Time and Concentration Responses of HepaRG.

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Abstract

This database contains raw and processed data which were produced within our work concerning an *in vitro* exploratory study, in which concentration-time-effect responses were measured and used for both the extrapolation of points of departure from an acute to chronic exposure, and the determination of a chronicity index that provides a quantitative measure of a chemical's potential to cause cumulative effects over time. Details of this research can be found in our publication P. Macko, T. Palosaari, M. Whelan, Extrapolating from acute to chronic toxicity in vitro, *Toxicology in Vitro* (2021) 105206 doi.org/10.1016/j.tiv.2021.105206.

1. Introduction

In this *in vitro* exploratory study, the viability of HepaRG cells was measured in live-cell mode as a function of both, the chemical concentration and the exposure time. The cells were seeded into black 96-well plate with a transparent flat bottom. The cells were treated with 5 different test chemicals: Methylene bis(thiocyanate); Tamoxifen; Cadmium Chloride; Aflatoxin B1 and Rotenone, with 8 different concentrations for a maximum duration of 86 hours. The test plate contained two replicate wells for each chemical at each concentration as well as a number of non-treated (seeded) wells used as negative controls. Immediately after exposure to the test chemicals, the cells were stained with Image-iT® DEAD Green™ (Thermo Fisher Scientific) viability stain. The well plate was imaged on the High Content Imaging (HCI) Cellomics ArrayScan® VTI instrument (Thermo Fisher Scientific) equipped with a live cell chamber to maintain the cells at 37°C in humidified atmosphere with 5% of CO_2 over the total exposure time of 86 hours. The fluorescence images of the stained cell culture were

acquired with a 20x objective. All wells on the plate were scanned and in each well four different fields of view were acquired. This was repeated every 30 min over the first 14 hours and then every 60 min until 74 hours. The final acquisition was made at 86 hours.

In each image the bright objects, corresponding to dead or dying cells stained with DEAD Green™, were identified and counted, with the total count serving as a measure for overall cell death. After the experiment, the cells in all wells were fixed and stained with Hoechst 33342 stain. The plate was scanned once more, but now two images for each field of view were recorded, one in the spectral range of Hoechst (XF93 - Hoechst filter set) and one of DEAD Green™. The Hoechst images were analysed and provided the total number of cells in the field of view and the DEAD Green™ image provided the number of dead cells. This provided the basis for data normalisation.

2. Database description

The Database is available at the JRC Data Catalogue
data.jrc.ec.europa.eu/dataset/79989ace-e09e-46c6-a8fc-2e076f23954f.

The database has two main entries available for download: 'Raw data (images)' and 'Experimental results'. The Raw data (images) entry contains a set of 34176 microscopic images, which are all compressed into one HepaRGTiffs.rar file (size 37GB). The Experimental results entry contains a file entitled DatabaseHepaRG.xlsx with the results of the image analysis, thus the number of dead cells per experimental condition, the normalised time and concentration responses, and the description of the experimental condition such as the plate layout, concentrations, and exposure time points.

2.1 Raw data (images)

The microscopic images were acquired using the High Content Imaging (HCI) Cellomics ArrayScan® VTI instrument (Thermo Fisher Scientific), equipped with Photometrics™ X1 CCD camera with 14-bit dynamic range. The grayscale images were stored in the uncompressed Tiff format (2x2 binning mode, size 1104 x 1104 pixels, 16 bits per pixel).

There are two groups of images. The first group of images, named DeadGreen_*.tiff, corresponds to the first phase of the experiment, in which the wells were repeatedly imaged

over the total exposure time of 86 hours in live-cell mode. The second group of images, named Hoechst&DeadGreen_*.tiff, corresponds to the second phase of the experiment, when the cells were fixed, stained with Hoechst 33342 stain, and two images for each field of view were recorded, one in the spectral range of Hoechst (XF93 - Hoechst filter set) and one of DEAD Green™.

The image file names use the Cellomics ArrayScan® VTI instrument (Thermo Fisher Scientific) file naming convention:

- For repeated plate scanning in time in the first phase of the experiment (96 wells x 4 fields of view x 87 time points = 33408 tiff images), the file name structure is as follow:

DeadGreen_i3t<TimePoint><WellName>f<Field>d<Dye>.tiff

where:

<TimePoint> is the three-digit number corresponding to the time point: 001, 002, and so on until 087.

<WellName> is the letter and number corresponding to the microplate row letter and column number of the well: A01 … H12.

<Field> is the two-digit field number, starting at 1. In each well, four fields of view f01, f02, f03 and f04 were recorded.

<Dye> is the channel number. Only d1 was used here, which corresponds to the DeadGreen channel.

Examples:

DeadGreen_i3t001A01f01d1.tiff – time point t001, well A01, field of view f01

DeadGreen_i3t087H12f04d1.tiff – time point t087, well H12, field of view f04

- For fixed endpoint plate scan in the second phase of the experiment (96 wells x 4 fields of view x 2 channels = 768 tiff images), the file name structure is as follow:

Hoechst&DeadGreen_<WellName>f<Field>d<Dye>.tiff

where:

<WellName> is the letter and number corresponding to the microplate row letter and column number of the well, A01 … H12.

<Field> is the two-digit field number, starting at 0. In each well, four fields of view f00, f01, f02 and f03 were recorded. (Note the shift in the field number index compared to the repeated plate scanning in time, the field of view f00 here corresponds to f01 in the repeated plate scanning in time, ect.)

<Dye> is the channel number, d0 is Hoechst and d1 is DeadGreen channel.

Examples:

Hoechst&DeadGreen_A01f00d0.tiff – well A01, field of view f00, channel d0 (Hoechst)

Hoechst&DeadGreen_A01f00d1.tiff – well A01, field of view f00, channel d1 (DeadGreen)

2.2 Experimental results

The images were analysed by the Compartmental Analysis v.4 bio-application, which is one of the image analysis tools available in the HCS Studio™ Cell Analysis Software. In each image the bright objects, corresponding to dead or dying cells stained with DEAD Green™, were identified and counted. Their counts per well and per timepoint are stores in the file DatabaseHepaRG.xlsx, sheet 'RawData'. Those data were used to create normalised concentration and time responses, which are stored in the sheet 'Normalized responses'. To calculate these normalised responses, we used an averaged signal from the control wells as a background which was subtracted from the treated wells. The total numbers of cells per well, which were captured in the second phase of the experiment using the Hoechst images, were used for data normalisation. The normalised responses represent the fraction of dead cells, the value zero means no cell death and the value one means that all the cells were dead. The description of the experimental conditions is in the sheet 'Experimental layout'. It contains the well plate layout, the concentrations of the chemicals and the content of the DMSO.