Cardiotoxicity arises from interference with normal cellular electrophysiology and contractility as well as toxicological effects. Hence, there is a pressing need to develop a high-throughput application module. Measurements assessed included: Granularity, Total Number Viable and Hoechst (nuclear stain, Invitrogen). Images were acquired using the ImageXpress® Micro XL system using a 10x objective. Analysis was done with MetaXpress® software using the Granularity module. Left: Images of cardiomyocytes from mitochondria healthy cells stained with JC-10 dye. Right: Mitochondria healthy cells exhibit red-green nuclei while cells with mitochondrial damage exhibit red nuclei only. 50% Distribution of Toxic Potency scores (ToxPi Score)

In vitro Cardiotoxicity Assay

Interruption of the sarcolemmal Na+/Ca2+ exchanger causes a net influx of Ca2+ into the sarcoplasmic reticulum leading to a need for an improved analysis method. To overcome this limitation, we applied the Benchmark Concentration (ToxPi) method for screening environmental chemicals. Each compound’s ToxPi values for the effects on beat rate and peak shape (spacing, amplitude, rise, decay, width) were analyzed and visualized using the ToxPi approach, which generates transparent graphics rankings to facilitate decision making (see Figure 5). The larger the area under the toxicity score compared to 1 (ToxPi at min concentration), the greater the effect the compound had on the endpoint measured. The numbers are ranked across classes to give an overall ToxPi cardiotoxicity score from 0 (predicted to be more cardiotoxic) to 6 (predicted to be more cardiotoxic).

Bench Mark Concentration Analysis

Dose-response information is critical for ranking compounds for their toxicity or safety. However, C50/EC50 analysis is limited by the need for high-low asymptotes. In toxicity assessment, high concentration response profiles are often not present, leading to a need for an improved analysis method. To overcome this limitation, we applied the Benchmark Concentration (ToxPi) analysis method developed by DDI for analysis of toxicity testing2 to the multi-parametric beating cardiomyocyte assay. A number of physiological parameters of cardiomyocyte beating, such as beat rate, peak shape (amplitude, width, rise, decay) and regularity were collected. Viability was assessed at 60 min and 24 h using the number of Calcium AM positive cells to yield a cardiotoxicity score based on standard deviations below the mean. Data points with viability below the cutoff were omitted from BAC analysis.

Logistic Model: Concentration-response profiles were defined using logistic modeling to derive a ToxPi value, defined as the concentration giving a response based on 20% above or below the mean of the vehicle (DMSO)-treated cells.


cell viability

\( f(x) = \frac{max - min}{max - min} + \frac{min}{max - min} \cdot \left(1 + e^{-(x-c)/\mu} \right) \)

\( c \) - midpoint of concentration-response profile

\( \mu \) - slope of concentration-response profile

Cell Viability/ln

Effects of compound on cell viability was assessed using high-content imaging of cardiomyocytes stained with the mitochondrial membrane potential dye 10-20 nM JC-10 dye. Viability was assessed in the 60 min after treatment on an ImageXpress Micro XL system using a 10x objective. Analysis was done with MetaXpress® software using the Multi-Well Imaging Imaging application module. Measurements assessed included: Total Number Viable and Percent Viable cells.

References


Cardiotoxicity Scores & ToxPi Toxicity Ranking

Comparison of in vitro derived effect levels to Citoxa has been suggested as a surrogate metric for ranking compounds with respect to their potential in vivo cardiotoxicity9. However, this approach is not amenable to drugs or chemicals that lack the necessary pharmacokinetic studies. We used an alternative approach to integrate multiple parameters collected in this study to rank chemicals in the screened library for their overall cardiotoxicity. Each compound’s ToxPi values for the effects on beat rate and peak shape (spacing, amplitude, rise, decay, width) were analyzed and visualized using the ToxPi approach, which generates transparent graphics rankings to facilitate decision making. Each compound's ToxPi value was calculated and compared to 1 (ToxPi at min concentration), the greater the effect the compound had on the endpoint measured. The numbers are ranked across classes to give an overall ToxPi cardiotoxicity score from 0 (predicted to be more cardiotoxic) to 6 (predicted to be more cardiotoxic).

Chemicals in the screened library for their overall cardiotoxicity. Each compound’s ToxPi values for the effects on beat rate and peak shape (spacing, amplitude, rise, decay, width) were analyzed and visualized using the ToxPi approach, which generates transparent graphics rankings to facilitate decision making. Each compound's ToxPi value was calculated and compared to 1 (ToxPi at min concentration), the greater the effect the compound had on the endpoint measured. The numbers are ranked across classes to give an overall ToxPi cardiotoxicity score from 0 (predicted to be more cardiotoxic) to 6 (predicted to be more cardiotoxic).

Statistical analysis revealed that a logistic model is more suited for automated screening of environmental chemicals.

Summary

We present here an assay model for the in vitro assessment of cardiotoxicity hazards that is well-suited for automated screening of environmental chemicals.

The assay system is amenable to concentration-response modeling and can be used to prioritize suspected cardiotoxicants for in vivo characterization and targeted/mechanistic follow-up studies.

In general, flame retardants and pesticides appear to be more toxic at 30 min compared to PAHs and drugs based on this analysis. A more distributed pattern of toxicity was observed among chemical classes at 24 hours.

Top 10 Inhibitors of Mitochondrial Membrane Potential

Distribution of ToxPi Scores BAC; chemical library

Distribution of ToxPi Scores BAC; chemical library

Toxicity Library Screening

A diverse set of 80 environmental chemicals (e.g., flame retardants, PAHs, pesticides, mitochondrial toxicants) and drugs were screened for cardiotoxicity across a 7-point concentration response in duplicates after 30 min and 24 h of treatment. After controlling for general cytotoxicity, a number of environmental chemicals were identified that affected in vitro cardiomyocytes. Such data is amenable to concentration-response modeling and can be used to prioritize suspected cardiotoxicants for in vivo hazard characterization and mechanistic follow-up studies.