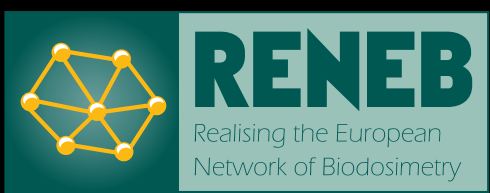
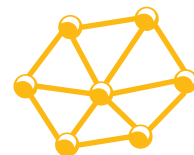


Realizing the EUROPEAN Network of BIODOSIMETRY



BULLETIN
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Progress of the work in RENEB Work Packages (WPs)

WP1: operational basis of the network

Inter-comparison exercises

During the time period between summer 2012 and summer 2013 RENEB partners carried out a large inter-comparison exercise with six established biodosimetric tools: 1) the dicentric assay, 2) the fluorescent in situ hybridisation (FISH) assay, 3) the micronucleus assay, 4) the premature chromosome condensation (PCC) assay, 5) the gamma-H2AX assay, 6) electron paramagnetic resonance (EPR) and optically stimulated luminescence (OSL). The aim of the exercise was to analyse the performance of the partners and identify areas where training is required in order to setup an operational network of biodosimetry laboratories. It should be stressed that this was the largest exercise of this kind ever performed.

The general strategy behind the exercise was to irradiate samples – blood in the case of the first five assays and telephone components in the case of EPR and OSL – code them and send to the partners for analysis and dose estimation. 19 partners participated in the dicentric exercise, 10 partners in the FISH exercise, 12 partners in the micronucleus exercise, 4 partners in the PCC exercise, 8 partners in the gamma-H2AX exercise and 3 partners in the EPR/OSL exercise. 13 partners of the European Radiation Dosimetry Group (EURADOS) joined the EPR exercise and 9 EURADOS partners the OSL exercise.

Overall, the exercise showed that irradiated samples can be quickly and reliably sent across Europe for analysis by different laboratories. This is an important prerequisite for an operational network. A good agreement between most partners was achieved in the precision of dose estimate by the different biodosimetric tools. Areas where training is required were identified and training activity will now start. The different parts of the exercise and the results are described in more detail below.

The dicentric assay

The exercise consisted of 2 parts: A and B. In Part A, the exercise was based on scoring of chromosome images provided through the internet, so that they could be analysed directly on-screen with a standard internet browser. No dedicated software was needed. In Part B the exercise simulated a realistic accidental situation: 4 coded blood samples were distributed. Lymphocytes were cultured, aberrations analysed the doses plus 95 % confidence intervals estimated.

What is RENEB?

Realizing the **E**uropean **N**etwork of **B**iodosimetry (RENEB) is a project that has the aim to establish sustainable network in biodosimetry in Europe. Such a network will significantly improve dose assessment capacity.

RENEB is a Coordination Action (CA) project funded within the 7th EU framework EURATOM Fission Programme. The project was launched on January 1st 2012, and it is planned to be carried out until the end of the year 2015. The project is coordinated by the Federal Office for Radiation Protection (BfS), the German Radiation Protection Authority.

More information of the project on the project webpage: www.reneb.eu

The exercise was successfully performed in time. The web based scoring provided very good results (figure 1). The estimated doses were close to the true doses. The data obtained demonstrated the suitability of web based scoring for inter comparisons and investigations of scoring criteria.

The results with the distributed blood samples demonstrated a higher variability between the partners, due most probably to differences in cell culture and slide preparation protocols. There is a need for standardisation. Each laboratory was able to correctly identify the non-exposed sample, the low or the high dose sample as well as the partial body sample. The accuracy of the dose estimations needs to be improved and this will be the aim of the coming tasks. In total, the data obtained so far in this first exercise look very promising for using the dicentric assay in an operational network.

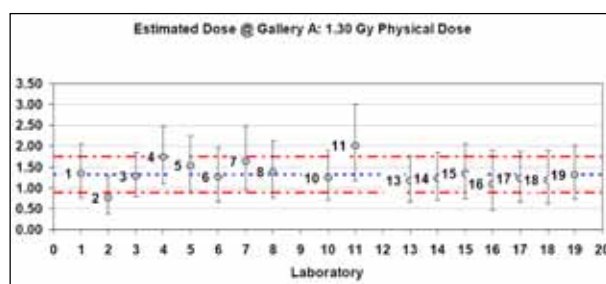


Figure 1. The dose estimates and 95 % confidence intervals provided by laboratories based on web-based scoring. The robust mean 1.32 ± 0.43 Gy is given as dotted lines. The physical dose was 1.3 Gy.



The FISH assay

Blood samples were irradiated with 2 Gy and sent to partner laboratories where lymphocytes were cultured, harvested, chromosomes painted, analysed and the dose estimated.

In total 13 dose estimations have been done, one for each partner except for laboratory 6 (three) and laboratory 20 (two) because they have dose-effect curves for more than one type of translocation. As can be seen in figure 2 two estimations are questionable and one is unsatisfactory (probably due to the low number of cells scored).

In total, the obtained results look promising but clearly show the need for further improvement of coordination and this will be the goal of the coming work.

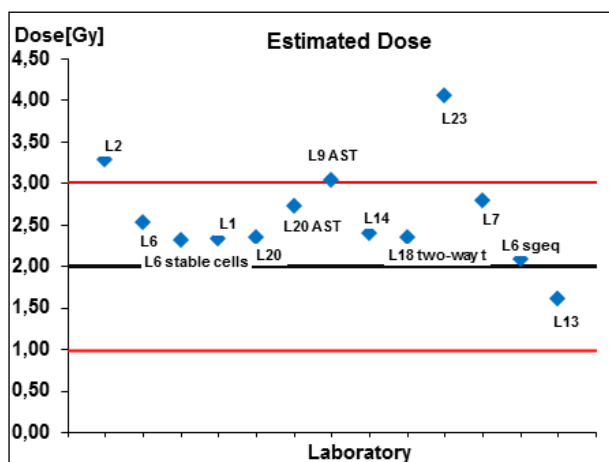


Figure 2. Dose estimations and confidence intervals after robust analysis with the z-score.

The micronucleus assay

4 blood samples were irradiated and sent to the 12 partners for blind scoring and dose assessment. Different exposure conditions were applied (acute whole body, partial body) and MN analysis was performed by automated, semi-automated or manual scoring of slides. Based on the results obtained with the different scoring methods, areas where training is required should then be identified.

An important result is that all participants were able to correctly identify the ranking of the samples, i.e. from control to the highest dose and the sample representing partial body exposure. Although the deviations of the estimated doses from the true doses are rather broad, quite comparable and satisfactory results were obtained by the different labs and scoring methods used. The accuracy of the dose estimations should be improved but the results look very promising for using the micronucleus assay in an operational network.

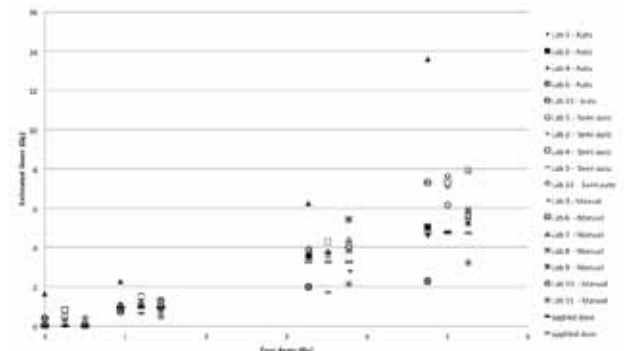


Figure 3. Estimated doses of the 4 blind samples relative to the true absorbed dose. Different scoring techniques are marked.

The PCC assay

Taking into account that two participants of this task group had no previous experience with PCC a booklet on PCC technique was prepared by the task leader and distributed among all participants. Thereafter blood was irradiated with different doses of gamma radiation, PCC slides prepared both by fusion and chemical techniques and sent to partner laboratories for analysis. The results show good dose estimates in two labs with experience with PCC. The estimates from the two newcomers require improvement. At the next stage of the project, in order to completely unify the PCC protocols among all participants, the task leader will organise a PCC training course where all technical issues as well as scoring criteria will be discussed and implemented.

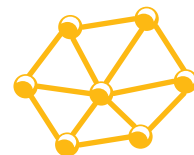
The gammaH2AX assay

The exercise was preceded by a two-day training course for those laboratories that had no or little previous experience with the assay, in order to provide a sound methodological basis for accurate analysis and future dose estimations.

The exercise consisted of two parts: A) telescoring, where partners analysed foci on images provided by the task leader, and B) where blood samples were collected, lymphocytes isolated, exposed to γ -rays, incubated at 37 °C for 4 or 24 hours, packed and shipped to partner laboratories. Calibration samples (0, 1, 2, 3, 4 Gy) or, for laboratories with existing calibration curves, negative and positive controls (0 and 2 Gy samples) were included in the shipments, in addition to the coded intercomparison samples.

Results indicated considerable discrepancies between laboratories regarding radiation-induced foci yields obtained by manual and automated scoring. However, samples could still be ranked in order of lowest to highest estimated radiation exposure based purely on mean foci/cell counts, and these could then be correlated for all participants.

Dose estimates were used to assign samples to triage categories of <1 Gy, 1-2 Gy and >2 Gy. Manual scoring of



4 h samples achieved the most accurate assignment of triage categories. Dose estimates reported for 24 h samples and those based on automated scoring tended to show more deviation from the correct triage categories. Additional training and frequent calibration of the assay may help improve the accuracy of dose estimations. Reducing the number of cells scored manually from 50 to 20 or automatically from 200 to 50 did not significantly affect dose estimates or assigned triage categories and could therefore be considered in a large scale emergency, in order to increase the assay throughput.

Overall, despite large variations between laboratories in the dose response relationship for foci induction, the obtained results indicate that the network should be able to use the gamma-H2AX assay for rapidly identifying the most severely exposed individuals within a cohort who could then be prioritised for accurate chromosome dosimetry.

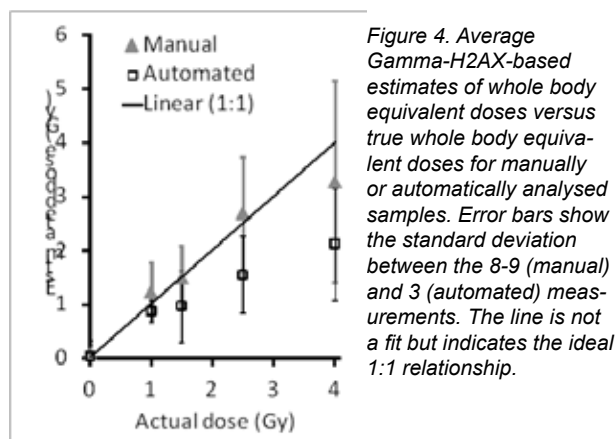


Figure 4. Average Gamma-H2AX-based estimates of whole body equivalent doses versus true whole body equivalent doses for manually or automatically analysed samples. Error bars show the standard deviation between the 8-9 (manual) and 3 (automated) measurements. The line is not a fit but indicates the ideal 1:1 relationship.

The EPR/OSL assays

EPR intercomparison

The EPR intercomparison was carried out in two parallel groups of laboratories: Group A with three experienced participants and group B with eight less experienced partners. Members of group A received fragments of glass displays from different smartphones. These samples could then be considered uniform. Members of group B received samples from individual smartphones were individually prepared from different smartphones and therefore were not uniform.

The results from group A were very satisfactory (figure 5). The three participants were able to identify correctly all dose categories and the agreement between the measured and the actual doses was also very high. The difference between mean dose and actual dose was less than twice its standard uncertainty. The results of group B were less satisfactory. The calibration curves were affected by a large uncertainty. Nevertheless 5 laboratories out of 8 were able to identify the correct category for the intermediate dose range. All except one participant were able to identify the

correct category for the high dose range and for the non irradiated samples. It is possible that shipping and storing conditions as well as the non uniformity of samples are responsible for the discrepancies. Work will now focus on finding solutions that will improve the performance of the network.

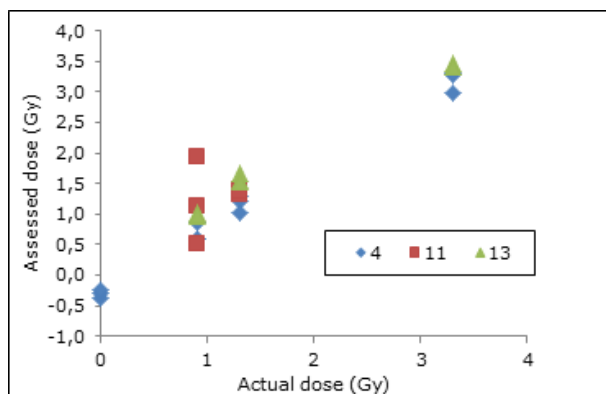


Figure 5. Estimated doses by the three laboratories of group A. Number represent the laboratory.

OSL intercomparison

The OSL intercomparison was carried out by 12 partners, using two different protocols: a “fast mode” protocol and a “full mode” protocol. With the fast-mode protocol no preheat process is performed on the sample, so that measurements are much faster; this protocol could be suitable for a first triage in a radiological mass casualty. In the full-mode protocol a preheat process on the sample aims to make the signal more stable. In principle this protocol should be more appropriate for an accurate dose assessment process.

The capability of the two protocols in identifying the correct triage dose range and in assessing the actual natural dose delivered to the sample was tested for three different doses delivered to cell phone semiconductor components: a low dose (0-1 Gy), a medium dose (1-2 Gy) and a high dose (>2 Gy).

Concerning the triage categorisation, results were very satisfactory with both protocols and for all dose ranges (figure 6). In all cases the mean of the doses measured by the labs fell in the correct range (see Table 1 and Table 5). Although the results are promising, some skills need to be improved. In particular, the biggest difficulties encountered by participants came from possible misidentifications of electronic components on the circuit board. Spending more time on a training process possibly involving more people for a same lab may help to partially solve this problem. It is suggested that corrective actions are designed that will be carried out now.

Overall, the results show that both EPR and OSL can be used for mass casualty triage in a concerted manner.

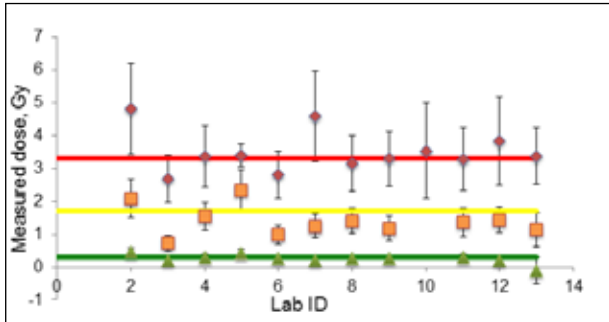


Figure 6. Doses estimated by the 12 labs using the fast-mode protocol. The green, yellow and red lines represent the 3 nominal doses: 0.3 Gy (green), 1.7 Gy (yellow) and 3.3 Gy (red). Error bars for each measured dose are also shown.

what happens in the project?

Second annual meeting in Nice, France



The second Annual meeting of RENEb took place at Maison du Seminaire, Nice, France from 18th to 20 February 2013. The meeting was organised by RENEb consortium members from IRSN and members of WP6. During the meeting the progress and the plans for the future were presented by the project coordinator Ulrike Kulka of BFS and the leaders of the WPs 1-5.

General Assembly meeting concluded that the project is performing according to the plan, and no major changes are needed. It was decided that the next meeting will take place in Valencia, Spain.

There were separate meetings for the Executive and Advisory Board of RENEb.

The consortium members would like to thank IRSN for the invitation to the magnificent Corso fleurie parade that took place on the afternoon of 20th of February, and that was the memorable social event that everybody enjoyed very much.

RENEb publications and presentations

RENEb publications:

The article about RENEb had been published in Radiation Protection Dosimetry journal in 2012 (Rad. Prot. Dosimetry (2012) 151(4), 621-625), and two papers were published in the proceedings; one from the **IRPA 13 congress** <http://www.irpa13glasgow.com/information/downloads/>, and one from **NATO HFM-223 Symposium on Biological Effects of Ionizing Radiation Exposure and Countermeasures** (2012).

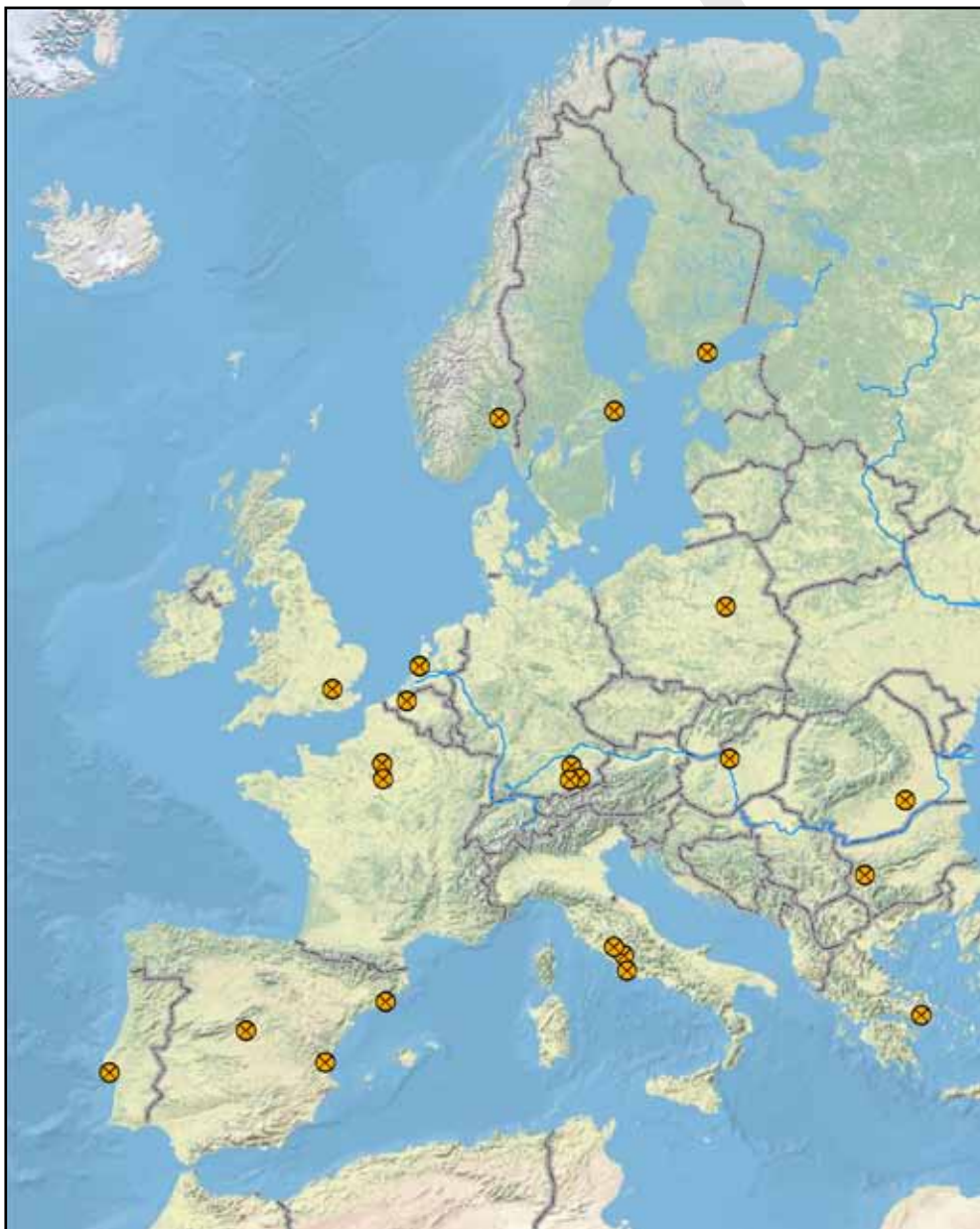
RENEb had been presented on the following international meetings (information about meetings taking place later than in August 2012):

- Oral presentations titled: RENEb – *Realizing the European Network of Biological Dosimetry* had been presented:
by U. Kulka on **EPRBioDose2013 symposium** in Leiden 24rd-28th March 2013,
- **International Conference 7th Dresden Symposium, Hazard - Detection and Management**, March 3 – 8, 2013, Dresden, Germany; on 20th
- **Nuclear Medical Defense Conference ConRad: 13 – 16 May 2013**, Munich, Germany;
- and by P. Voisin on **NATO HFM-223 Symposium on Biological Effects of Ionizing Radiation Exposure and Countermeasures: Current Status And Future Perspectives**, 8-10 October 2012, Ljubljana, Slovenia.

Posters: RENEb - Realising the European Network of Biodosimetry

- **4th International MELODI Workshop** 12 –14 September 2012, , August 2012, Helsinki, Finland
- **European Radiation Research 2012**, Vietri sul Mare, Italy, 15-19 October 2012
- **20th Nuclear Medical Defense Conference ConRad: 13 – 16 May 2013**, Munich, Germany. At this conference another poster (titled *Critical parameters that influence efficient cooperation inside the biological dosimetry network (RENEb) in an emergency situation*) was presented by task leader S. Sommer.
- Additionally, during the EPRBiodose conference in Leiden, there were several RENEb project related activities moderated by the meeting organisers.

RENEb project has been presented with oral presentation or posters on several national meetings like meetings of German and French Radiation Protection Associations, the Spanish Association of Medical services of the Nuclear power plants (UNESA), and in the medical Bulgarian newspaper *Forum Medicus*.



consortium members institutions:

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| Bundesamt für Strahlenschutz (BfS), Germany | Leiden University Medical Center (LUMC), The Netherlands |
| Bundeswehr Institut für Radiologie in Verbindung mit der Universität Ulm (BIR), Germany | National Center for Radiobiology and Radiation Protection (NCRRP), Bulgaria |
| Commissariat à l'Énergie Atomique(CEA), France | National Centre for Scientific Research "Demokritos" (NCSR), Greece |
| Agenzia Nazionale per le Nuove Tecnologie, L'Energia e lo Sviluppo Economico Sostenibile (ENEA), Italy | National Research Institute for Radiobiology & Radiohygiene (NRIRR), Hungary |
| Helmholtz Zentrum München (HMGU), Germany | Norwegian Radiation Protection Authority (NRPA), Norway |
| Health Protection Agency (HPA), United Kingdom | Radiation and Nuclear Safety Protection (STUK), Finland |
| Institute of Nuclear Chemistry and Technology (INCT), Poland | Stockholm University (SU), Sweden |
| Institutul National de Sanatate Publica (INSP), Romania | Universitat Autònoma de Barcelona (UAB), Spain |
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