Multi-disciplinary biodosimetric tools to manage high scale radiological casualties

Rendicontazione

Informazioni relative al progetto

MULTIBIODOSE

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Final Report Summary - MULTIBIODOSE (Multi-disciplinary biodosimetric tools to manage high scale radiological casualties)

Executive Summary:
In the event of a large scale radiological emergency biological dosimetry is an essential tool that can identify those exposed individuals who should receive immediate medical treatment. A number of biodosimetric tools are available, but they have to be adapted and tested for a large-scale emergency scenario. The aim of MULTIBIODOSE was to analyse a variety of biodosimetric tools and adapt them to different mass casualty scenarios. The assays were chosen because they complement each other with respect to sensitivity, specificity to radiation and the exposure scenario as well as speed of performance. The project involved key European players with extensive experience in biological dosimetry and was completed on time in April 2013.
Within MULTIBIODOSE we tested and validated the following dosimetric assays for their suitability as tools to triage exposed people in case of a large-scale radiological emergency:
1. Manual and automated dicentric assay
2. Automated micronucleus assay
3. Gamma-H2AX assay
4. Skin speckle assay (SSA)
5. Serum protein assay (SPA)
6. Electron paramagnetic resonance (EPR)
7. Optically stimulated luminescence (OSL)
The assays 4 (SSA) and 5 (SPA) were excluded from the battery of tools because they were found unsuitable for triage due to: (1) a long latency period between exposure and signal manifestation (SSA) and (2): large individual variability (SPA).
The other assays were standardised in the participating laboratories and training was provided. They were then tested for their ability to identify a person exposed to a dose higher than 1 Gy of gamma radiation. A statistic software was developed that allows collating the results from all assays.
Inter-laboratory exercises were carried out to ensure that the all participating laboratories assessed the dose correctly. Blood samples and mobile phone elements were irradiated and shipped to participating laboratories. Three triage categories were aimed at: 0-1 Gy (low dose); 1-2 Gy (median dose) and 2-4 Gy (high dose). The results show that, on the whole, the methods and assays are very good at identifying doses within the correct triage category. Categorisation was without exception most successful based on dose estimates alone, i.e. ignoring the standard errors associated with the estimates. This was somewhat unexpected, as it was initially hypothesised that it would be necessary to use the upper standard error or confidence limit to ensure that some doses weren’t missed. However, this result strongly demonstrates that the assays actually do what they are supposed to be doing, and that the dose estimates provided are indeed a good indication of the actual exposure dose.
A guidance document for radiation emergency responders was developed with information about the possibilities and limitations of the triage tools and about the capacity of the participating laboratories to carry out biodosimetric triage in a mass casualty emergency. The guidance document can be downloaded from the project web page.

In summary, the Multibiodose consortium successfully tested, adapted, and validated five biodosimetry assays for their use in triage biodosimetry in a mass casualty situation. In an emergency situation a MULTIBIODOSE partner laboratory in the affected country should act as the “core” or “administrative” laboratory that will be in charge of the decision which assays to use and how to involve other laboratories. The laboratory will give advice to the health and radiation protection authorities about collection of samples and it will collect the results from other laboratories. In the end, this laboratory will provide the health and radiation protection authorities with dosimetric and radiological triage categorisation results to support medical and public health decisions.

Project Context and Objectives:
In the event of a large scale radiological emergency, the triage of patients according to their degree of exposure forms an important initial step. Although clinical signs and symptoms of a serious exposure are used for that purpose, there are a number of reasons against their exclusive use as the basis for medical decision making: clinical symptoms are dynamic, show a strong degree of individual variability and are not specific to radiation. A good example is vomiting, which can be psychogenic, whereas its absence does not exclude significant overexposure. Biological dosimetry is an essential tool in the management of a radiological mass casualty, which can provide timely assessment of radiation exposure to the general population and enables the identification of those exposed people and specifically who should receive medical treatment.
A number of biodosimetric tools are currently in use or potentially available, but all of these must be adapted and tested for a large-scale emergency scenario. These methods differ in their specificity and sensitivity to radiation, the stability of signal and speed of performance. A large scale radiological emergency can take different forms. Based on the emergency scenario, different biodosimetric tools should be applied so that the dose information can be made available with optimal speed and precision.
The aims of this multi-disciplinary collaborative project were:
1. To analyse a variety of biodosimetric tools and adapt them to different mass casualty scenarios. The following biodosimetric tools were validated: the dicentric assay, the micronucleus assay, the gamma-H2AX assay, the skin speckle assay (SSA), the blood serum protein assay (SPA) and electron paramagnetic resonance/optically stimulated luminescence (EPR/OSL) dosimetry in components of portable electronic devices.
2. To validate and then embed the techniques in a number of European laboratories. The project involved the key European players with extensive experience in biological dosimetry. The staff of the participating laboratories were trained to perform dosimetric triage in a timely and reliable manner and a programme of on-going training (including other relevant laboratories) was established in collaboration with the EU-funded network RENEB.
3. To develop an operational guide and disseminate it among emergency preparedness and radiation protection organisations. This activity will make the stakeholders aware of the existence of the operational biodosimetric network and will inform them about the possibilities and costs of applying the biodosimetric triage tools in a mass casualty situation.
4. To develop an algorithm, based on the TMT-handbook, that will be helpful in deciding which tools should be applied for optimal triage and are best related to the type/circumstances of the emergency.
5. Automation and commercialisation was pursued and a software package was developed for integrated statistical analysis of data from each of the assays.
The assays were chosen because they complement each other with respect to sensitivity, specificity to

radiation and the exposure scenario as well as speed of performance. Some of the assays were well established as biodosimetric tools and only needed to be adapted to a mass casualty scenario, while other assays needed validation. This approach guaranteed that the final important deliverable of this project is the establishment of a biodosimetric network that is fully functional and ready to respond in case of a mass casualty situation. Thus, the project strengthens the European security capabilities by achieving tangible technical and operational results.

Project Results:
THE OVERAL DESCRIPTION OF THE PROJECT RESULTS
THE BIODOSIMETRIC TOOLS AND THEIR APPLICABILITY FOR TRIAGE
The following dosimetric assays were tested and validated for their suitability as tools to triage exposed individuals in case of a large-scale radiological emergency:
• Manual and automated dicentric (Dic) assay
• Automated micronucleus (MN) assay
• Gamma-H2AX assay
• Skin speckle assay (SSA)
• Serum protein assay (SPA)
• Electron paramagnetic resonance (EPR)
• Optically stimulated luminescence (OSL)
In the following, the tools are shortly presented and their applicability for biodosimetric triage described as resulting froom the work done during the project. The tools were tested for their ability to identify a person exposed to a dose higher than 1 Gy of gamma radiation.
Manual and automated dicentric assay (Dic)
The dicentric assay is based on assessing the frequency of dicentric chromosomes in peripheral blood lymphocytes (PBL) of an exposed person. The dicentric chromosome is specific for ionising radiation and the spontaneous frequency is very low in the healthy population (Romm et al, 2009). It is internationally standardised (ISO 19238 and ISO 21243) and regarded as the gold standard of biological dosimetry (Blakely et al, 2005). The assay requires ca. 5 ml venous blood and subsequent in vitro culturing of PBL for a 48 h period for visualisation of chromosomes. Dicentrics can be scored manually under the microscope or automatically/semi-automatically with the help of an image analysis system coupled to a microscope equipped with a motorised stage. The MULTIBIODOSE team relied on the image analysis system distributed by MetaSystems, Germany (Schunck et al, 2004). Following this approach the absorbed doses can be assessed up to few months after exposure (IAEA 2011).
It was found that manual scoring of 50 cells per donor or automatic scoring of 150 cells per donor is sufficient to identify a person exposed to a dose higher than 1 Gy. Partial body exposure covering 50% of the cells and protracted exposure (irradiation time of 16 hours) were also tested and found to be detectable (Romm et al, 2012, Romm et al, 2013).
Automated micronucleus assay (MN)
The micronucleus assay is based on assessing the frequency of micronuclei in PBL of the exposed person. Micronuclei (MN) are not specific for ionising radiation and the spontaneous frequency is much higher than that of dicentrics. However, radiation is a very potent inducer of MN, so a high frequency of MN strongly indicates radiation exposure. A large number of cells can be scored within a time shorter than that required for scoring dicentrics. International standardisation is in progress (ISO 17099). This assay also requires ca. 5 ml venous blood and subsequent in vitro culturing of PBL for ca. 72 hours. The big

advantage of MN is that they can be scored automatically with high speed using an image analysis system coupled to a microscope equipped with a motorised stage. The MULTIBIODOSE team relied on the image analysis system distributed by MetaSystems, Germany (Schunck et al. 2004). The absorbed dose can be assessed up to few months after exposure (IAEA 2011).

It was found that automatic scoring of 1000 cells per donor with the MN assay is sufficient to identify a person exposed to a dose higher than 1 Gy with a high precision. Protracted (irradiation time of 16 hours) whole body, and partial body exposure were also tested and found to be detectable.

Gamma-H2AX assay

The gamma-H2AX assay is based on analysing the formation of DNA repair protein clusters – called gamma-H2AX “foci” - in peripheral blood lymphocytes of an exposed person (Rothkamm and Loebrich 2003, Rothkamm and Horn 2009). Similar to micronuclei, the foci are not specific to but indicative of ionising radiation exposure and the spontaneous frequency is quite low. The analysis can be done manually or automatically using a microscope or automatically using a flow cytometer or fluidic device (Pope et al, 2011). The advantages of the assay are its high sensitivity (if used within a few hours post exposure), that it can be used with only a drop of blood (finger prick) and that it does not require culturing of lymphocytes as in the case of the dicentric and MN assays and therefore provides results within a few hours. However, the absorbed dose (whole and partial body) can only be assessed up to a few days after exposure (Horn et al. 2011, Lassmann et al, 2010). It was found that automatic scoring of 50 cells per donor with the gamma-H2AX assay is sufficient to identify a person exposed to a dose higher than 1 Gy. Protracted (irradiation time of 16 hours) whole body exposure was detectable, but doses could not be accurately estimated (Rothkamm et al, 2013). Partial body exposure was also tested and found to be detectable only using manual, but not automated analysis (Horn et al, 2011; Rothkamm et al, 2013).

Skin speckle assay (SSA)

The skin speckle assay is based on the ability to detect radiation-induced speckle patterns in the skin. The assay is specific to radiation-induced skin damage and its unique advantage is the possibility to detect a dose to a small area of the skin in a totally non-invasive and very fast way. However, the results obtained in this project suggest that at least one month must pass between radiation exposure and analysis before a radiation-induced skin speckle pattern is detectable. For this reason this assay was found not suitable for a timely triage of people exposed in a large-scale radiation emergency.

Serum protein assay (SPA)

The change of concentration of selected proteins in serum following localised exposure of skin to radiation was tested in samples collected form patients treated by external beam radiotherapy as a tool for identifying partial-body exposure. Although promising results were obtained in earlier mouse experiments, the changes in protein concentration in patients showed very strong individual variability that makes the assay unsuitable for use in emergency situations, when the individual levels of proteins before radiation exposure are not known.

Electron paramagnetic resonance (EPR)

EPR is a spectroscopic technique for studying radiation-induced radicals in biological or artificial materials (IAEA 2002). Ionising radiation induces radicals in glass displays of portable electronic devices such as smart phones. Consequently, these can be used as individual dosimeters, but must be removed and destroyed for measurement. The advantage of EPR is its high radiation specificity, good signal linearity in the high dose range (>1 Gy) and long signal stability (several months). Its detection threshold is 1 Gy. Analysis must be carried out in a laboratory equipped with an EPR spectrometer.

Optically stimulated luminescence (OSL)

OSL assesses the dose of ionising radiation by measuring light emitted from irradiated objects following optical stimulation. Electronic elements used in mobile phones have luminescent properties and can be used as individual dosimeters, but require removal and destruction of the phone's electronic circuitry board. The advantage of OSL is its very high specificity and sensitivity to radiation (from several mGy to several Gy). There is a signal loss of 50% in the first 10 days after irradiation and fading correction must be applied. Analysis must be carried out in a laboratory equipped with an OSL reader.

Comparison of the performance of the tools through an exercise

In order to validate the tools an exercise was performed where blood samples and elements of mobile phones were irradiated and shipped to project partners for analysis. For the biological assays, blood collected from a total of 8 volunteers was exposed to high dose rate Co-60 gamma irradiation at STUK and UGent. A total of 5 doses/irradiation schemes were included: A control (0 Gy); a medium acute high-dose-rate dose (1.5 Gy); a high acute high-dose-rate dose (2.75 Gy); a simulated medium partial body exposure (1.5 Gy, mixed 1:2 with 0 Gy blood from the same donor); a simulated high partial body exposure (2.75 Gy, mixed 2:1 with 0 Gy blood from the same donor). A total of 8 samples of blood and/or separated lymphocytes (with a 4 hr timepoint) were then shipped to each participating laboratory. 8 labs participated in the exercise: 6 laboratories carried out the dicentric assay (BfS; BIR; HPA; INCT; IRSN; STUK); 5 laboratories carried out the micronucleus assay (BfS; BIR; HPA; INCT; UGent) and 5 laboratories carried out the gamma-H2AX foci assay (BIR; HPA; IRSN; STUK; UGent). The dicentric assay was run in manual triage mode (at least 50 cells or 30 dicentrics scored) and/or automatic mode (at least 150 captured metaphases scored). The micronucleus assay was run in automated mode with cut-off> 4 MN and without cut-off as well as in semi-automated mode and participants were asked to score at least two slides and two cultures per sample. The foci assay was carried out in manual (≥20 cells scored) and/or automatic (200 cells scored) mode, and participants were asked to use a positive control to test the ongoing validity of their calibration curve. For all three assays, standardised scoring sheets were provided which contained the method for calculating standard errors which was defined in deliverable 6.4. Further, each participating laboratory was asked to use their own most appropriate calibration curve. The exercise was run in 'real time,' so the return of results was timed, in order to simulate and test a realistic accident response as far as possible. The triage results were based on the labs' first reported result for each sample, using their own preference for which method (automatic or manual) to apply in triage mode, i.e. to apply first.

For the physical assays, 11 and 13 institutions took part in the EPR and OSL intercomparisons, respectively. During a two-day preparatory meeting, organized at IRSN, the participants were trained on the use of the OSL and EPR protocols and on the various steps of the method, i.e. sample preparation, measurement, signal evaluation and uncertainty assessment. During this meeting, the participants also received the blind dose samples, i.e. mobile phone touchscreens for EPR and mobile phones for the OSL. Irradiations at 3 unknown doses were performed at IRSN in terms of air kerma using a cobalt source. Each of the 3 unknown doses fell within one of the 3 triage dose ranges: 0-1 Gy (low dose), 1-2 Gy (medium dose) and >2 Gy (high dose). For EPR, participants also received a set of calibration samples to determine the calibration curve and a software for the evaluation of the signal intensity. Participants were asked to measure the blind dosed samples in their own laboratories following the MULTIBIODOSE protocol (described in D 5.2). Nine and eleven participants reported the final results for EPR and OSL. The EPR intercomparison was carried out in parallel in two groups of laboratories: in the first group, formed by three participants, samples were taken from a bulk of glass fragments of three smart phones of the same model and stored in the same conditions at one laboratory for the first week, whereas for the second group, made of eight participants, the samples were individually prepared from different smartphones. Shipping and

storing conditions were different among laboratories and therefore on average the samples were less uniform than those of of the first group (but more similar to a “real” situation). For OSL, the intercomparison was carried out using two different protocols: a “fast mode” protocol, where no preheating is applied so that measurements are faster and a “full mode” protocol, where preheating leads to the isolation of a more stable signal at the expense of longer measurement times. The “fast mode” protocol could thus be suitable for a first triage in a radiological mass casualty, whereas in “full-mode” a more accurate dose assessment should be possible.

As the ‘administrating laboratory,’ i.e. the laboratory in the country in which the simulated radiation emergency occurred, HPA collated the results of each assay from each lab, and entered the data into the MULTIBIODOSE software, in order to assign a triage status to each simulated exposed individual. Once the results were collated, the codes on the samples were broken, in order to ascertain the success of the triage categorisation exercise. In addition to direct comparison of the experimental results with the irradiation doses, statistical analysis of all the experimental variables was carried out using General Linear Model Analysis of Variance (ANOVA) and post-hoc testing where appropriate. In addition, a number of alternative methods of triage categorisation were tested: categorisation based on upper 95% confidence limit; categorisation based on upper standard error and categorisation based on mean dose value alone, and for either whole body equivalent or actual measured (whole body or partial body doses).

Results

For the biological assays, for the initial, timed, triage, 124 of the 128 expected results (dicentric assay: 6 labs; micronucleus assay: 5 labs; foci assay: 5 labs; 8 samples for each assay and lab) were returned within 9 days of the initial irradiation. For the full exercise, a total of 245 results were reported within a period of two weeks following initial radiation. The data consisted of both manual and automated scoring results from each of the laboratories carrying out the dicentric assay; automatic, semi-automatic and automatic with cut-off modes from each of the laboratories carrying out the micronucleus assay and manual mode, automated mode or both, from each of the laboratories carrying out the foci assay. The data are summarised in Figure 1.

Figure 1. Measured and actual (whole body equivalent) doses for each sample for each assay, organised by participating laboratory. Lab 1 = BfS; Lab 2 = BIR; Lab 3 = HPA; Lab 4 = INCT; Lab 5 = IRSN; Lab 6 = STUK; Lab 7 = UGent. Assay 1 = Dicentric assay; Assay 2 = Micronucleus assay; Assay 3 = Foci assay.

General Linear Model ANOVA was carried out in order to investigate the contribution and interaction of the individual experimental factors. Overall, there was no evidence of any significant effect of irradiating lab ($p = 0.473$) scoring lab ($p = 0.659$) scoring mode ($p = 0.106$) or assay ($p = 0.140$) in the final results. z-test statistic values were carried out for comparison of the individual laboratories’ mean estimate with the actual whole body equivalent dose (results not shown). The results demonstrate that although there is quite a large amount of inter-laboratory variation across the full range of samples, it was samples S4 and U4, the high partial body doses that showed the highest degree of variation. In fact all labs had a tendency to over-estimate doses however, again, it was the highest dose partial body samples which were over-estimated the most.

The best results in terms of triage categorisation arose from using whole body equivalent (WB) doses, and categorising the samples based on un-weighted dose estimate alone, i.e. ignoring the magnitude of the standard errors and where the upper standard error and upper confidence limit fall (data shown in Appendix 3).

Table 1: Combined (un-weighted) triage categorisation results for all data from the dicentric, micronucleus

and foci assays.

Actual WB equivalent dose, Gy Actual triage category Mean Measured Dose, Gy SD*, Gy Triage category Correct category?

0 0-1 Gy 0.224 0.069 0-1 Gy Yes

0.495 0-1 Gy 0.953 0.183 0-1 Gy Yes

1.5 1-2 Gy 1.727 0.263 1-2 Gy Yes

1.815 1-2 Gy 2.515 0.281 2+ Gy No (High)

0 0-1 Gy 0.054 0.059 0-1 Gy Yes

1.5 1-2 Gy 1.731 0.237 1-2 Gy Yes

2.75 2+ Gy 2.966 0.301 2+ Gy Yes

1.815 1-2 Gy 2.149 0.263 2+ Gy No (High)

*SD: Standard deviation of samples between participating laboratories

Dicentric assay

Using whole body equivalent (WB) doses, and categorising the samples based on dose estimate alone: Out of a total of 48 samples (8 in each of 6 labs), 32 (67%) were categorised correctly and 47 (98%) were either correct or placed in a category that was too high. Of the 30 HDR samples, 27 (90%) were correct and 29 (97%) were correct or too high. Of the 18 PAR samples, 5 (28%) were correct and 18 (100%) were correct or too high.

Table 2: Combined (un-weighted) triage categorisation results for all data from the dicentric assay.

Actual WB equivalent dose, Gy Actual triage category Mean Measured Dose, Gy SD*, Gy Triage category Correct category?

0 0-1 Gy 0.104 0.046 0-1 Gy Yes

0.495 0-1 Gy 0.775 0.237 0-1 Gy Yes

1.5 1-2 Gy 1.654 0.307 1-2 Gy Yes

1.815 1-2 Gy 2.361 0.273 2+ Gy No (High)

0 0-1 Gy 0.026 0.047 0-1 Gy Yes

1.5 1-2 Gy 1.635 0.279 1-2 Gy Yes

2.75 2+ Gy 3.172 0.284 2+ Gy Yes

1.815 1-2 Gy 2.244 0.284 2+ Gy No (High)

*SD: Standard deviation of samples between participating laboratories

Micronucleus assay

Using whole body equivalent (WB) doses, and categorising the samples based on dose estimate alone: Out of a total of 36 samples (8 in each of 4 labs and 4 from 1 lab), 27 (75%) were categorised correctly and 36 (100%) were either correct or placed in a category that was too high. Of the 22 HDR samples, 18 (82%) were correct and 22 (100%) were correct or too high. Of the 14 PAR samples, 9 (64%) were correct and 14 (100%) were correct or too high.

Table 3: Combined (unweighted) triage categorisation results for all data from the micronucleus assay.

Actual WB equivalent dose, Gy Actual triage category Mean Measured Dose, Gy SD*, Gy Triage category Correct category?

0 0-1 Gy 0.038 0.061 0-1 Gy Yes

0.495 0-1 Gy 0.316 0.205 0-1 Gy Yes

1.5 1-2 Gy 1.485 0.288 1-2 Gy Yes

1.815 1-2 Gy 1.931 0.383 1-2 Gy Yes

0 0-1 Gy 0.095 0.120 0-1 Gy Yes

1.5 1-2 Gy 1.757 0.306 1-2 Gy Yes
2.75 2+ Gy 3.090 0.405 2+ Gy Yes
1.815 1-2 Gy 1.614 0.376 1-2 Gy Yes
*SD: Standard deviation of samples between participating laboratories
Gamma H2AX foci assay
Using whole body equivalent (WB) doses, and categorising the samples based on dose estimate alone: Out of a total of 40 samples (8 in each of 5 labs), 28 (70%) were categorised correctly and 34 (85%) were either correct or placed in a category that was too high. Of the 25 acute, high dose rate, whole body simulated (HDR) samples, 19 (76%) were correct and 21 (84%) were correct or too high. Of the 15 simulated partial body exposures (PAR) samples, 9 (60%) were correct and 13 (87%) were correct or too high.
Table 4: Combined (un-weighted) triage categorisation results for all data from the foci assay.
Actual WB equivalent dose, Gy Actual triage category Mean Measured Dose, Gy SD*, Gy Triage category Correct category?
0 0-1 Gy 0.087 0.019 0-1 Gy Yes
0.495 0-1 Gy 0.736 0.042 0-1 Gy Yes
1.5 1-2 Gy 1.561 0.093 1-2 Gy Yes
1.815 1-2 Gy 1.873 0.123 1-2 Gy Yes
0 0-1 Gy 0.054 0.025 0-1 Gy Yes
1.5 1-2 Gy 1.606 0.131 1-2 Gy Yes
2.75 2+ Gy 2.400 0.237 2+ Gy Yes
1.815 1-2 Gy 1.656 0.147 1-2 Gy Yes
*SD: Standard deviation of samples between participating laboratories
Triage categorisation with all biological data
Although it is unrealistic to expect that, in a triage situation, multiple laboratories will use multiple assays and operational methods to estimate doses for individuals, for validation purposes it is interesting to see how the triage categorisation results compare for the full data set.
Table 5: Combined (un-weighted) triage categorisation results for the full set of data from all the assays employed during the exercise (a total of 245 data points).
Actual WB equivalent dose, Gy Actual triage category Mean Measured Dose, Gy SD*, Gy Triage category Correct category?
0 0-1 Gy 0.377 0.136 0-1 Gy Yes
0.495 0-1 Gy 0.978 0.175 0-1 Gy Yes
1.5 1-2 Gy 1.754 0.170 1-2 Gy Yes
1.815 1-2 Gy 2.714 0.169 2+ Gy No (High)
0 0-1 Gy 0.280 0.149 0-1 Gy Yes
1.5 1-2 Gy 1.882 0.282 1-2 Gy Yes
2.75 2+ Gy 3.073 0.383 2+ Gy Yes
1.815 1-2 Gy 2.529 0.159 2+ Gy No (High)
*SD: Standard deviation of samples between participating laboratories
The results in table 5 demonstrate that the total results from all participating laboratories do not differ from the initial triage results collected, again indicating consensus between the participating laboratories, assays and methods used.
Electron paramagnetic resonance assay

Using a homogeneous set of samples, each of the mean values of the respective measured doses fell into the correct triage category (<0 Gy, 0-1 Gy, 1-2 Gy and >2 Gy; Table 6). Looking at the results on an individual level, a correct identification of the triage category was achieved for 26 out of 27 measurements (96%).

Table 6. Mean values of the doses measured for the full set of data from Group A per triage category

Nominal dose, Gy Triage category Mean measured dose ± SD*, Gy Correct category?

0.9 0-1 Gy 0.96 ± 0.26 Yes

1.3 1-2 Gy 1.37 ± 0.21 Yes

3.3 2-4 Gy 3.31 ± 0.17 Yes

0 0 -0.31 Yes

*SD: Standard deviation of samples between participating laboratories

Using a non homogeneous set of samples, the mean values of the respective measured doses fall into the correct triage category only for 1-2 Gy and >2 Gy(Table 7). Looking at the results on an individual level, no measurement fell in the 0-1 Gy category. For the intermediate and the high dose, a correct identification of the triage category was achieved for 13 ouf of 24 samples (54%) and for 24 out of 24 samples (100 %) using the full mode protocol. All participants, but one, were able to identify the non irradiated sample.

Table 7. Mean values of the doses measured for the full set of data by Group B per triage category

Nominal dose, Gy Triage category Mean measured dose ± SD*, Gy Correct category?

0.9 0-1 Gy 1.8 ± 0.4 No (High)

1.3 1-2 Gy 1.6 ± 0.6 Yes

3.3 2-4 Gy 3.9 ± 0.8 Yes

0 0 -0.8±0.8 Yes

Optically stimulated luminescence assay

For both, the fast mode and the full mode protocol, each of the mean values of the respective measured doses fall into the correct triage category (0-1 Gy, 1-2 Gy and >2 Gy; Tables 8 and 9). Looking at the results on an individual level, a correct identification of the triage category was achieved for 35 ouf of 40 samples (88%) using the fast mode protocol and for 40 out of 44 samples (91 %) using the full mode protocol.

Table 8: Summary of the results of the OSL inter-comparison using the “fast-mode” protocol. For each nominal dose, the mean value of the measured doses between all the labs is reported.

Nominal dose, Gy Triage category Mean measured dose ± SD, Gy Correct category?

0.3 0-1 Gy 0.25 ± 0.15 Yes

1.7 1-2 Gy 1.41 ± 0.46 Yes

3.3 >2 Gy 3.48 ± 0.69 Yes

*SD: Standard deviation of samples between participating laboratories

Table 9. Summary of the results of the OSL inter-comparison using the “full-mode” protocol. For each nominal dose, the mean value of the measured doses between all the labs is reported.

Nominal dose, Gy Triage category Mean measured dose ± SD, Gy Correct category?

0.3 <1 Gy 0.36 ± 0.36 Yes

1.7 1-2 Gy 1.50 ± 0.60 Yes

3.3 >2 Gy 3.26 ± 0.76 Yes

*SD: Standard deviation of samples between participating laboratories

Conclusions – comparisons of the assays

The ANOVA results, which show no overall differences between labs, gives a very clear indication that the MULTIBIODOSE approach of applying multiparametric tools to radiation emergencies is valid and effective.

The triage categorisation results show that, on the whole, the methods and assays are very good at identifying doses within the correct triage category. An exception to this could be for the high dose partial body exposure, for which the whole body equivalent dose is estimated to be in the 2 Gy+ triage category. In terms of the z-test results, the samples in this exercise which fall into this category (U4 and S4) were shown to elicit the most variation in estimates between laboratories and the largest deviation from the actual experimental whole body equivalent dose. However, it is obvious from the triage categorisation results of the individual biological assays (tables 2, 3 and 4) that it is the dicentric assay results that are driving this overestimation. With a whole body dose equivalent of 1.815 Gy, and an actual simulated partial body dose of 2.5 Gy to ~67% of the body, it could be argued that a triage categorisation of > 2 Gy would be appropriate. This approach has therefore been adopted for Multibiodose.

For the biodosimetry assays, categorisation was without exception most successful based on dose estimates alone, i.e. ignoring the standard errors associated with the estimates. This is somewhat unexpected, as it was initially hypothesised that it would be necessary to use the upper standard error or confidence limit to ensure that some doses weren't missed. However, this result strongly demonstrates that the assays actually do what they are supposed to be doing, and that the dose estimates provided are indeed a good indication of the actual exposure dose. One limitation that should not be forgotten, however, is that we do only have a small number of doses and simulated exposure scenarios: further validation, testing and perhaps redefinition of the categorisation scheme should also be recommended.

In terms of timing, by day 3 for the dicentric and foci assays, and day 4 for the micronucleus assays, all samples were correctly categorised: adding new data didn't change the category, because categorisation was done by dose alone (and worked best this way for all assays and for all data combined). In fact, it is the results that arrived in the last few days that changed the categorisation of the high dose partial body samples from the correct category to too high. Again, this highlights the need to look further at the question of partial body exposures.

The EPR intercomparison was carried out in parallel in two groups of laboratories with the different aims of validating the method and to simulate a real accident situation. The results of the first group were very satisfactory. The three participants were able to identify correctly all dose categories. The difference between actual and measured dose was less than twice standard uncertainty for all the participants. The results of the second group were less straightforward. The calibration curves were affected by a large uncertainty, due to the non uniformity of the calibration samples. In some cases an unexpected influence of the shipping and storing conditions was hypothesized. Nevertheless 5 laboratories out of 8 were able to identify the correct category for the intermediate dose range. All participants were able to identify the correct category for the high dose range and for the unirradiated samples. The results from the first group confirmed that the method that was set up during the Multibiodose project has the potential to work as a method for radiological triage. The results from the second group indicated possible pitfalls of the method that deserve further investigation. Nevertheless, even with non uniform samples, the method is able to discriminate between irradiated and non irradiated samples and between high and low doses. It is suggested that corrective actions are designed and that a new intercomparison is carried out within the RENEB project.

For the OSL intercomparison, results were very satisfactory with both protocols and for all dose ranges in terms of triage categorisation. In all cases the mean of the doses measured by the labs fell in the correct

range (see Table 1 and Table 5) and correctly estimated the nominal dose within error bars (1 SD). Considering fast- and full-mode separately, there is a slight prevalence of correct categorisations in the case of the preheated samples (91 %) with respect to non-preheated ones (88 %), but it is not significant. On the other hand, if one considers dose assessment, slightly better results were obtained using the fast-mode protocol. All in all, the method appears to be very promising, nevertheless 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 and that a new intercomparison is carried out within the RENEB project for those partners where wrong sampling turned out to be the main source of error.

Finally, it should be kept in mind that for both the EPR and OSL assay, the majority of participants did not have any previous expertise in the methods and received only a relatively short training of two days prior to the exercise.

Overall, the results of the exercise show good consistency in dose estimates provided between laboratories and dosimetry methods, although some variation has been detected. However, importantly, consistently successful triage categorisation has been demonstrated for the full range of doses and irradiation schemes tested as part of the exercise.

OVERALL CHARACTERISTICS OF THE TOOLS

The assays were characterised not only with respect to their dosimetric performance but also their time performance. Table 1o shows the approximate duration (in days) between the time point of sample arrival at the laboratory and the completion of dose estimations, calculated for different numbers of samples analysed by one or five laboratories. The calculation was made for one person per lab working 8 hours per day. In case of automatic scoring it was assumed that the scoring system works 24h per day.

Table 10. Approximate duration (in days) between the time point of sample arrival to the laboratory and the completion of dose estimation for the purpose of triage.

Biodosimetric
tool Total time in days to analyse samples
(excluding time of shipment)
1 sample
1 lab 50 samples
1 lab 100 samples
1 lab 100 samples
5 labs 1000 samples
1 lab 1000 samples
5 labs
Dic manual 2.5 6 9 5 65 16
Dic automated 2.5 3 4 3 19 6
MN automated 3.5 4 5 4 20 6
Gamma_H2AX < 1 1 1 1 3 3
EPR < 1 1 4 1 40 14
OSL < 1 1 4 1 40 14

The aim of the table is not to provide precise time estimates, but rather to give a comparative overview of the characteristic of each method. The stated times may change based on the momentary capacity and

work load of a laboratory. It is evident that the fastest method is the gamma-H2AX assay, followed by EPR and OSL. It must be remembered that, due to signal loss, gamma-H2AX can only be used up to a few days post exposure. The speed of analysis decreases for EPR and OSL as the number of samples increases. This is due to the fact that there is no possibility to analyse samples in parallel in a laboratory equipped with a single EPR and OSL reader. In contrast, the preparation of samples for the analysis of dicentric chromosomes and micronuclei can be done in parallel and the analysis itself is automated, so many samples can be analysed in a reasonable period of time.

OVERALL CAPACITY OF THE MULTIBIODOSE LABORATORIES

A major aim of MULTIBIODOSE was to set up networking of the partner laboratories so that in case of a large radiological emergency samples can be shared and analysed in parallel, leading to a large capacity. This overall capacity was assessed and the results are summarised in table 11, expressed as number of samples that can be processed per week and per month.

Table 11. Approximate total capacity of the MULTIBIODOSE partner laboratories expressed as number of samples that can be analysed for the purpose of triage per week and per month.

Tool: Dic and MN Gamma- H2AX EPR and OSL

Time period: Week Month Week Month Week Month

1100 4200 2300 9100 4200 17900

The major reason why the capacity for the dicentric and micronucleus assays is lowest lies in the necessity to culture lymphocytes for 48 hours (Dic) and 72 hours (MN). The highest capacity for EPR and OSL results from the possibility to mobilise a large number of competent laboratories that are incorporated in the EURADOS network of retrospective dosimetry. It is important to bear in mind that the values represent approximations and that the numbers can change based on the momentary personal capacity and work load of a laboratory.

CONCLUSIONS

The Multibiodose consortium successfully tested, adapted, and validated five biodosimetry assays for their use in triage biodosimetry in a mass casualty situation. The automation of the assays has also been validated. The application of EPR and OSL assays in portable electronic devices was developed and validated not only among MULTIBIODOSE laboratories but also in 27 EURADOS associated laboratories, some of which are placed outside the EU. The intercomparison exercises and validation of results between the laboratories have made it possible to act in a concerted way in case of a mass casualty accident. As a result of this, both the speed of performing the assays and the throughput of the laboratories have been optimised.

In an emergency situation a MULTIBIODOSE partner laboratory in the affected country (or another national laboratory designated to perform biodosimetry) should act as the “core” or “administrative” laboratory that will be in charge of the decision which assays to use and how to involve other laboratories. The laboratory will give advice to the health and radiation protection authorities about collection of samples and it will collect the results from other laboratories. In the end, this laboratory will provide the health and radiation protection authorities with dosimetric and radiological triage categorisation results to support medical and public health decisions.

REFERENCES

Alexander GA et al. BiodosEPR-2006 Meeting: Acute dosimetry consensus committee recommendations on biodosimetry applications in events involving uses of radiation by terrorists and radiation accidents. Radiation Measurements (2007) 42:972 – 996.

Blakely WF, Salter CA, Prasanna PG.. Early-response biological dosimetry - recommended

countermeasure enhancements for mass-casualty radiological incidents and terrorism. Health Phys (2005) 89:494-504.
Fattibene P, Wojcik A. (Eds.), Biodosimetric tools for a fast triage of people accidentally exposed to ionising radiation. Ann Ist Super Sanità (2009); 45(3).
Horn, S., Barnard, S. and Rothkamm, K. Gamma-H2AX-based dose estimation for whole and partial body radiation exposure. PLoS One (2011), 6, e25113.
IAEA, Cytogenetic dosimetry: Applications in preparedness for and response to radiation emergencies. International Atomic Energy Agency (2011), Vienna, Austria.
IAEA, Use of electron paramagnetic resonance dosimetry with tooth enamel for retrospective dose assessment. Report IAEA-TECDOC-1331, International Atomic Energy Agency (2002), Vienna, Austria.
Lassmann, M., Hanscheid H, Gassen D, Biko J, Meineke V, Reiners C and Scherthan H. In vivo formation of gamma-H2AX and 53BP1 DNA repair foci in blood cells after radioiodine therapy of differentiated thyroid cancer. J Nuc Med (2010); 51, 1318-1325.
Pope, I.A. Barber, P.R. Horn, K., Ainsbury, E., Rothkamm, K. and Vojnovic, B. A portable microfluidic fluorescence spectrometer device for gamma-H2AX-based biological dosimetry. Radiat Meas (2011), 46, 907-911.
Romm H, Oestreicher U, Kulka U. Cytogenetic damage analysed by the dicentric assay. In: Biodosimetric tools for a fast triage of people accidentally exposed to ionising radiation. Fattibene P, Wojcik A. (Eds.) Ann Ist Super Sanità (2009); 45(3): 251-259
Romm H., Ainsbury E, Bajinskis A, Barnard S, Barquinero J-F, Beinke C, Puig-Casanovas R, Deperas-Kaminska M, Gregoire E, Kulka U, Oestreicher U, Lindholm C, Moquet J, Rothkamm K, Sommer S, Thierens H, Vral A, Vandersickel V, Wojcik A. The dicentric assay in triage mode as a reliable biodosimetric scoring strategy for population triage in large scale radiation accidents. Proc IRPA (2012); 13:TS 2c
Romm H, Ainsbury E, Barnard S, Barrios L, Barquinero JF, Beinke C, Deperas M, Gregoire E, Koivistoinen A, Lindholm C, Moquet J, Oestreicher U, Puig R, Rothkamm K, Sommer S, Thierens H, Vandersickel V, Vral A, Wojcik A. Automatic scoring of dicentric chromosomes as a tool in large scale radiation accidents. Mutation Research (2013) in press.
Rothkamm, K. and Horn, S. γ-H2AX as protein biomarker for radiation exposure. Ann Ist Super Sanità (2009), 45:265-271.
Rothkamm K, Barnard S, Ainsbury EA, Al-hafidh J, Barquinero JF, Lindholm C, Moquet J, Perälä M, Roch-Lefèvre S, Scherthan H, Thierens H, Vral A, Vandersickel V (2013). Manual versus automated gamma-H2AX foci analysis across five European laboratories: can this assay be used for rapid biodosimetry in a large scale radiation accident? Mutat Res (2013) , doi: 10.1016/j.mrgentox.2013.04.012.
Rothkamm K and Loebrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Natl Acad Sci U S A (2003), 100:5057–5062.
Schunck C et al. New developments in automated cytogenetic imaging: unattended scoring of dicentric chromosomes, micronuclei, single cell gel electrophoresis, and fluorescence signals. Cytogenet Genome Res (2004), 104:383-389.

CONSISE DESCRIPTION OF RESULTS OBTAINED WITHIN EACH WP

WP 1

The work plan of WP 1 was is split into 6 tasks. The progress made in these tasks will be described shortly. Details are given in the appropriate deliverables.

Task 1.1 involved validation of the established, conventional dicentric assay in the participating laboratories. This step was necessary to ensure the homogeneity of dose estimation between the laboratories. Three types of irradiation scenarios were involved: acute whole body exposure, protracted exposure and acute partial body exposure. Each laboratory set up cultures of irradiated blood with respect to the ISO Standards and estimated a dose in a number of blinded samples. In total, all labs were well trained during this exercise and showed their experience for the different scenarios by using the corresponding cytogenetic tools to provide helpful dose estimations. Furthermore, some limitations to scoring in triage mode, when a reduced number of cells are evaluated, were observed. With respect to the homogeneity of the conventional scoring results, it may be concluded, that each lab was able to demonstrate great expertise in managing the biological dosimetry challenge of this exercise.
Task 1.2 was to investigate and validate the usefulness of a semi-automatic dicentric scoring system. The aim was to demonstrate whether the automated system is able to distinguish between different doses and exposure conditions, relevant for triage. Initially, the scoring criteria for dicentrics were adjusted between the beneficiaries using a gallery of 80 high resolution images containing dicentrics. Semi-automation of the dicentric assay was successfully introduced at 6 laboratories. In each laboratory a calibration curve for gamma radiation was established. It has been shown that the dicentrics detected by the software follow a Poison distribution (which is relevant for the dose effect curves). Validation of the curves has demonstrated very promising results for blood samples of simulated acute whole body exposures. Semi-automated scoring has also shown to allow the detection of inhomogeneous and protracted exposures. In addition, the throughput is massively increased compared to manual scoring. It is therefore concluded that semi-automated scoring can be used in a large scale radiation accident as a pre-screening (triage) tool.
Task 1.3 was to investigate and validate the usefulness of “telescoring” or “web based scoring” of dicentric assay. The aim was to investigate whether it is possible to analyse high resolution images of metaphase spreads in the internet. The idea behind this method stemmed from new technical possibilities arising from the internet, which can be used in the case of a large scale accident, to share images with trained scorers anywhere in the world. The open question is, how reliable will the results be. It is known that there are big variations concerning scoring between the labs - therefore it is recommended that each lab should have its own calibration curve. Furthermore, two different methods of web based scoring were applied: 1) counting of clearly visible dicentrics in apparently complete cells (quick scoring) and 2) analysis of complete cells (46 chromosomes, conventional scoring on the screen). The experience with the web based scoring has shown that the 8 participating labs were able to achieve comparable results. The obtained yields of dicentrics were similar enough to establish a dose effect curve, which could be used for reliable dose estimations with blind samples after whole body exposure. Furthermore, it was possible with images of metaphase spreads received from 4 labs with different slide preparations and two different colcemid treatments. The applied quick scan scoring strategy, which is about six times faster than the restrictive conventional triage mode did not show a reduced accuracy. The estimated doses of the whole body exposed samples were in triage mode in 21 of 24 cases within ± 0.5 Gy of the true dose, 1 time within ± 1.0 Gy and 2 times > 1.0 Gy of the true dose. In quick scan mode the dose estimated were 18 times within ± 0.5 Gy of the true dose and 6 times within ± 1.0 Gy of the true dose.
Task 1.4 was to compare the dicentric assay with the other bioassays. The performance of all assays was compared through an exercise. The results are described above (Comparison of the performance of the tools through an exercise). The performance of the assays respective the time needed to analyse a certain number of samples is given in the table below:

Task 1.5 was to establish contact with MetaSystems with the intention of improving the performance of the Metafer image analysis system for automatic dicentric scoring. The intercomparison and training exercise performed by the partners of WP 1 in task 1.2 of the MULTIBIODOSE project produced satisfactory results which demonstrate the suitability of the Metafer image analysis system for semi-automatic dicentric scoring for population triage after a large scale radiation accident. Dicentric chromosome candidates were detected by the DCScore software in high resolution images, which were captured in the automated mode by “Autocapture”. Results were obtained by 6 laboratories using their own standards for slide preparation and scoring and three different classifiers for dicentric classification. We contacted MetaSystems (Dr. Christian Schunck) with the intention to further improve the automatic scoring of dicentrics. The discussion of different items with MetaSystems resulted in different possibilities for the iterative design of a user-friendly and intuitive interface for automated scoring of dicentric chromosomes.
Task 1.6 was to develop a future training program. When MULTIBIODOSE kicked off in May 2010 it was the only project that received any funding to create a network of laboratories dealing with biological dosimetry in Europe. In January 2012 the project RENEB (www.reneb.eu) kicked off which is a Coordination Action (CSA-CA) founded within the 7th EU framework EURATOM Fission Programme. The goal of RENEB is to establish a sustainable European network in biological dosimetry involving laboratories and organisations from 16 European countries. RENEB will develop an operational structure including a long term funding strategy that will guarantee sustainability once the EU funding is over. All MULTIBIODOSE partners are members of RENEB and many have leading positions (all WP leaders of RENEB are members of MULTIBIODOSE). All biodosimetric tools developed and validated in MULTIBIODOSE are also included in RENEB. Within RENEB an exercise and training program was developed that will be pursued in the future.

WP2
The work plan of WP 2 was is split into 7 tasks. The progress made in these tasks will be described shortly. Details are given in the appropriate deliverables.

Task 2.1 was to standardise the automated scoring of micronuclei. Parameter settings of the Metasystems MSearch image analysis system were optimised by every beneficiary so that the same performance characteristics of automated scoring were obtained. This procedure was based on visual inspection of the objects in the image gallery and manual scoring of false positive binucleated cells. A standardised protocol for the micronucleus assay for automated scoring was devised in collaboration between BfS and UGent. With respect to image analysis the classifier settings for automated micronucleus scoring with the Metafer 4 system (MetaSystems) were optimized and included in a protocol that forms the content of deliverable 2.1.
Task 2.2 was to set up dose response curves for different exposure conditions. In vitro irradiated blood samples were transported from UGent to Bfs, BIR, HPA and INCT. In each participating center at least two blood cultures of each sample were set up and the micronucleus assay was performed following the protocol described in deliverable 2.1. Using the local computerized image analysis Metafer 4 system (MetaSystems) automated and semi-automated scoring was performed for at least two slides for each sample. Micronucleus yields of all partners were collected, which allowed an interlaboratory comparison. Common dose response curves were obtained by averaging the data over all partners except for HPA data. The data set of HPA showed no clear dose response in contrast to the data of the other partners, which were generally in good agreement.

Task 2.3 was to set up a database of spontaneous micronucleus yields. Blood samples were collected by all WP partners. At least two blood cultures of each sample were set up and the micronucleus assay was performed by all partners following the protocol described in deliverable 2.1. Using the local computerized image analysis Metafer 4 system (MetaSystems) with local classifier settings in the MSearch software three types of scoring were performed for at least two slides for each sample: complete automated scoring, automated scoring with cut off at MN frequency of 4 MN/1000 binucleated (BN) cells and semi-automated scoring with manual inspection of the gallery of selected BN cells. All data gathered by the partners were pooled for analysis. For the interpretation of the results the donors were asked to fill in a questionnaire with the following information: gender, age, smoking or non-smoking. The pooled population of 202 individuals consisted of 83 males and 119 females.
Task 2.4 was to organise a large scale accident training exercise. In vitro irradiated blood samples were sent to all partners of WP2 for blind scoring and dose assessment. Different exposure conditions (acute whole body, partial body) were applied. The dose estimates, obtained by the partners, were sent directly to E.A. Ainsbury (HPA) for intercomparison and statistical analysis. The training exercise has produced satisfactory results which demonstrate the suitability of the automated micronucleus assay to deliver reliable dose estimates for population triage within 4-5 days after a large scale radiation accident. The exercise shows also the consistency of the dose estimates, obtained by participating laboratories, supporting the network as platform to tackle biodosimetry of large populations in radiation accidents using automated micronucleus scoring. The observed agreement is the result of the standardization of the automated micronucleus assay and systematic training , which has taken place during the project.
Task 2.5 was to compare the micronucleus assay with the other bioassays. The outcome of this deliverable is described under Task 1.4 above.
Task 2.6 was to establish contact with MetaSystems with the intention of improving the performance of the Metafer image analysis system for automatic micronucleus scoring. Dr. Christian Schunck from Metasystems was conacted with the intention to further improve the automatic scoring of micronuclei. The discussion of different items with MetaSystems has resulted in different possibilities for the iterative design of a user-friendly and intuitive interface for automated scoring of MN.
Task 2.7 was to develop a future training program. Please see task 1.6 for the outcome of this task.

WP 3
The work plan of WP 3 was is split into 8 tasks. The progress made in these tasks will be described shortly. Details are given in the appropriate deliverables.

Task 3.1 was to optimise blood sampling, storage, transport and processing for maximum reproducibility, sensitivity and throughput. Inter-laboratory comparison, experimental testing and optimisation have been performed for the individual steps involved in blood sampling, transport and processing for gamma-H2AX biodosimetry. Suitable parameters and conditions have been established for the different steps, processing time has been shortened from ~3.5 h at the outset to ~2.5 h even for the standard protocol with no significant loss of sensitivity. A protocol facilitating high throughput processing has been established which should reduce the time required for processing 96 blood samples to ~4 hours. A separate protocol for processing samples for fluidic gamma-H2AX analysis has been developed which, however, cannot offer the same sensitivity as the microscopy-based assays, thus potentially limiting its usefulness to blood samples obtained within a few hours after the radiation exposure.
Task 3.2 was to develop high throughput fluidic fluorescence intensity and automated microscopic foci

analysis systems and procedures suitable for γH2AX-based triage and identify the potential for commercial exploitation. A fluidic approach was developed and tested. It did not fulfil the requirements in terms of sensitivity to be useful in a radiation accident scenario where first blood samples are likely to be taken many hours or days after the exposure. As a consequence, no further effort has been made towards its commercial exploitation. The microscopic approach looks much more promising but variable staining quality of samples complicates automated image analysis procedures available in commercial systems.
Task 3.3 was to obtain γ-H2AX reference data sets for different doses and time points following acute whole body, acute partial body and chronic whole body irradiation. Reference data sets for γ-H2AX analysis were generated by in vitro irradiation of peripheral blood lymphocytes obtained from healthy volunteers. Data were obtained for different exposure scenarios. These data were used to establish dose response calibration curves for i) X-rays and ii) gamma-rays at two different time points after exposure. For the Co-60 gamma ray exposures, any changes caused by overnight sample shipment – which would be a likely scenario in the event of a real large scale radiation accident – were taken into account. The obtained results suggest that the higher speed and convenience of automated relative to manual foci scoring needs to be balanced against its compromised accuracy, stronger dependence on reproducible sample staining quality and inability to detect partial body exposure.
Task 3.4 was to assess inter-individual variation of baseline and in vitro irradiation-induced gamma-H2AX levels in 25 volunteers. The spontaneous level of foci was estimated in lymphocytes from 25 healthy donors. Radiation induced foci were analysed in lymphocytes of between 16 and 25 donors (different numbers for different levels of dose). The results suggest that inter-individual variation in i) spontaneous foci, ii) their induction yields following irradiation, and iii) residual levels following 24 h repair incubation are not a major source of uncertainty when using gamma-H2AX as a quantitative biomarker of radiation exposure.
Task 3.5 was to test the performance of the γ-H2AX assay following in vivo exposure. Data for γ-H2AX foci induction in peripheral blood T lymphocytes from 20 prostate cancer patients receiving different types of radiotherapy confirm the linear dose response for in vivo exposures, with a similar foci yield as observed for ex vivo-irradiated blood. Good correlation of foci frequencies with mirconucleus counts obtained for the same patients provides additional evidence that the γ-H2AX foci assay can be used for biological dosimetry. The good consistency between in vivo and ex vivo responses observed so far provides support for the use of calibration curves for different radiation exposure scenarios established with ex vivo samples for conversion of foci counts into dose estimates. Nonetheless, additional in vivo data should be obtained at higher doses and other time points to further validate the assumption of equivalence in the γ-H2AX response in vivo and ex vivo.
Task 3.6 was to organise a large scale accident training exercise. The training exercise for the gamma-H2AX assay has produced encouraging results which demonstrate the suitability of this method to deliver rough dose estimates within a few hours with good throughput compared to conventional cytogenetic assays. More work is needed to reduce uncertainties associated with the variability of foci yields and to address the detection of partial body exposures. It will also be important to assess the accuracy of dose estimations at different time points post exposure in future inter-comparison exercises. More results from the exercise are given above, in the chapter “Comparison of the performance of the tools through an exercise”.
Task 3.7 was to compare the gamma-H2AX assay with the other bioassays. The outcome of this deliverable is described under Task 1.4 above.
Task 3.8 was to develop a future training program. Please see task 1.6 for the outcome of this task.

WP 4
The work plan of WP 4 was is split into 10 tasks. The progress made in these tasks will be described shortly. Details are given in the appropriate deliverables.

Task 4.1 was to obtain an ethical permission for collecting blood samples. The permission was obtained.
Task 4.2 was to select optimal parameters to quantify the dose to the skin in the pig model for the SSA assay. To meet the objectives of the contract, experimental strategy was adapted in reference to the initial plan. In a first step, a model of localized irradiation was implemented in rats for a rapid screening of a larger number of animals needed to define the reproducibility of the signal, the minimum time after exposure at which the signals become detectable, the establishment of the signal persistence for different dose threshold. This model was used to conduct experiments on a larger number of animals. Rats were irradiated at different dose including 0 Gy (self control), 15 and 25 Gy. 3 rats were irradiated for each experimental condition. 3 polarisation states corresponding to 3 level of depth were investigated. 6 time points (day 0 to day 8) were measured. In a second step, a mini-pig model of localized irradiation close to the accidental situation, i.e irradiation over a relatively small area (<20% body surface) with a sharp dose gradient was set up. Two animals were irradiated.
Task 4.3 was to obtain 2DG scans of serum proteins from mice exposed to radiation. The sensitivity and reproducibility of the radiation signal was determined in serum of 20 mice per dose (0, 20, 40 and 80 Gy) and per blood collection time (day 1 and day 3 post-irradiation), representing a total number of 160 mice. Changes in the expression of serum proteins were investigated using proteomic tools (2DG electrophoresis). 34 candidate proteins were identified by mass spectrometry.
Task 4.4 was to obtain a proteomic signature of skin exposure. Results from task 4.3 were analysed by multivariate analysis. 8 proteins were identified that allow discriminating exposed from non-exposed animals:
APOE_MOUSE Apolipoprotein E
APOH_MOUSE Apolipoprotein H
CO7_HUMAN Complement component 7
FA10_MOUSE Coagulation factor X
FETUB_MOUSE Fetuin B
MUG1_MOUSE Murinoglobulin 1
PANK4_MOUSE Pantothenate kinase 4
SPA3K_MOUSE Serine protease inhibitor A3K

Task 4.5 was to validate SSA on skin of patients receiving radiotherapy. Due to their significant anatomical and physiological similarities, porcine skin is the reference biological model of human skin. Therefore, we used pigs in order to validate SSA. The use of pig skin is described in the DoW part B1.2.5. In the DoW table B1.3.5.4 (Workpackage 4 description) and in the table B1.3.4 List of Deliverables it is wrongly stated that we shall test the SSA on patients undergoing radiotherapy. Two animals were irradiated. 4 skin areas constituted the dose gradient of 0 to 40 Gy. The higher the dose, the earlier was the discrimination by the speckle technique. Nevertheless, discrimination of the 40 Gy irradiation was not possible before days 33 – 39 after irradiation. From the point of view of triage – this time is too long to be of any practical use. Given this result, it did not appear worthwhile to continue research on the skin speckle assay for the purpose of triage dosimetry. This conclusion was reached by the MULTIBIODOSE consortium after presentation of

the results during the annual meeting in Lilehammer (May 2012).
Task 4.6 was to collect serum from patients receiving radiotherapy. Blood collection started in December 2011. This delay was caused by organisational problems at the Karolinska hospital in Stockholm, where patients are treated. Blood was collected before radiotherapy, at 3 time points during therapy (after 2 Gy, after 10 Gy and after 20 Gy – blood was collected on the day of completion the dose) and one month after radiotherapy. Against earlier expectations, it was only possible to collect blood samples from a total of 16 patients.
Task 4.7 was to validate the SPA in serum of patients undergoing therapy collected during task 4.6. Human analogues to the proteins listed under task 4.4 were found and antibodies for ELISA says obtained. Serum from each probe was diluted and applied in triplicate to 96-well plates pre-coated with antibody against a candidate protein . The standard protein dilutions were run in parallel, the absorbance was measured on a microplate reader at the wavelength of 450 nm. A standard concentration curve was constructed for each protein and the corresponding absorbance was obtained from triplicates. The concentrations of proteins in the serum samples were determined from the standard curves. The results indicated significant difference between patients for all proteins, significant difference between repeats for A2M and alpha-1-ac, no significant effect of dose for APOE, C7 and FETUB. Posthoc testing demonstrates significant difference between all dose levels for APOH, FX and PANK4. Some other pair-wise significances were observed, but without overall trend. No improvements in the above when likely endpoints are combined – actually in some cases it causes the dose to be no longer significant. In view of this it was decided not to include the serum protein assay in the battery of MULTIBODOSE dosimetric tools.
Task 4.8 was to compare the SSA and SPA assays with the other biodosimeters. This task was abandoned in view of the decision not to include SSA and SPA in the battery of MULTIBODOSE dosimetric tools.
Task 4.9 was to develop a future training program for SSA and SPA. This task was abandoned in view of the decision not to include SSA and SPA in the battery of MULTIBODOSE dosimetric tools.
Task 4.10 was to identify opportunities for commercial exploitation of SPA. This task was abandoned in view of the decision not to include SSA and SPA in the battery of MULTIBODOSE dosimetric tools.

WP 5
The work plan of WP 5 was is split into 6 tasks. The progress made in these tasks will be described shortly. Details are given in the appropriate deliverables.
Task 5.1 was to analyse elements from mobile phones for their suitability to be used for dose assessment by EPR and OSL. The analysis included three criteria: (1) percent of availability in portable electronic devices (PED), (2) the presence of a radiation specific signal immediately after 10 Gy irradiation; (3) the presence of a remnant radiation induced signal 10 days after irradiation. This was done by measurement of display windows glass and alumina based resistors and capacitors in 25 mobile phones in each lab. Each lab measured 25 mobile phones (MP): (1) 7 MP: similar at all partners' labs. They were used MP (produced before 2009) provided by IRSN, (2) 18 MP: different models at each partner's lab. Purchased under Multibiodose and produced after 2010. Total numbers of MP measured: 75.
For OSL the results showed that:
• All electronic components (EC) showed a radiation-induced OSL signal.
• If present, all EC show detection limits well below 1 Gy.
• Inductors seem to be the most sensitive and the capacitors the least sensitive.

• All EC showed fading: typically 50% in 10 days, except small inductors (30% signal loss in 10 days)
• Intermediate size EC are present in sufficient quantities in all mobile phones (normal and smart phones)
• Small size EC are present in a smaller percentage, but the scenario might change quickly with the technology advancement
• Capacitors are present in about half of all mobile phones
For EPR the results showed that:
• 5 types of EPR spectra were identified of which all but one were sensitive to radiation
• Out of the 5 radiation sensitive, two were clearly specific of radiation (i.e. distinguishable from the signal in non irradiated glass). These spectra were found mostly in the oldest and in the newest models of mobile phones.
• Radiation specific signals are not clear in the other EPR spectra, but might be identified by spectrum analysis (e.g. deconvolution).
Task 5.2 was to develop optimized EPR/OSL procedures for the components selected in Task 5.1. The protocols were developed and the deliverable was divided in two parts:
1: Operative protocol for the identification of irradiated resistors by Optically Stimulated Luminescence (OSL) and determination of the dose received by the resistors.
2: Operative protocol for the identification of irradiated glass by Electron Paramagnetic Resonance (EPR) spectroscopy and determination of the dose received.
Task 5.3 was to evaluate performance parameters of EPR /glass and OSL/resistors methods and to communicate them to WP6. Following performance parameters were evaluated:
OSL: (1): fading correction, (2): Dose assessment. It was concluded that when the exposure history is unknown a conservative universal fading correction with larger uncertainty has to be applied.
EPR: The parameters critical dose (amplitude) and limit of detection were chosen for analysis of the uncertainty of EPR measurements. The critical dose and the limit of detection were defined. It was decided that for signals larger than the critical amplitude, the uncertainty in assessed dose in glass is estimated as the confidence interval for the dose value determined with the calibration curve from replicate signal measurements of the test sample. For signals having amplitude smaller than the critical amplitude, the signal is considered not detected and it is given the upper limit of the confidence interval.
Task 5.4 was to carry out an inter-laboratory comparison with EPR and OSL. The EPR inter-comparison was carried out in parallel in two groups of laboratories:
1) Group A: this was formed by three participants. The samples were taken by a bulk of glass fragments of various smartphones. These samples could then be considered uniform. The storing conditions and the acquisition parameters were the same for the three participants. The results of this comparison were aimed at demonstrating that the method is effective.
2) Group B: this group was formed by eight participants. For this group samples were individually prepared from different smartphones and therefore were not uniform. Shipping and storing conditions and acquisition parameters were different among laboratories. This comparison was aimed at evaluating the method performance in a situation close to the one which could occur in a real accidental situation.
The results of the first group were very satisfactory. 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 then twice its standard uncertainty for all the participants.
The results of the second group 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 participants, but one, were able to identify the correct category for the high dose range and for the non irradiated samples. It was hypothesized an unexpected influence of the shipping and storing conditions and of the non uniform samples.
The OSL intercomparison was carried out 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.
Concerning the triage categorisation, results were very satisfactory with both protocols and for all dose ranges. In all cases the mean of the doses measured by the labs fell in the correct range and correctly estimated the nominal dose within error bars (1 SD).
Even if the method appears to be very 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 and that a new inter-comparison is carried out within the RENEB project for those partners where wrong sampling turned out to be the main source of error.
Task 5.5 was to compare EPR and OSL with other biodosemeters. The outcome of this deliverable is described under Task 1.4 above.
Task 5.6 was to develop a future training program for EPR/OSL. Please see task 1.6 for the outcome of this task.

WP 6
The work plan of WP 6 was is split into 6 tasks. The progress made in these tasks will be described shortly. Details are given in the appropriate deliverables.
Task 6.1 was to compare existing data analysis methods to identify the state of the art and the current shortcomings for the assays. It was concluded that the sources of uncertainty of the assays are well understood, whereas for assays such as the SSA and SPA, more work is required to finalise the techniques themselves before work can begin in identifying and characterising the likely sources of error in each case. Quantification of the uncertainties associated with all the individual assays must then be carried out. It is intended that this will be done to the high standard of the dicentric assay – thus varying degrees of work are required in each case depending on the overall level of development of each assay. In all cases, the main sources of uncertainty are likely to be from the comparison of results of the different laboratories and of the different techniques. These factors will need to be considered in detail in order for the proposed software to be able to produce the planned 'single' dose estimate in each case. For instance, each of the assays has different detection limits and ranges of sensitivity, and this must be explicitly taken into account within the software. It is thus likely that it will be most appropriate to give the results in terms of a range of doses, which are dependent on confidence limits. The existing programs that have been developed by MULTIBIODOSE partners and others can be built upon to produce the software. Overall, this method should allow us to meet the final aim of the project, which is, as discussed in the proposal, assignment of a patient into one of (e.g.) three triage categories for radiation exposure.
Task 6.2 was “where shortcomings are identified or analysis methods are not universally defined, develop the methodology so that data analysis for each method is comparable, with reference to the dicentric assay. As discussed, in many cases, more work is required to finalise the techniques themselves before

work can begin in identifying and characterising the likely sources of error in each case. Final quantification of the uncertainties associated with all the individual assays must then be carried out. It is intended that this will be done to the high standard of the dicentric assay – thus varying degrees of work are required in each case depending on the overall level of development of each assay.
Once the sources of error are fully understood and characterised for each assay, work on task 6.3 statistical combination of the uncertainties, will commence. By its very nature, combination of sources of error is a complex task, and combining the many different uncertainties involved in the seven extremely different techniques involved in the MULTIBIODOSE project will not be trivial. However, a pragmatic approach is planned, whereby the most important sources of uncertainty are identified for each assay, and the more trivial uncertainties are ignored. This will allow the calculations to be somewhat simplified. In all cases, the main sources of uncertainty are likely to be from the comparison of results of the different laboratories and of the different techniques with, for instance, different detection ranges and sensitivities, and these will need to be considered in the final uncertainty calculations. Additional sources of error will also be induced by triage conditions and assay automation, where this is implemented.
Task 6.3 was to report on methods developed for statistical combination of data and decisions regarding assignation of triage status (high, medium, low exposure). The deliverable contains details of the statistical combination of data and decisions. In order to provide a single dose category estimate, there will need to be a number of decisions taken within the software. The intended process of using the software will be as follows:
1) Mode selected – Group analysis mode or Individual analysis mode
2) Information input – Type of radiation; type of exposure; likely highest and lowest doses, numbers to be analysed (in group mode); information regarding localisation for each individual
3) Dosimetry input – Enter doses calculated using each dosimetry method used in each case
4) Calculation of triage category
For step 1, if group mode is selected, a data base will be set up to record individual doses as they are entered. The software will then continue as normal with individual analyses. The information provided in step 2, if given, will allow automated assessment of the most appropriate method(s) of dosimetry, based on the following criteria:
- Is the dose acute?
- Is the dose homogenous (whole body) or localised (partial body)?
- What is the likely dose range (link to operationally detectable ranges of different methods)?
For step 3, dose (and uncertainty) calculations will be carried out by individual laboratories, according to the standardised methods given in the guidance document, so doses, rather than, for example, individual counts of dicentrics, will be entered into the software.
At step 4, the software will carry out combination of the results from each of the assays, weighting according to the data entered in step 2. The weighting procedure will be defined as part of task 6.4 tested as part of the comparison of the assays, and refined accordingly. However, if no information is available/provided, then the dosimetry methods will be given equal weight according to their associated uncertainty.
Task 6.4 was implementation of the data analysis methods into a decision making software platform and details of planned exercise. The methods of calculation of standard error (se) on dose for the dicentric, micronucleus and gamma-H2AX foci assays have been finalised and agreed. The participants of WPs 4 and 5 have separately developed analysis methods that are applicable for their assays, which contain a comparable level of consideration of the associated uncertainties. It has been agreed that for purposes of

MULTIBIODOSE, the agreed uncertainty analysis methods will be applied at individual laboratory level, so that in the case of an incident, reporting of doses to the coordinating laboratory will simply involve reporting dose +/- se. The Multibiodose software was been created in Java, using java development kit (JDK) 1.7.0_03 and has been tested with java runtime environment (JRE) 7. The software creates a link to a data bases, created in SQLite and linked to the software with the aid of the SQLite java database connectivity library (JDBC). The following methods have been implemented in the software: (1): Database creation/administration (and associated procedures) for previous/new incidents, (2): Group or individual analysis based on assay type, case ID, or pooled for the groups as a whole, with weighting by se, according to the procedures detailed in deliverable 6.3.
The exercise will be carried out as follows: Blood will be collected from volunteers and irradiated at three of the participating laboratories. The irradiations will be carried out blind – only the operators and the exercise administrator know the details of the doses/irradiation schemes that will be included. A total of 12 samples of blood or separated lymphocytes will then be shipped to each participating laboratory. 6 laboratories will carry out the dicentric assay; 6 laboratories will carry out the micronucleus assay and 5 laboratories will carry out the foci assay. HPA will act as the administrating laboratory, so the results will therefore be returned to HPA for analysis and assignment of triage status, using the MULTIBIODOSE software. Once the results have been finalised, the codes on the samples will be broken in order to ascertain the success of the assays and software.
Task 6.5 was implementation of the software onto the website, production of training document. The software has been updated to include a front end of triage questions, according to the MULTIBIODOSE guidance. In addition, weighting of the assay results for triage categorisation is now implemented, so that the assays which are recommended for use in the guidance document are prioritised for triage categorisation. Full details of the software functions are given in the manual which is available for download with the software from the web page of MULTIBIODOSE.
To ensure all project participants are trained in its use, and that the program is as reliable and user friendly as possible, the software and manual were finalised and tested with the assistance of all project participants. At least one representative from each work package tested the software and in addition, eight individuals from across the different work packages were selected as ‘debugging’ testers. These individuals thoroughly tested the software on a number of different platforms (Windows, Mac and Linux systems) and attempted to identify problems with the functionality. A number of issues were identified and resolved, as a result of which the final version of the software is now extremely stable.
Task 6.6 was to validate the software through comparison of individual assays. The details of this task are given in the chapter above “Comparison of the performance of the tools through an exercise”.

WP 7
The work plan of WP 7 was split into 4 tasks. The progress made in these tasks will be described shortly. Details are given in the appropriate deliverables.

Task 7.1 was to set up a webpage. The web page was launched in July 2010 and was updated several times. The updates and improvements were necessary in order to make consortium part of the home page more user friendly; to better structure the news page and to find out how to solve the problem of linking to statistic software etc.
Task 7.2 was to print bulletins describing the progress of work. The first bulletin issued in October 2010 and was sent to radiation protection authorities in all members´ countries. Further bulletins were printed

according to plan and were distributed among radiation protection authorities and at meetings relevant to radiation research and emergency preparedness. Pdf files of the bulletins can be downloaded from the MULTIBIODOSE web page.
Task 7.3 was to present the results at meetings. MULTIBIODOSE was presented at numerous meetings listed in TABLE A2: LIST OF DISSEMINATION ACTIVITIES.
Task 7.4 was to develop guidance on using the tools. The guidance document was developed, printed in 600 copies and distributed among radiation protection authorities. A pdf file with the guidance document can be downloaded from the MULTIBIODOSE web page.

WP 8
The work plan of WP 8 was split into 4 tasks. The progress made in these tasks will be described shortly. Details are given in the appropriate deliverables.

Task 8.1 was to prepare the consortium agreement. The agreements was prepared and signed by all participants.
Task 8.2 was financial management of the project. This task was carried out according to plan.
Task 8.3 was organisation of meetings. This task was carried out according to plan.
Task 8.4 was reporting. This task was carried out according to plan.

Potential Impact:
The socio-economic impact and the wider societal implications of the project
MULTIBIODOSE was a research project with tangible results. In this context, the obtained results can be regarded from two points of view: the added scientific value and the added societal value.
The added scientific value of the project is given by the results of the study. The experiments that were carried out with each WP resulted in a significant increase of our knowledge about the assays and their application for biological dosimetry. A description of this is provided in the chapter “CONSISE DESCRIPTION OF RESULTS OBTAINED WITHIN EACH WP” above. A number of publication and presentations were prepared and these are listed in the Section A below.
The added societal value is given by the fact that MULTIBIODOSE resulted in the creation of a working network of laboratories that are prepared to carry out biological dosimetry in case of a mass casualty radiological emergency. This is the largest network in the world with this type of competence and capable of applying such a high number of different biodosimetric tools. From the perspective of biological dosimetry the EU is now very well prepared to face a radiological mass casualty.

Main dissemination activities
The main dissemination activities were publications and lectures. These are listed in the Section A below. From the perspective of the bodies engaged in radiation protection and emergency preparedness an important dissemination activity was the publication and spreading of the bulletins and of the guidance document. These documents, written in a non-scientific language, contribute towards a better understanding of the possibilities and limits of biological dosimetry and, most importantly, inform the bodies of the existing laboratories that can be contacted in case of an emergency.

Documenti correlati

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Permalink: https://cordis.europa.eu/project/id/241536/reporting/it