biodosimetric laboratories to perform dosimetric triage of accident victims. The European Commission funded a feasibility study to assess the viability of a European Network of Excellence (NoE) for biological dosimetry (cytogenetic and related assays) to assess mass radiation casualties. The results of the study are presented here.

2. Methodology of the viability study

Technical details on the different biodosimetric methods discussed below can be found in (1,5,6).

A letter was sent to the radiation protection authorities of the 27 member states of the European Union plus Switzerland and Norway. The letter included a technical questionnaire to be passed on to the appropriate laboratories. In order to be sure that the questionnaire reached as many laboratories as possible, it was independently sent to 23 laboratories known personally to us. The questionnaire elicited information about the following assays: dicentric, micronucleus, fluorescence in-situ hybridisation (FISH) translocation, premature chromosome condensation (PCC), gamma H2AX and any others established or under development. In addition, we asked for the implementation of quality assurance (QA)/ quality control (QC) programmes, teaching capacity, networking, participation in national emergency preparedness programmes and sources of funding.

Questions about the assays included details on calibration curves, numbers of experienced staff, experience in handling of accidents, computer-aided scoring systems and capacity to perform biological dosimetry in triage as well as full mode.

Following the reception of the answered questionnaires, the directors of the laboratories authorities were asked to issue statements that biological dosimetry will remain or become a continuing activity in their institution.

3. Results and discussion of the viability study

No response was received from Austria, Cyprus, Ireland, Latvia, Luxembourg, Slovenia and Switzerland. The radiation protection authorities of Denmark, Estonia, Malta and Norway wrote that their countries have no capacity for biological dosimetry and no plans to implement it. Biodosimetric laboratories are currently being set up in Lithuania, Slovakia and Sweden, but they are not yet operational. It is worth mentioning that some of the countries nevertheless do have standing arrangements to carry out biological dosimetry in other European countries, although to date, these have only been for occasional accidents involving one or a few cases. Biology dosimetry is established in 15 countries, as listed in table 1. The laboratories have either an academic or governmental status, with only one military laboratory in Germany. In some countries, more than one laboratory is operational (Germany: 2; Italy: 2; Poland: 3; Spain: 5; UK: 2). One of the laboratories in Italy specialises in biodosimetry based on electron paramagnetic resonance (EPR) and optically stimulated luminescence (OSL), without any capacity in the methods listed in table 1. Even if *sensu stricto* biological dosimetry is not performed in this laboratory it was included in our survey because it is clearly identified as performing retrospective dose assessment.

The dicentric assay is established in all laboratories except for the one in Romania. Half of the laboratories have a computer-aided metaphase finding system. The micronucleus assay is established in 11 countries and, except for Romania and one laboratory in Spain, is obviously used as a complementary assay to the dicentric test. This is reasonable, given the lower sensitivity to radiation of the micronucleus assay (1). Computer-aided metaphase finding is only present in 5 laboratories. Stockpiling for reagents and single-use plastic ware is larger for the dicentric assay than for micronuclei. However, it can be noticed that the stockpiles would enable 1000 samples to be processed, whereas the overall capacity of the network is 1500 cases. Nevertheless most of the labs have rapid access to more consumable in order to be able to handle 1500 blood samples. Generally, more dose estimations following radiation accidents were performed with the dicentric assay than with the micronucleus assay. However, because of a large number of samples analysed by one laboratory in Spain, the total of analysed cases

is larger for micronuclei than for dicentrics. It should be noted that the numbers of analysed cases given by some laboratories include both samples analysed for accidental overexposures as well as for screening purposes.

It was interesting to assess the capacity of laboratories to score dicentrics in the triage mode (50 metaphases per donor) and the full mode (500 metaphases per donor) as well as the capacity to score 500 binucleated cells per donor for micronuclei. The capacity is expressed as number of analysed cases per week, excluding the time needed for lymphocyte culturing. For dicentrics, the European total scoring capacity in the triage mode is 1493 and 187 for the full mode. The time needed for scoring a given number of cells was judged independently by each laboratory. Consequently, the ratio of triage mode capacity to full mode capacity is not consistently 10, as would be expected based on scored metaphase numbers. The highest capacity is at present in Italy, followed by the UK, Spain, Bulgaria and Poland. The lowest capacities are in Romania, Portugal and the Czech Republic.

For micronuclei the total scoring capacity is 811, which is approximately half of the capacity for dicentrics. This is mainly due to a lower number of laboratories where the micronucleus assay is established. However, except for Belgium and Portugal, all laboratories where both assays are established estimated a lower triage capacity for micronuclei than for dicentrics. A computer-aided scoring system is present in 5 laboratories. Interestingly, there is no simple correlation between the presence of an image analysis system and the capacity to score micronuclei.

Chromosome painting is established in 13 laboratories. The largest experience is present in the UK, Netherlands and Portugal. The method is mainly used for screening purposes and only to a small extent for assessing the doses in victims of radiation accidents. Both the PCC and gamma-H2AX methods are applied in 9 out of 24 labs, however for research purposes and not yet for dose estimation. An exception is the laboratory in Greece, with extensive experience in PCC.

Ten out of 24 laboratories operate a quality assurance scheme. Twenty-one laboratories declared the capacity to host trainees and 12 laboratories are involved in a network, mainly BioDoseNet of the WHO (7).

The main aim of the survey was to assess the capacity of the laboratories to perform biological dosimetry in the case of mass casualties, when the speed of analysis is of utmost importance. Although the given numbers of cases that can be handled per week must be regarded as approximations, they do allow an estimate of the available capacity in the EU. Presently, the capacity is about 1500 cases per week analysed with the dicentric assay or about 800 cases analysed with the micronucleus assay. From the perspective of preparedness for a mass casualties event, these numbers appear encouraging, especially since the capacity will probably increase when the laboratories in Lithuania, Slovakia and Sweden become operational. Nevertheless some accident and credible terrorist attack scenarios that are contemplated by emergency response agencies do involve a larger number irradiated persons (4), although at the same time it is impossible to predict the maximum number of possible cases for which one should be prepared. Furthermore, in the triage mode only 50 cells per case are scored for the dicentric assay. This provides an approximate dose estimate and usually without discriminating whether the exposure was to the whole or to a part of the body. Thus, after the triage step a more precise dose estimation may be necessary. This requires scoring in the full mode, for which the weekly EU capacity is only 187 cases. The capacity for analysis by micronuclei is about 800, but this is based on scoring 500 binucleated cells which may not be enough for a precise dose estimation. Moreover, the micronucleus assay is not suited for assessing partial body exposure, thus the use of the dicentric assay for a full doses assessment is indispensable.

An important aspect of preparedness for mass casualties at a national or international scale is networking. In a large-scale radiological accident or terrorist outrage the number of people that may need to be screened could easily exceed 280, the largest capacity declared by one of the EU laboratories (table 1, column 5). The result of the survey clearly shows that there is not much collaboration between the EU laboratories. The major networking in which the laboratories participate is BioDoseNet of WHO, which has a global and not an EU character. Regional networks exist within the BioDoseNet framework, notable well established examples are in Japan, Canada and a consortium of Latin American countries. In Europe networking exists between France, Germany and UK where an inter-institutional agreement allows for surge assistance to cover large radiological events in just those three countries. What is obviously required is an active network at the EU level. The survey has shown that in the EU there is sufficient capacity to create a strong and sustainable biodosimetry network. The basis is already established by the network of France, Germany and UK and this tripartite arrangement might form a nucleus that could be enlarged to an EU-wide networking, capable of rendering assistance to all countries, including those without a national capability. Moreover, many laboratories are integrated in national governmental structures and possess long-term expertise. Therefore, the next step should be to initiate and support cooperation between the laboratories in the framework of an EU Network of Excellence, to establish in form of a coordinated action the logistic structure and to consolidate the standardisation and harmonisation of the core assays which, based on the survey, appear assorted at the moment. Furthermore, the network should include regular intercomparison studies and accident exercises that would guarantee rapid response and reliability of dose estimates.

Previous experience in EURATOM and other EC-programmes has shown that the main difficulty when establishing a NoE is in moving towards a sustainable integration. Reaching this objective will be dependent on the willingness of the partners to sustain biological dosimetry beyond the duration of a NoE. Therefore, we asked the directors of authorities/laboratories in the 18 countries which recognise the necessity to perform biological dosimetry to confirm that biological dosimetry will remain or become a continuing activity in the addressed institution. We received positive statements from 18 institutions. These statements along with the long term expertise and the integration of the laboratories in their national governmental structures makes it highly probable that a network will be sustained beyond the period when EC funding has ceased.

4. Conclusions

The European Community has a large number of biological dosimetry laboratories but nevertheless, each laboratory has a limited capacity and can not handle a mass casualty event. It is only possible for a surge to be handled by European cooperation. There is a strong willingness among the European laboratories to come together as a network to prepare for a mass casualty event somewhere in Europe. Funding of most laboratories seems to be assured. This is an essential prerequisite for a sustained interacting network across Europe.

5. References

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6. Fattibene, P. and Wojcik, A. (eds) Biosimetric tools for a fast triage of people accidentally exposed to ionising radiation. Rome: Annals of the Istituto Superiore di Sanita (ISS) 45(3), 2009.
7. Blakely, W.F. et al. WHO 1st consultation on the development of a global biosimetry laboratories network for radiation emergencies (BioDoseNet), Radiat. Res. 171 127-139, 2009.

6. Table 1. Results of the survey on biodosimetric capacity in the European Union. Information is given for different assays as listed in the top row. Information is also given on quality assurance (QA), teaching experience (TE) and networking (NET). CAP: capacity given as number of cases analysed per week; CAS: computer aided system; EST: established, given as number of labs per country; FU: full mode - 500 cells per donor, EXP: experience given as number of cases analysed until today; STO: given as number of cases for which consumables are stored; TR: triage mode - 50 cells per donor.

Country	Labs	Dicentrics (21 labs)					Micronuclei (13 labs)				FISH		PCC		g-H2AX		QA	TE	NET
		CAS	STO	Capacity		EXP	CAS	STO	CAP	EXP	EST	EXP	EST	EXP	EST	EXP			
				TR	FU														
Belgium	1	1	50	50	5	20	1	50	150	50	-	-	-	-	1	-	-	1	1
Bulgaria	1	-	25	160	24	100	-	25	160	10	1	-	-	-	-	-	-	1	1
Czech Rep.	1	-	4	10	2	100	-	-	-	-	1	10	-	-	-	-	-	-	-
Finland	1	1	100	80	8	300	-	-	-	-	1	10	1	-	1	-	1	1	1
France	1	1	100	100	10	200	-	-	-	-	1	20	1	-	1	-	1	1	1
Germany	2	1	125	130	10	336	1	125	53	6	1	25	-	-	1	1	1	1	1
Greece	1	1	50	100	20	100	-	-	-	-	1	20	1	50	-	-	1	1	-
Hungary	1	-	10	20	2	40	-	10	10	40	-	-	1	-	-	-	-	1	1
Italy	2	1	10	280	28	0	-	10	200	-	-	-	1	-	1	-	-	1	1
Netherlands	1	1	50	60	20	300	1	50	30	-	1	150	1	-	1	-	1	1	1
Poland	3	2	67	150	18	508	1	67	120	8	2	-	2	-	1	-	1	3	-
Portugal	1	1	50	10	2	50	-	60	10	-	1	90	-	-	1	-	-	1	1
Romania	1	-	-	-	-	-	-	3	-	800	-	-	-	-	-	-	-	1	-
Spain	5	-	217	168	20	161	-	37	28	4500	1	15	-	-	-	-	2	5	1
UK	2	2	125	175	18	1213	1	100	50	5	2	470	1	-	1	-	2	2	2
Total	24	12	983	1493	187	3428	5	537	811	5419	13	810	9	50	9	1	10	21	12

7. List of beneficiaries with the corresponding contact name and associated coordinates

The list includes radiation protection authorities and laboratories performing biological dosimetry in the EU.

Member state of the EU	Authority	Biodosimetry laboratory
Austria	Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft Abt. V/7 Strahlenschutz Stubenring 1 1010 WIEN ÖSTERREICH www.lebensministerium.at	
	Bundesministerium für Gesundheit Abteilung III/B/5 Strahlenschutz Radetzkystraße 2 1030 WIEN ÖSTERREICH www.bmgfj.gv.at	
Belgium	An Fremout Federaal Agentschap voor Nucleaire Controle 36 Ravensteinstraat 1000 BRUSSEL BELGIUM www.fanc.fgov.be	Prof. Hubert Thierens Prof. Anne Vral University of Ghent (UG) Department of Basic Medical Sciences De Pintelaan 185 Building 5B3 9000 GENT BELGIUM Hubert.Thierens@rug.ac.be Anne.Vral@UGent.be
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Czech Republic	State Office for Nuclear Safety Senovážné namesti 9 PRAHA 1	

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Luxembourg	Division de la Radioprotection Ministre de la Santé Villa Louvigny - Allée Marconi 2120 LUXEMBOURG LUXEMBOURG http://www.drp.etat.lu/contact.htm	
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