Section A

Institution: Durham University

Unit of Assessment: Mathematical Sciences UoA 10

Title of case study: Statistical methods for biological dosimetry

Period when the underpinning research was undertaken: Feb 2014 to now

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Dr Jochen Einbeck	Associate Professor (Reader)	Since Oct 2006
Dr Maria Oliveira	Postdoctoral researcher	Feb-Oct 2014

Period when the claimed impact occurred: 2015 to now

Is this case study continued from a case study submitted in 2014? N

Section B

1. Summary of the impact (indicative maximum 100 words)

Durham University's Statistics group have produced cutting-edge models, methods and software for estimating levels of radiation doses in those exposed in a radiological incident. Their work involved developing statistical methodology that goes beyond traditional procedures, specifically overdispersed count data biomarkers. The procedures developed are referenced in an ISO standard and incorporated into the emergency response plans under RENEB, a European network specialising in radiation dose estimates. The team have developed an Uncertainty Quantification (UQ) framework for the innovative gamma-H2AX protein biomarker – enabling quicker triage compared to other biomarkers, and hence improved preparedness in case of a mass exposure scenario – that has been adopted by Public Health England (PHE) into the standard operating procedures for its commercial biodosimetry unit.

2. Underpinning research (indicative maximum 500 words)

Following a radiation incident involving exposure or suspected exposure of individuals to ionizing radiation, a fast and reliable assessment of the contracted dose is essential for effective diagnosis and treatment. Such an assessment is possible through biomarkers, which allow the contracted dose to be deduced from the damage which the radiation has caused inside human blood cells. The 'gold standard' for this purpose, based on counts of dicentric chromosomes, currently has a total global capacity of a few thousand samples a week, which would clearly be inadequate in the case of a large-scale nuclear accident. Recently, alternative biomarkers based on proteins (specifically, the phosphorylated gamma-H2AX histone) have been developed. While biomarkers of this new generation are considerably quicker to process and allow for larger throughput, no statistical routines were available (prior to our work) to infer the dose estimate and its uncertainty from the biomarker measurement.

In this context, the contribution of the research underpinning this case study is twofold:

(1) Publications **[R1-R3]** develop and discuss, in the context of a wide range of cytogenetic and biomolecular radiation biomarkers, modelling strategies for a correct representation of the uncertainty inherent in such biomarkers. Specifically, publications **[R1, R3]** demonstrate that a commonly made assumption (that of equidispersion, or mean=variance, underlying the Poisson distribution) is wrong or misleading for most biomarkers apart from a few idealized scenarios, and recommend specific alternative models to be used under the violation of this assumption. While a misspecification of this distributional assumption has only a minor impact on the dose estimates themselves, it has a strong impact on the uncertainty assessment. Publication **[R2]** critically assesses the state of the art in uncertainty quantification for radiation biomarkers; specifically it shows that ignorance of this uncertainty can lead to incorrect conclusions (triage classifications, treatments, etc), with potentially severe consequences for the individuals concerned.

(2) Our recent work **[R4],** building on exploratory work in **[R3]**, focuses on the development of statistical methodology for dose estimation and uncertainty quantification for the innovative gamma-H2AX assay, which has so far rarely been

used in laboratories due to the lack of available software and agreed standards. The H2AX histone is a DNA-repair protein, that is, once a cell gets exposed to ionizing radiation and a double-strand break has occurred, it coordinates the repair of the damaged DNA and in this process phosphorylates, becoming gamma-H2AX. This phosphorylation leads, after addition of fluorophore-labelled antibodies, to fluorescent dots which can be counted under a microscope, and then related to radiation dose via statistical models. The specific challenge in the implementation of the gamma-H2AX biomarker is the presence of multiple types of uncertainties (inter-and intra-individual variation, inter-laboratory variation, dependence on factors such as temperature at irradiation, scorer, shipment, etc), resulting in a large overall uncertainty in comparison to cytogenetic biomarkers. The work in **[R4]** develops statistical methodology to take the different layers of uncertainty into account, and comes with a web applet which implements this methodology (http://shinur.unirioja.es/apps/h2axDE/). It is shown that, even if the uncertainty is large but quantifiable, the biomarker is still useful for triage purposes, for instance to distinguish a severely irradiated individual requiring urgent medical treatment from a 'worried well'.

3. References to the research (indicative maximum of six references)

In the publications below, contributions of authors belonging to the Department of Mathematical Sciences at Durham University are given in square brackets.

[R1] Oliveira, María [40%], Einbeck, Jochen [30%], Higueras, Manuel, Ainsbury, Elizabeth, Puig, Pedro & Rothkamm, Kai (2016). Zero-inflated regression models for radiation-induced chromosome aberration data: A comparative study. *Biometrical Journal* **58***:* 259-279, *http://dro.dur.ac.uk/17160/, https://doi.org/10.1002/bimj.201400233*

[R2] Ainsbury, Elizabeth A., Higueras, Manuel, Puig, Pedro, Einbeck, Jochen [10%], Samaga, Daniel, Barquinero, Joan F., Barrios, Lleonard, Brzozowska, Beata, Fattibene, Paola, Gregoire, Eric, Jaworska, Alicija, Lloyd, David, Oestreicher, Ursula, Romm, Horst, Rothkamm, Kai, Roy, Lawrence, Sommer, Sylwester, Terzoudi, Georgia, Thierens, Hubert, Trompier, Francois, Vral, Anne & Woda, Clemens (2017). Uncertainty of fast biological radiation dose assessment for emergency response scenarios. *International Journal of Radiation Biology* **93***: 127-135,*
http://dro.dur.ac.uk/19789/, https://doi.org/10.1080/09553002.2016.1227106

[R3] Einbeck, Jochen [50%], Ainsbury, Elizabeth, Barnard, Stephen, Oliveira, Maria [10%], Manning, Grainne, Puig, Pere & Badie, Christophe *(2017).* On the Use of Random Effect Models for Radiation Biodosimetry. *In: Extended Abstracts Fall 2015.* Ainsbury, E., Calle, M., Cardis, E., Einbeck, J., Gómez, G. & Puig, P., *Research Perspectives CRM Barcelona,* Springer.**7**: 89-94*, http://dro.dur.ac.uk/21801/, https://doi.org/10.1007/978-3-319-55639-0_15*

[R4] Einbeck, Jochen [40%], Ainsbury, Elizabeth, Sales, Rachel [20%], Barnard, Stephen, Kaestle, Felix, and Higueras, Manuel (2018): A statistical framework for radiation dose estimation and uncertainty quantification from the gamma-H2AX assay. *PloS One* **13**(11): e0207464, *http://dro.dur.ac.uk/27000/, https://doi.org/10.1371/journal.pone.0207464*

This body of work was instigated by a 29K NIHR grant 'Random effects modelling for radiation biodiosimetry' (Principal investigator: Einbeck; postdoctoral researcher: Oliveira; run time: Feb-Dec 2014, Ref. NIHR-RMOFS-2013-03-04; panel feedback: 'A strong application that brings a talented researcher into the medical field. The project innovates by transferring known models into a new area. The research plan was plausible for the timescale and will result in new collaborations'.) This work resulted in **[R1]** and led to an invitation to the Centre de Recerca Matematica (Barcelona) in November 2015 as a Visiting Researcher, where **[R3]** was produced. The work on **[R4]** was supported by Horizon 2020 COST Action IC1408 'Computationally-intensive methods for the robust analysis of non-standard data (CRoNoS)'. All publications involve co-authors from Public Health England; **[R2]** and **[R4]** involve co-authors from at least two Public Health Institutions inside and outside the UK. Publications **[R1]** and **[R4]** are Q1 journals on Scimago.

4. Details of the impact (indicative maximum 750 words).

Practice and policy impact

The work in **[R1]** to **[R4]** shaped guidelines and procedures concerning the choice of models and the quantification of uncertainty for count data biomarkers. Citing the letter from Public Health England, 'the methods … have been incorporated into the retrospective dosimetry elements of the EU radiation emergency response plans under the RENEB network' **[E1]**. RENEB (Running the European Network of Biological and retrospective Physical dosimetry) is a major network of public health organizations and research institutes/laboratories funded by the 7th EU framework EURATOM Fission Programme **[E1, E2]**, with the mission to provide 'rapid, comprehensive and standardised methodology for individualised dose estimation in case of large-scale radiological events in Europe and beyond' (http://www.reneb.net/). As a further activity linked to RENEB, 'as a collaboration between Universitat Autònoma de Barcelona (UAB), Bundesamt für Strahlenschutz (BfS), Durham University (DU), Institut de Radioprotection et de Sûreté Nucléaire (IRSN), Universidad de la Rioja (UdR), and Public Health England (PHE)' **[E4],** dosimetry software for multiple biomarkers, including the code produced for **[R1]**, have been developed into a 'BioDose Tools' package **[E2]**. This tool not only provides an easy-to-use web-applet (available at https://aldomann.shinyapps.io/biodosetools-v3/) for biological dosimetry laboratories worldwide **[E8]**, but also serves to 'simplify and standardize uncertainty estimation in biological dosimetry', hence making an important contribution to the 'international community of biological dosimetry' **[E2].**

In ISO standard 20046:2019(en) **[E3],** Subsection 12.1.3., **[R2]** is given as the reference for methods to derive confidence limits for overdispersed count data. That subsection discusses how to correctly derive the sampling distribution of the biomarker count under violation of the Poisson assumption, and, exclusively referring to our work, which distributions should be employed by laboratories in this case. Standards play a crucial role to 'harmonize the procedure of biological and retrospective physical dose assessments', and ISO standards are for instance used by RENEB for 'certification of the laboratory/department/institute' or 'accreditation/certification of one or several techniques according to standard ISO' (Gregoire et al, 2016).

While the technological feasibility of the gamma-H2AX histone as a radiation biomarker was established in the relevant literature about a decade ago, its practical implementation by laboratories had so far been hampered by a lack of methodology to transfer the biomarker measurement into a dose estimate. Our promising initial results **[R3]** using H2AX biomarker data, which were previously collected by PHE but had been left unpublished (due to lack of an obvious methodology to analyse them), convinced PHE that this is an area which required further resource and research, and led 'to a revised assessment of dose estimation techniques and biomarkers' **[E1]**. One of the actions arising from this was a research visit of Felix Kaestle from the German Federal Office for Radiation Protection (Bundesamt für Strahlenschutz; BfS) to PHE in the summer of 2017 in order to carry out a substantial experimental study using this biomarker **[E1]**. These data, along with other existing data sets, were used in DU to devise a new methodological framework for radiation dose estimation from this assay, including quantification of uncertainty **[R4]**. This methodology is not yet part of the BioTools package, but we have produced an applet 'DoseEstimateH2AX' alongside publication **[R4]** which is available online (since November 2018). The methods are now included in PHE's standard operating procedures **[E1].** The letter **[E1]** also expresses that this collaboration had been 'hugely important and influential' and of 'direct benefit' to PHE.

We are in contact, through LD-RadStatsNet, the BioDose project, and via RENEB, with essentially every laboratory or public health institution in Europe which is able to carry out gamma-H2AX-foci analyses (many of these are co-authoring **[R2]**.). So, the reach of our methodology, across Europe, is close to 100%. The letter by the Bundesamt für Strahlenschutz **[E2]** exemplifies the significance of our work to public health institutions, and the explicit mentioning in a NATO report **[E7]** its visibility.

Societal and economic impact

This body of work has contributed to an increased appreciation in the field that dose estimation from biomarkers requires sophisticated statistical methodology **[E1, E5].** Specifically, **[R1]** has raised awareness that the inferences obtained from cytogenetic radiation biomarkers will depend on the assumed response distribution**,** and, by extension, that the resulting dose estimates carry uncertainties, which can lead to incorrect triage **[R2],** hence urgently requiring methods to quantify these uncertainties **[E1, E5].** However, towards the middle of the last decade, there was a clearly identifiable skills gap in the field of dosimetry, with 'the proportion of individuals with formal mathematical and statistical training [in laboratories or public health institutions] relatively low' **[E5].** PHE and DU were involved in several joint activities to fix this skills gap, including the organization of a workshop aimed at Statisticians (2015, Barcelona) **[E5, E6]** and the creation of a network (LD-RadStatsNet) **[E5, E6],** which in turn was also involved in planning training activities **[E6]**. These activities have contributed to establishing biodosimetry as a statistical discipline, as evidenced for instance by a dedicated session 'Statistical Methods in Radiation Research' at the CMStatistics 2018 conference (http://www.cmstatistics.org/CMStatistics2018/), and several students working on (statistical) PhD projects in this field (some based at public health institutions, such as BfS, and some in academia, including DU), thereby producing a generation of skilled researchers in statistical biodosimetry.

According to **[E1],** the methods developed in the framework of the collaborative work with PHE 'are now used by PHE for commercial biological dose estimation' in PHE's Cytogenetics and Pathology Group, which runs the UK's commercial Chromosome Dosimetry Service (https://www.phe-protectionservices.org.uk/cds/). This unit has responsibility for emergency preparedness in retrospective biological dosimetry for triage purposes in the case of a large-scale radiation accident or incident.

The impact of this work, especially **[R4],** on the general public is a better preparedness in the case of a mass radiation casualty scenario, by providing methodology for the production of meaningful dose estimates and uncertainty bounds, hence enabling effective triage in a much shorter time than through previously existing methods. While the full impact of this innovation will hopefully never be realised, recent events have shown that emergency response preparedness, and in particular testing capability, is vitally important. This holds both for the biomedical technology on which such diagnostics are built, and the computational and statistical routines which give meaning to, and allow principled decisions based on, the raw results of the biomarker. The importance of statistical biodosimetry for emergency response preparedness is, for instance, highlighted in **[E8]** (page 19, 1st column, of the 2018/19 report), referring to potential large-scale irradiation scenarios caused by terror attacks or nuclear weapons.

References

Gregoire E., et al. (2016). The harmonization process to set up and maintain an operational biological and physical retrospective dosimetry network: QA QM applied to the RENEB network, *International Journal of Radiation Biology*, **93**, 81-86, https://www.tandfonline.com/doi/full/10.1080/09553002.2016.1206232

5. Sources to corroborate the impact (indicative maximum of ten references)

E1. Letter from Public Health England (Dr Elizabeth Ainsbury), dated 18/07/2019
E2. Letter from Bundesamt für Strahlenschutz (Dr Ulrike Kulka), dated 12/12/2019
E3. ISO Standard **20046:2019(en)**, Radiological protection — Performance criteria for laboratories using Fluorescence In Situ Hybridization (FISH) translocation assay for assessment of exposure to ionizing radiation, page 19.
E4. BioDoseTools contributors, Screenshot from https://rdrr.io/github/biodosimetry-uab/biodosetools/f/inst/app/www/contributors_app.md

E5. LDRadStats-2015 final meeting report, including workshop programme, from melodi-online.eu
E6. AIR2 bulletin, including evidence for the creation of a network (page 4), also citing **[R1]**
E7. North Atlantic Treaty Organization (NATO), STO Technical Report TR-HFM-222, Biological Effects of Ionising Radiation, page 1-9, also citing **[R1]**
E8. Annual reports of the Bundesamt für Strahlenschutz [in German; 2 merged documents]. Annual report 2017/18 cites **[R2]** on page 61 (relating to content on page 19, 3rd column, where also a reference to the 'Online-Software Tool' **[E4]** is made); Annual report 2018/19 cites **[R4]** on page 76 (relating to content on page 72, 1st column)