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MULTIBIODOSE - Summary of results from each WP and task

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:

	Total time to analyse samples*					
	Time in days					
	1 sample 1 lab	50 samples 1 lab	100 samples		1000 samples	
			1 lab	5 labs	1 lab	5 labs
Dicentrics manual	2.5	6	9	5	65	16
Dicentrics automated	2.5	4	5	3	24	7
Dicentrics telescoring	2.5	6	9	4	65	13
Micronuclei	3.5	4	5	4	20	6
Gamma H2AX	<1	1	1	1	3	3
EPR (ped)[2]	<1	1	4	1	40	14
OSL (ped)	<1	1	4	1	40	14

*: does not include time for shipment of samples
Calculation made for one person per lab working 8 hours per day
In case of telescoring all cultures are done by one lab
In case of automatic scoring the machine works 24h /day
ped: portable electronic devices

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 (www.multibiodose.eu) 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.

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.