Biodose Tools

User Manual & Documentation

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Contents

Preface

This project in an app to be used by biological dosimetry laboratories. Biodose Tools is an open-source project that aims to be a tool to perform all different tests and calculations needed. The app is developed with R (R Core Team 2020) together with Shiny (Chang et al. 2020) to offer an on-line, easy-to-use solution. Although the intention is to provide the application as a website, all R routines can be downloaded for improvement or personal use.

We also aim to clarify and explain the tests used and to propose those considered most appropriate. Each laboratory in its routine work should choose the optimum method, but the project aims to reach a consensus that will help us in case of mutual assistance or intercomparisons.

The project is initially developed by RENEB association, but contributions are always welcome.

Structure of the book

Chapter 1 gives a brief background on the software used in the past in Biological Dosimetry and outlines the reasons to use R and R Shiny as the basis of Biodose Tools.

Chapter 2 introduces the user to Biodose Tools and how to use it either online or locally in RStudio. Chapter **??** introduces the basic design principles behind the user interface, and the usage of the different modules. These two chapters should be sufficient to get most readers comfortable with Biodose Tools itself.

Chapter 3 is a comprehensive review of the different statistical methods and tests used and available throughout Biodose Tools, geared towards mathematicians and biologists alike. In particular, 4 discusses the methods used for dicentrics analysis; 5, the methods used for translocations; and 6, the methods used for micronuclei.

Appendix A explains how to give feedback of the app. In Appendix B, a brief technical review of the implementation of Biodose Tools is discussed. In Appendix C we provide a small guide to cite Biodose Tools.

To sum it up, this book is a comprehensive reference of Biodose Tools. You can follow the 80/20 rule when reading it. Some sections are there for the sake of completeness, and not all sections are equally useful to the particular use case your laboratory or institution may have to Biodose Tools.

About the authors

The project is initially developed by RENEB association, 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).

Principal contributors

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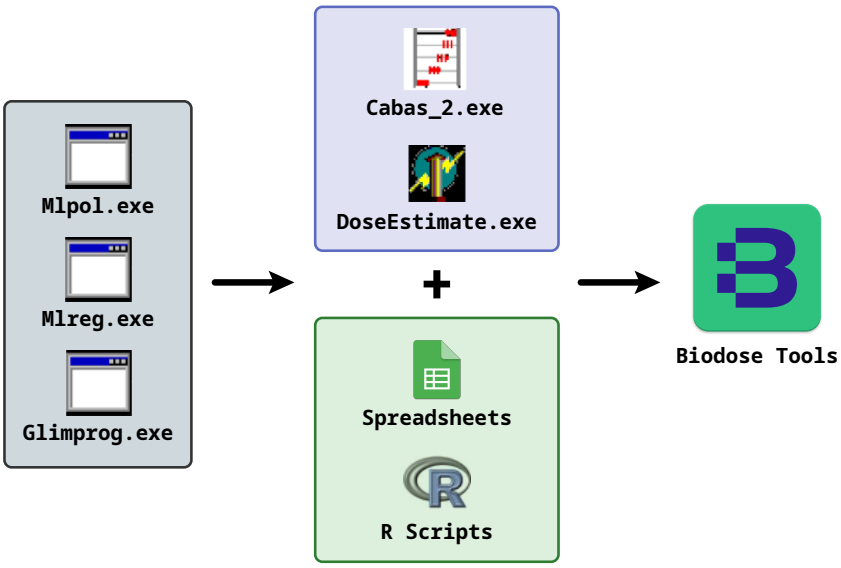
1. Introduction

1.1. Biological dosimetry

The aim of biological dosimetry is to estimate the absorbed dose in a suspected individual exposed to ionizing radiation (IR), by means of analysing biomarkers (International Atomic Energy Agency 2011). A great majority of biomarkers of dose exposition are those coming from the DNA damage induced by IR, most of them analysed using cytogenetic techniques such as dicentric chromosomes, translocations or micronuclei. Dose-assessment is based in converting and an observed yield of damage (i.e. the frequency of dicentrics present in peripheral blood lymphocytes) using a pre-established calibration curve. If

physical and biological procedures involved in biological dosimetry are not considered, the process assumes mathematical models and statistical probability distributions. First, to elaborate a calibration curve blood samples are uniformly irradiated at several doses, and the observed aberration cell distribution is confronted generally to a Poisson distribution. It is assumed that for low-LET radiation types uniform exposures result in a dicentric cell distribution that agrees with Poisson (Edwards, Lloyd, and Purrott 1979). High-LET radiation types tend to show overdispersed distributions. Then the observed yields at different doses are used to construct a calibration curve assuming a Linear-Quadratic model or Linear, depending on the radiation quality. High-LET radiation types tend to be linear (Edwards, Lloyd, and Purrott 1980). The coefficients of these models are obtained using maximum likelihood or iteratively reweighted least squares approaches. Then, in case of an accident the observed cell distribution of aberrations is confronted to the Poisson distribution to distinguish between homogeneous exposures from heterogeneous ones. If homogeneous exposure is accepted. To estimate a dose and its uncertainty, the observed yield is interpolated to the curve and uncertainties are usually calculated considering only considering the uncertainty relative to the yield observed or considering also uncertainties coming from the calibration curve (Edwards 1978; Merkle 1983; Savage et al. 2000). Procedures to consider both sources of uncertainties are not simple because mixes confidence intervals coming from Poisson distribution, and confidence intervals from the normal distribution arisen from the LQ or L models of the calibration (Merkle 1983; Savage et al. 2000). If heterogeneous exposures is determined, other approaches are then applied (Dolphin 1969; Sasaki and Miyata 1968; Pujol et al. 2016). Bayesian methods have been recently proposed to consider both sources of uncertainty, and for both, whole-body and partial-body dose assessment (Ainsbury et al. 2014; Moriña et al. 2015; Higueras et al. 2015).

1.2. Background



The tools to deal with some or all this statistical procedures have evolved (Ainsbury and Barquinero 2009). Initially in MS-DOS environment, there were some routines based on commercial software like SASR or Generalized Linear Interactive Modelling (GLIM), and specially developed programs, such as MLPOL from the former National Radiological Protection Board (NRPB) nowadays Public Health England (PHE, UK) or MLREG from the Bundesamt für Strahlenschutz (BfS, Germany). All these routines or programs were used to estimate the coefficients of the dose-effect curves. Laboratories also used some Microsoft Excel based spreadsheets for both calibration fitting and dose assessment, like the ones used at the Autonomous University of Barcelona (UAB, Spain) and at the Institut de Radioprotection et de Sûreté Nucléaire (IRSN, France).

The first user-friendly with a graphic user interface programmes were CABAS (Deperas et al. 2007) developed by Institute of Nuclear Chemistry and Technology (INCT, Poland) and DOSGEN (Valdes Ramos et al. 2010) developed by the Centro de Protección e Higiene de las Radiaciones (CPHR, Cuba). DOSGEN calculated calibration curve coefficients were also used to evaluate partial-body irradiations. With CABAS it is possible to obtain the coefficients for LQ models and calculate dose-assessment under different scenarios, such as partial or delayed.

A more use-friendly sophisticated program is Dose Estimate (Ainsbury and Lloyd 2010). It allows calculating L and LQ models, and for dose assessment after whole dose exposures considers both, the error of the yield observed and the error from the curve. Dose Estimate also includes specific solutions for FISH based dosimetry, and can be used under different scenarios. Both CABAS and Dose Estimate are very useful to compare results among laboratories, and nowadays-interlaboratory comparisons instigate the use of a single program to have harmonized results. These programs are based on closed source software

and, for example, use a single methodology to calculate uncertainties. So improvements are subjected to those who manage the source code, and end user have no possibility to modify or implement any improvement.

1.2.1. R Shiny

Recently, using the R project for Statistical computing (R Core Team 2020) some scripts have been written for biological dosimetry purposes. Although R programming is based on an open source code, its use needs skills in R language, and in the case of biological dosimetry it is also needed knowledge on the mathematical and statistical assumptions accepted. However, the R package shiny makes it easy to build interactive web apps straight from R, and the creation of a friendly user application based on open source codes.

Our aim is to present the app Biodose Tools, which has been developed with R together with Shiny, to offer an on-line and easy-to-use solution to be used by biological dosimetry laboratories. Biodose Tools is an open-source program and all R routines can be downloaded for improvement or personal use. We also aim to clarify and explain the tests used and to propose those considered most appropriate. This app has been developed in the frame of the RENEB association.

1.3. R Shiny as a statistical tool

1.3.1. R

R is a general purpose package that includes support for a wide variety of modern statistical and graphical methods (many of which have been contributed by users). It is available for GNU/Linux, Mac OS X, and Windows. The R Foundation for Statistical Computing holds and administers the copyright of the R software and documentation. R is available under the terms of the Free Software Foundation's GNU General Public License in source code form.

1.3.2. R Shiny

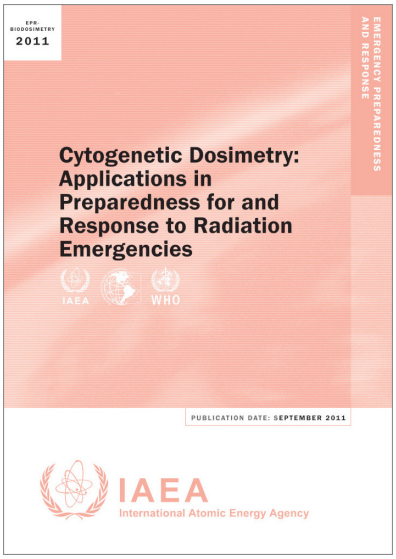
Shiny is an R package that makes it easy to build interactive web apps straight from R, combining the computational power of R with the interactivity of the modern web.

- Access to a powerful web framework for building web applications using R.
- Being in complete control of the mathematics and statistics behind.
- Rethink what biologists and laboratories need in their everyday workflow.
- Build a complete, fully documented tool.

- Provide an Open Source tool to the community.

1.3.3. Modules

Different modules can be built for each function (fitting, dose estimation, ...) while being totally independent from one another.



This opens up the possibility to implement statistical methods not included in the IAEA Manual (2011).

Part I.
Using Biodose Tools

2. Getting Biodose Tools

There are mainly two methods to get Biodose Tools.

- Online on a web browser.
- Locally on your computer using RStudio.

This chapter will go over the details and steps required to get Biodose Tools running for each method.

2.1. Online

During the beta testing phase, the application is hosted on Shinyapps.io: https://aldomann.shinyapps.io/biodose-tools-beta/.

Biodose Tools will be later hosted at one of our institution's servers.

Biodose Tools has been througly tested to be supported by the following web browsers:

- Google Chrome.
- Firefox.
- Microsoft Edge.
- Safari.

2.2. On RStudio

Many laboratories will prefer to use their own computers to run the app instead of relying on an external server, either for security reasons or better reliability.

To run Biodose Tools on your local machine, you need to install R (R Core Team 2020). Additionally, we recommend to install RStudio (RStudio Team 2015).

2.2.1. Installing R

Under Windows Versions of R for Windows XP and later, including 64-bit versions, are available at CRAN. The distribution includes `Rgui.exe`, which launches a self-contained windowing system that includes a command-line interface, `Rterm.exe` for a command-line interface only, Rscript.exe for batch processing only, and `R.exe`, which is suitable for batch or command-line use.

More information on Windows-specific issues can be found in the CRAN R for Windows FAQ.

Under macOS A version of R for macOS 10.6 and higher is available at CRAN. This is distributed as a disk image containing the installer. In addition to the graphical interface version, a command line version (particularly useful for batch operations) can be run as the command `R`.

More information on Macintosh-specific issues can be found in the CRAN R for Mac OS X FAQ.

Under GNU/Linux R is available for most Linux distributions through your distribution's repositories. For example, R is provided on Debian-based distributions like Ubuntu by the `r-base` package. Many additional packages, such as `r-cran-rpart`, are provided at the maintainer's discretion.

To install R on Ubuntu, run the following commands on the Terminal:

```
sudo apt-get update
sudo apt-get install r-base r-base-dev
```

To install R on Fedora, run the following command on the Terminal:

```
dnf --refresh install R
```

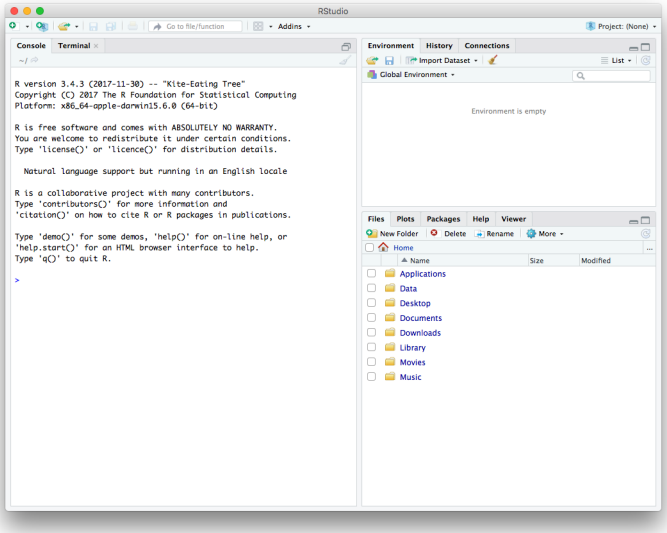
To install R on Arch Linux, run the following command on the Terminal:

```
sudo pacman -S r
```

2.2.2. Installing RStudio

RStudio for Windows, macOS, or GNU/Linux can be downloaded from https://www.rstudio.com/products/rs
RStudio requires R to be installed on the local machine, so make sure to install it first.

Once installation is complete, the recommended next step for a new user would be to start RStudio and run a sample session.



The > character is the command prompt, and commands are executed once the user presses the RETURN or ENTER key.

2.2.3. Installing Biodose Tools

To download the development version, you can just run

```
# install.packages("devtools")
devtools::install_github("biodosetools-team/biodosetools")
```

In the future, Biodose Tools will be available on CRAN.

2.2.4. Running Biodose Tools

Once it is installed, Biodose Tools can be easily run by typing

```
biodosetools::runApp()
```

Part II.
Statistical Methods

3. Introduction

This chapter presents a review of the existing statistical methods for the different implemented modules, i.e.,

- Dicentric analysis.
- Translocation analysis.
- Micronuclei analysis (*work in progress*).

The primary objective of this section is to provide biologists with technical information about the statistical methods and tests used on Biodose Tools. The main source are (International Atomic Energy Agency 2001), (International Atomic Energy Agency 2011) and the papers the statistical analyses are based on.

3.1. Statistical considerations

There is a strong evidence that the yields of chromosome aberrations (dicentrics, translocations) or micronuclei (Y) are related to exposed dose (D) by the linear quadratic equation

$$Y = C + \alpha D + \beta D^2 \tag{1}$$

or, for high LET radiation, the α-term becomes large and eventually the β-term becomes biologically less relevant and also statistically "masked." In this situation, the the dose response is approximated by the linear equation

$$Y = C + \alpha D \tag{2}$$

3.1.1. Dose-effect curve fitting

The objective of dose-effect or curve fitting is to determine those values of the coefficients C, α and β which best fit the data points. For dicentrics (see Chapter 4), irradiation with X or gamma rays produces a distribution of damage which is very well represented by the Poisson distribution (Edwards, Lloyd, and Purrott 1979). In contrast, neutrons and other types of high LET radiation produce distributions which display overdispersion, where the variance (σ^2) exceeds the mean ($\bar{y}$). Whether the ratio of variance to mean

$(\sigma^2/\bar{y})$ is a function of dose is at present an open question. For micronuclei (see Chapter 6) the data tend to overdispersion at all doses even with photon irradiation.

3.1.2. Poisson distribution

The Poisson distribution is named after Simmeon Poisson, a leading French mathematician during the early 1800s. The distribution applies when the random variable is discrete and represents the number of events that occur in a unit-of-scale, such as unit-or-time or unit-of-area. The rate of events occurring is constant and the number of events k are the integers of zero and larger.

Statistically, a discrete random variable Y is said to have a Poisson distribution with parameter $\lambda > 0$, if, for $k = 0, 1, 2, \ldots$ the probability mass function of Y is given by:

$$f(k;\lambda) = \Pr(Y = k) = \frac{\lambda^k e^{-\lambda}}{k!}, \tag{3}$$

where

- e is Euler's number ($e = 2.71828\ldots$),
- $k!$ is the factorial of k.

The positive real number λ is equal to the expected value of Y and also to its variance:

$$\bar{y} = \mathrm{E}(Y) = \lambda, \quad \sigma^2 = \mathrm{Var}(Y) = \lambda. \tag{4}$$

However, for real count data, it is recommended to calculate the variance defined as

$$\begin{aligned} \sigma^2 = \mathrm{Var}(Y) &= \frac{1}{N-1}\left[\sum_{k=1}^{N} k^2 C_k - N\bar{y}^2\right] \\ &= \frac{1}{N-1}\left[\sum_{k=1}^{N} k^2 C_k - \frac{1}{N}\left(\sum_{k=1}^{N} k C_k\right)^2\right], \end{aligned} \tag{5}$$

where

- N is the total number of events,
- $C_k = N \cdot \Pr(Y = k)$ is the occurrence count or frequency for event k.

3.1.3. Testing for Poisson

Since most curve fitting methods are based on Poisson statistics, the dicentric cell distribution should be tested for compliance with the Poisson distribution for each dose used to construct the calibration curve. Nowadays, the most widely used test is the u-test (Rao and Chakravarti 1956; Savage 1970). The u-test statistic is a normalized unit of the dispersion index ($\sigma^2/\bar{y}$), which for a Poisson distribution should be unity. u-values higher than 1.96 indicate overdispersion (with a two-sided significance level, $\alpha = 0.025$).

$$u = (\sigma^2/\bar{y} - 1)\sqrt{\frac{N-1}{2(1-1/X)}} \tag{6}$$

where

- $\sigma^2/\bar{y}$ is the dispersion index,
- u is the u-value, which for a Poisson distribution should be unity,
- N indicates the number of cells analysed,
- $X = \sum_{k=1}^{N} kC_k$ the number of dicentrics (or dicentrics plus rings) detected,
- C_k is the count of cells where k dicentrics were detected.

u-values of $<$ -1.96 indicate underdispersion. Biologically, underdispersion is very unlikely to occur so values of u lower than -1.96 may be indicative of a problem in data sampling.

3.1.4. Dose-effect calibration

Adequate curve fitting requires a sufficient number of degrees of freedom to minimize the error on the curve. Ideally, 10 or more doses should be used in the range 0.25–5.0 Gy. For low LET radiation it is not necessary to have data higher than approximately 5.0 Gy and, indeed, beyond this dose there is evidence of saturation of the aberration yield which will lead to a distortion of the β coefficient (Lloyd and Edwards 1983). For high LET radiation a maximum of 2.0 Gy is suggested.

As most radiation accidents involve doses of less than 1.0 Gy, the lower end of the curve is of particular importance in estimating doses. A significant effort should therefore be made to reduce the statistical uncertainty associated with the α coefficient of yield. It is suggested that several of the calibration doses, certainly a minimum of four, should be in the range of 0.25–1.0 Gy. If the laboratory is capable of obtaining data at doses below 0.25 Gy, this is very desirable. At higher doses, scoring should aim to detect 100 dicentrics at each dose. However at lower doses this is difficult to achieve and instead several thousand cells per point should be scored; a number between 3000 and 5000 is suggested. In all cases, the actual number of cells scored should be dependent on the number of dose points in the low dose region, with the focus on minimizing the error on the fitted curve.

3.1.5. Background level

Opinions vary on how to treat the background level of aberrations in fitting dose response data. In general there are three approaches: (a) a dose point at zero Gy is included in the curve fitting procedure, (b) the zero dose point is ignored, or else (c) the zero dose point is represented in every fitting procedure by a standard background value.

If the measured yield at zero dose is used as one of the data points for the curve fitting (as used in the curve fitting presented above), the background becomes a variable parameter. However, since the yield in unirradiated cells is usually low, often none are observed so the measured yield at zero dose is zero. As discussed, at low doses, the statistical resolution of the data points is generally low. Thus, including the zero dose point in the curve fitting procedure can sometimes lead to negative estimates of the background value (C) and negative linear coefficients (α), which obviously have no biological basis. Some investigators resolve this problem by ignoring zero dose data points and constraining the curve to pass through the origin (to be implemented in Biodose Tools in the future). There are, however, sufficient data published from surveys of subjects exposed only to background radiation to show that there is a small positive background level of aberrations. An alternative method adopted by some workers is therefore to use a small positive background value as a data point and to ascribe a large percentage of uncertainty to it. Ideally a laboratory should generate its own background data, although this requires the analysis of many thousands of cells. A consensus has emerged that the background level of dicentrics is ~0.5–1.0 per 1000 cells (Lloyd, Purrott, and Reeder 1980) whilst for translocations (Sigurdson et al. 2008) and micronuclei (Fenech 1993) the control values are higher.

4. Dicentric analysis

4.1. Dose-effect curve fitting

Table 3 gives example data used to construct dose-effect curves for low LET γ-radiation and high LET α radiation.

For each dose analysed, total number of cells scored (N), total number of dicentrics observed (X), cell distribution of dicentrics and dispersion index ($\sigma^2/\bar{y}$) and u-test (u) are presented. u-values greater than 1.96 indicate overdispersion.

4.1.1. Fitting method

The technique suggested for determining the best fit coefficients is that of maximum likelihood (Papworth 1975; Merkle 1983). Using this method, the best fit value for each coefficient is achieved by assuming a Poisson distribution and maximizing the likelihood of

Table 3: [Download dicentrics distribution.](https://biodosetools-team.github.io/documentation/data/count-data-IAEA.csv)

D	N	X	C0	C1	C2	C3	C4	C5	DI	u
0.00	5000	8	4992	8	0	0	0	0	0.9985997	-0.0748406
0.10	5002	14	4988	14	0	0	0	0	0.9974005	-0.1348939
0.25	2008	22	1987	20	1	0	0	0	1.0804910	2.6098032
0.50	2002	55	1947	55	0	0	0	0	0.9730135	-0.8614691
0.75	1832	100	1736	92	4	0	0	0	1.0259749	0.7898872
1.00	1168	109	1064	99	5	0	0	0	0.9992767	-0.0175514
1.50	562	100	474	76	12	0	0	0	1.0639572	1.0765604
2.00	333	103	251	63	17	2	0	0	1.1407182	1.8218931
3.00	193	108	104	72	15	2	0	0	0.8336227	-1.6377580
4.00	103	103	35	41	21	4	2	0	0.8823529	-0.8442765
5.00	59	107	11	19	11	9	6	3	1.1498550	0.8107914

Table 4: Dicentrics Coefficients

Coefficient	Estimate	Std. Error	t-statistic	p-value
C	0.00128	0.0004714	2.716	0.0066080
α	0.02104	0.0051580	4.079	0.0000452
β	0.06303	0.0040070	15.730	0.0000000

the observations by the method of iteratively reweighted least squares. For overdispersed (non-Poisson) distributions, as obtained after high LET radiation, the weights must take into account the overdispersion. If the data show a statistically significant trend of $\sigma^2/\bar{y}$ with dose, then that trend should be used. Otherwise, the Poisson weight on each data point should be divided by the average value of $\sigma^2/\bar{y}$.

4.1.2. Goodness of fit

The goodness of fit of the curve and significance of fitted α and β coefficients should then be tested, for instance using an appropriate form of the F-test (e.g. F-test, z-test or t-test) respectively. Biodose Tools implements the t-test.

Let $\hat{\theta}$ be an estimator of the parameter $\theta = \alpha, \beta, C$ in the fit model. Then the t-statistic for this parameter is defined as

$$t_{\hat{\theta}} = \frac{\hat{\theta}}{\widehat{se}(\hat{\theta})} \tag{7}$$

The p-values of the t-test shown in Table 4 indicate that the fitted data points were not statistically different from the observed ones confirming a good fit.

4.2. Dose estimation

5. Translocation analysis

5.1. Dose-effect curve fitting

5.2. Dose estimation

6. Micronuclei analysis

6.1. Dose-effect curve fitting

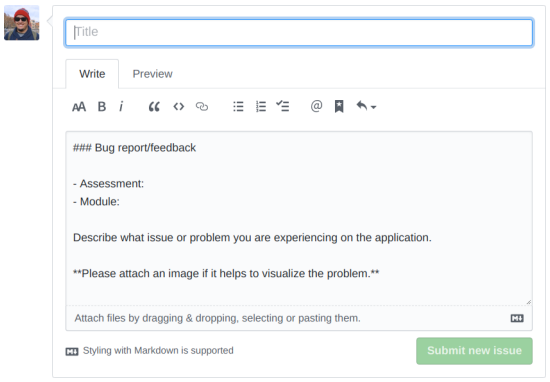
6.2. Dose estimation

A. Feedback

Please provide your suggestions and feedback to help us improve Biodose Tools. Use the following link to open a new issue on the project's issue tracker: https://github.com/biodosetools-team/biodose-tools/issues/new.

You can also use the "Give feedback" button on Biodose Tools' navbar (the location of the button is subject to change after the beta testing phase).

We provide a short template on the issue tracker (pictured below), but feel free to do your own thing:



Be as descriptive as possible and in case of an error or issue, explain the required steps to reproduce the experienced error. Notice that pictures can attached

B. Implementation details

B.1. Technologies

The Biodose Tools user interface is written in R Shiny (Chang et al. 2020) using Bootstrap 3 through the {shinydashboard} (Chang and Borges Ribeiro 2018) package, analyses are implemented in the R programming language (R Core Team 2020), with the resultant tables and plots rendered in HTML through JavaScript libraries. This is done by the browser of choice, or by an instance of QtWebKit if the app is run through RStudio.

C. How to cite Biodose Tools

If you want to cite Biodose Tools, you can use the following BibTeX entry:

```
@Manual{BiodoseTools2019,
  title = {{Biodose Tools}},
  author = {Alfredo Hernández and Joan Francesc Barquinero and
            David Endesfelder and Pere Puig and Lleonard Barrios and
            Jochen Einbeck and Amine Benadjaoud and Elizabeth Ainsbury
            and Ulrike Kulka and Eric Grègoire and Manuel Higueras and
            Gruel Gaëtan and Ursula Oestreicher and David Lloyd},
  year = {2019},
  url = {https://biodosetools-team.github.io/documentation/},
}
```

You can download the BibTex entry clicking here.

Ainsbury, Elizabeth A., and J. Francesc Barquinero. 2009. "Biodosimetric tools for a fast triage of people accidentally exposed to ionizing radiation. Statistical and computational aspects." *Annali Dell'Istituto Superiore Di Sanita* 45 (3): 307–12.

Ainsbury, Elizabeth A., and David C. Lloyd. 2010. "Dose estimation software for radiation biodosimetry." *Health Physics* 98 (2): 290–95. https://doi.org/10.1097/01.HP.0000346305.84577.b4.

Ainsbury, Elizabeth A., Volodymyr A. Vinnikov, Pedro Puig, Manuel Higueras, Nataliya A. Maznyk, David C. Lloyd, and Kai Rothkamm. 2014. "Review of bayesian statistical analysis methods for cytogenetic radiation biodosimetry, with a practical example."

Radiation Protection Dosimetry 162 (3): 185–96. https://doi.org/10.1093/rpd/nct301.

Chang, Winston, and Barbara Borges Ribeiro. 2018. *Shinydashboard: Create Dashboards with Shiny*. http://rstudio.github.io/shinydashboard/.

Chang, Winston, Joe Cheng, JJ Allaire, Yihui Xie, and Jonathan McPherson. 2020. *Shiny: Web Application Framework for r*. http://shiny.rstudio.com.

Deperas, Joanna, M. Szluinska, Marta Deperas-Kaminska, Alan Edwards, David Lloyd, Carita Lindholm, Horst Romm, et al. 2007. "CABAS: a freely available PC program for fitting calibration curves in chromosome aberration dosimetry." *Radiation Protection Dosimetry* 124 (2): 115–23. https://doi.org/10.1093/rpd/ncm137.

Dolphin, G. W. 1969. "Biological Dosimetry with Particular Reference to Chromosome Aberration Analysis: A Review of Methods." In *Handling of Radiation Accidents*, edited by International Atomic Energy Agency, 215–24. Vienna. https://inis.iaea.org/search/search.aspx?orig_q=RN:45029080.

Edwards, A. A. 1978. "The estimation of dose and its uncertainty." *NRPB*, 16–17.

Edwards, A. A., D. C. Lloyd, and R. J. Purrott. 1979. "Radiation induced chromosome aberrations and the poisson distribution." *Radiation and Environmental Biophysics* 16 (2): 89–100. https://doi.org/10.1007/BF01323216.

———. 1980. "Dicentric chromosome aberration yield in human lymphocytes and radiation quality; A resume including recent results using alpha-particles." In *Seventh Symposium on Microdosimetry, Report Eur7147*, 1263–73. Harwood Academic Publishers.

Fenech, Michael. 1993. "The cytokinesis-block micronucleus technique: A detailed description of the method and its application to genotoxicity studies in human populations." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 285 (1): 35–44. https://doi.org/10.1016/0027-5107(93)90049-L.

Higueras, Manuel, Pedro Puig, Elizabeth A. Ainsbury, Volodymyr A. Vinnikov, and Kai Rothkamm. 2015. "A new Bayesian model applied to cytogenetic partial body irradiation estimation." *Radiation Protection Dosimetry* 168 (3): ncv356. https://doi.org/10.1093/rpd/ncv356.

International Atomic Energy Agency. 2001. "Cytogenetic Analysis for Radiation Dose Assessment - A Manual." *Technical Report Series-International ...*, 138. http://www.teneb.eu/IAEA_report_405.pdf.

———. 2011. "Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies." *Manual Series*, 247.

Lloyd, D. C., and A. A. Edwards. 1983. "Chromosome aberrations in human lymphocytes : Effect of radiation quality, dose, and dose rate." *Radiation-Induced Chromosome Damage in Man*, 23–49. https://ci.nii.ac.jp/naid/10003527336/en/.

Lloyd, D. C., R. J. Purrott, and E. J. Reeder. 1980. "The incidence of unstable chromosome aberrations in peripheral blood lymphocytes from unirradiated and occupationally exposed people." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 72 (3): 523–32. https://doi.org/10.1016/0027-5107(80)90123-2.

Merkle, W. 1983. "Statistical methods in regression and calibration analysis of chromosome aberration data." *Radiation and Environmental Biophysics* 21 (3): 217–33. https://doi.org/10.1007/BF01323412.

Moriña, David, Manuel Higueras, Pedro Puig, Elizabeth A. Ainsbury, and Kai Rothkamm. 2015. "radir package: an R implementation for cytogenetic biodosimetry dose estimation." *Journal of Radiological Protection* 35 (3): 557–69. https://doi.org/10.1088/0952-4746/35/3/557.

Papworth, D. G. 1975. "Curve fitting by maximum likelihood." *Radiation Botany* 15: 127–40.

Pujol, Mònica, Leonardo Barrios, Pedro Puig, María Rosa Caballín, and Joan-Francesc Barquinero. 2016. "A New Model for Biological Dose Assessment in Cases of Heterogeneous Exposures to Ionizing Radiation." *Radiation Research* 185 (2): 151–62. https://doi.org/10.1667/RR14145.1.

R Core Team. 2020. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rao, C. Radhakrishna, and I. M. Chakravarti. 1956. "Some Small Sample Tests of Significance for a Poisson Distribution." *Biometrics* 12 (3): 264. https://doi.org/10.2307/3001466.

RStudio Team. 2015. *RStudio: Integrated Development Environment for r*. Boston, MA: RStudio, Inc. http://www.rstudio.com/.

Sasaki, Masao S., and Hisatoshi Miyata. 1968. "Biological Dosimetry in Atomic Bomb Survivors." *Nature* 220 (5173): 1189–93. https://doi.org/10.1038/2201189a0.

Savage, J. R. K. 1970. "Sites of radiation induced chromosome exchanges." *Current Topics in Radiation Research* 6: 129–94. https://inis.iaea.org/search/search.aspx?orig_q=RN:3026266.

Savage, J. R. K., D. G. Papworth, M. Bauchinger, A. T. Natarajan, G. E. Pantelias, C. S. Griffin, M. Figgitt, et al. 2000. "Constructing a 2B Calibration Curve for Retrospective Dose Reconstruction." *Radiation Protection Dosimetry* 88 (1): 69–76. https://doi.org/10.1093/oxfordjournals.rpd.a033022.

Sigurdson, Alice J., Mina Ha, Michael Hauptmann, Parveen Bhatti, Radim J. Sram, Olena Beskid, E. Janet Tawn, et al. 2008. "International study of factors affecting human chromosome translocations." *Mutation Research/Genetic Toxicology and*

Environmental Mutagenesis 652 (2): 112–21. https://doi.org/10.1016/j.mrgentox.2008.01.005.

Valdes Ramos, Maryzury, Juan Tomas Zerquera, Ana Ilsa Lamadrid Boada, Miguel Prendes Alonso, and Omar Garcia Lima. 2010. "DOSGEN: 'An integrated data and dose estimation system for the cytogenetic dosimetry'." In *Proceedings of Irpa12: 12. Congress of the International Radiation Protection Association: Strengthening Radiation Protection Worldwide - Highlights, Global Perspective and Future Trends*, edited by International Atomic Energy Agency. Buenos Aires. https://inis.iaea.org/search/search.aspx?orig_q=RN:42072497.