# Quantitative Estimates of Toxicodynamic Variability for New Approach Methodologies-Based Systemic Safety Toolbox Using a Population-Based Human in Vitro Model

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## ABSTRACT

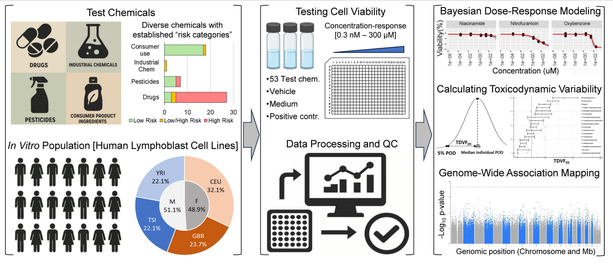
**Background and Purpose:** Next-Generation Risk Assessment (NGRA) frameworks leverage New Approach Methodologies (NAM) to support regulatory decision-making without animal testing. While NAM-based methods for hazard and dose-response assessment are widely used, considerations of chemical-specific evidence for inter-individual variability are still largely relying on default uncertainty factors for toxicokinetic and toxicodynamic variability. However, chemical-specific estimates of inter-individual variability can be derived using human cell-based in vitro models. This study tested the utility of a NAM-based approach to derive toxicodynamic variability factors in the context of NGRA. We tested a hypothesis that by including chemical-specific data on toxicodynamic variability into risk characterization step of NGRA, more protective estimates of human risk will be derived.

**Methods:** We used 131 human lymphoblastoid cells (LCLs) from four European and African subpopulations as the in vitro model of inter-individual variability. A broad range of chemicals (n = 53) to which humans are exposed in a variety of ways, including food additives, cosmetic ingredients, pharmaceuticals, vitamins, agricultural chemicals, and home care products were tested in concentration-response and viability measurements were collected. To derive quantitative estimates of adverse effects for each chemical, Bayesian concentration–response modeling was conducted to derive points of departure for each individual cell line.

**Results:** Overall, 18 out of 53 tested chemicals had effects below the highest concentration tested (300 μM) – from which a toxicodynamic variability factor ($TDVF_{05}$) was derived for these chemicals. The median $TDVF_{05}$ was 3.8 [range from 1 to 46], which is in line with the default human toxicodynamic variability factor of 3.16. In addition, we conducted an exploratory genome-wide association study (GWAS) analysis to identify potential genetic drivers of cytotoxic responses to further characterize the mechanisms of toxicity. This analysis showed that for most of the tested chemicals with inter-individual differences in cytotoxicity, genomic loci containing xenobiotic metabolism genes conferred genome-wide suggestive effects underlying such variability.

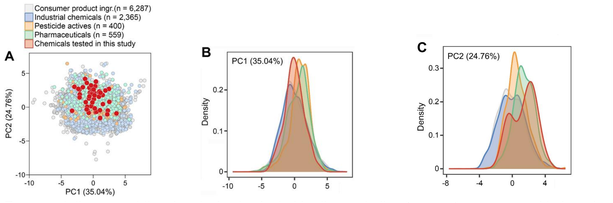
**Conclusions:** Overall, the study demonstrated that human LCLs can be used as an in vitro model to quantify inter-individual variability in the context of NGRA. These data will improve confidence in the overall risk predictions using NAM data and also enable the development of testable hypotheses regarding the mechanisms of inter-individual variability.

## MATERIALS AND METHODS




**Experimental design and main study elements for the project. First**, test chemicals (n = 53) from different classes were selected to represent various human exposure scenarios and risk categories. **Second**, a sample of race/ethnicity- and sex-balanced 131 human lymphoblast cell lines was selected from four subpopulations. **Third**, cells were exposed to test chemicals in concentration–response to determine effects on cell viability and the data was subject to rigorous quality control. **Fourth**, Bayesian dose-response modeling was used to derive points of departure (POD) for each chemical and individual cell line and toxicodynamic variability factor 5% ($TDVF_{05}$) was calculated for 18 chemicals with cytotoxic effects. **Finally**, GWAS analyses were performed using POD data.

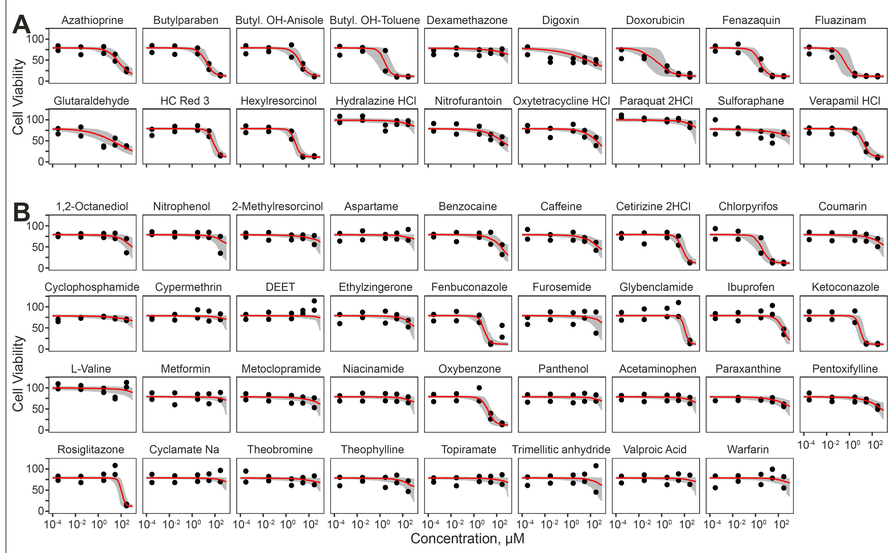
## Substances Tested in This Study




Representativeness of the chemical set selected for these studies (maroon) as compared to broader chemical types in the classes of consumer product ingredients (gray), industrial use chemicals (blue), pesticide actives (yellow), and pharmaceuticals (green). Shown are the results of the principal component analysis based on 11 physico-chemical parameters.
(**A**) displays a 2D plot of PC1 vs PC2 and (**B-C**) display 1D distributions in PC1 and PC2.

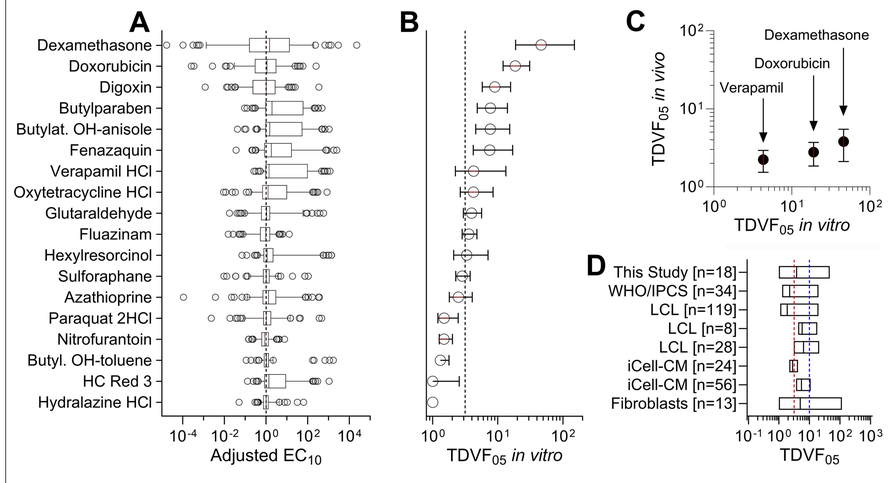
## RESULTS

### Bayesian Concentration-Response Modeling to Derive Points-of-Departure (Effective Concentration 10%, $EC_{10}$) and Toxicodynamic Variability Factors (TDVFs)



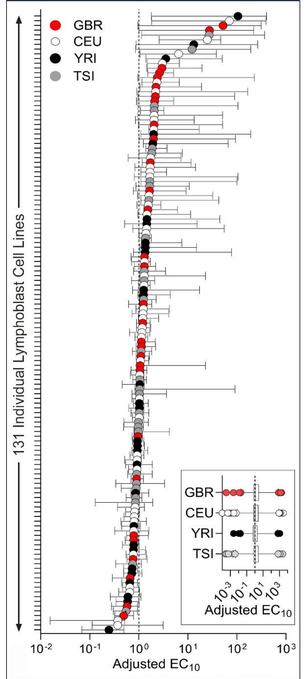

**Representative Bayesian curve-fitting examples of concentration–response profiles for tested chemicals in cell line 12275.** Red line is an overall population-based curve fit. Dots represent experimental data points. Gray lines represent individual simulated curves from the last 100 iterations. (A) Shows the data for 18 chemicals with an overall population-wide cytotoxic effect. (B) shows the for remaining 35 chemicals.

### Concentration–Response Analysis of The Inter-Individual Variability in Chemical Effects Across 131 Tested Lymphoblast Cell Lines



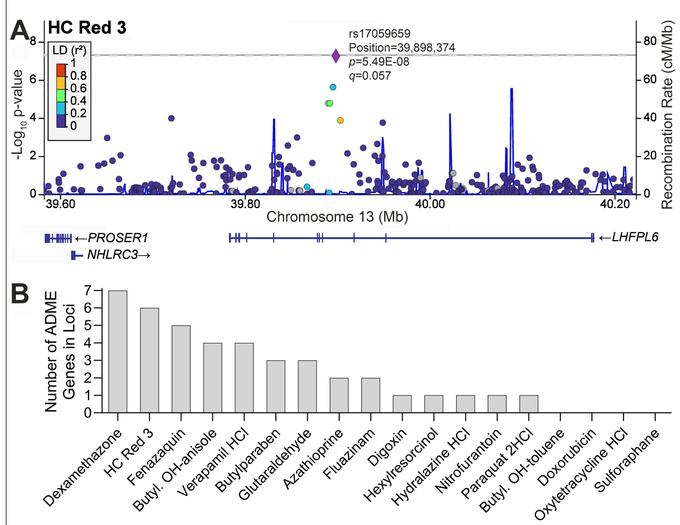

**Concentration–response analysis of the inter-individual variability in chemical effects across 131 tested lymphoblast cell lines. (A)** Distribution of the adjusted PODs (effective concentration 10%, $EC_{10}$) for the chemicals with population-wide cytotoxic effects is shown as box (interquartile range)-and-whiskers (5th and 95th percentiles) plots. Box colors represent chemicals classified as high (red) or low (white) risk. **(B)** $TDVF_{05}$ values calculated for chemicals with population variability. Dots represent central estimate, and whiskers represent upper and lower bound on $TDVF_{05}$. **(C)** Comparison of in vitro-derived (this study) and human in vivo $TDVF_{05}$ values for 3 substances for which suitable clinical data could be identified. **(D)** Ranges in reported $TDVF_{05}$ values from this study as compared to previous publications reporting data from humans (WHO/IPCS) and human cells (LCLs, induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CM), and dermal fibroblasts.

### Individual LCL-Specific Adjusted PODs ($EC_{10}$)




Dots represent central estimate; whiskers represent upper and lower bound on $EC_{10}$.

### Genome-Wide Associations Study (GWAS) Analysis: Generating Hypotheses About Mechanisms of Inter-Individual Variability




**GWAS analysis of population variability in cytotoxicity of 18 chemicals.** (**A**) A representative plot of the GWAS results for **HC Red 3** at the locus around the most significant single nucleotide polymorphism (SNP, rs17059659; purple diamond) on Chromosome 13. Other analyzed SNPs are shown as colored dots (according to linkage disequilibrium, LD, see top left inset) arranged by their genomic position and corresponding -Log10 p-value. Position of the genes located in the locus is also shown. (**B**) For each of 18 chemicals, shown are the numbers of absorption, distribution, metabolism and excretion (ADME)-related genes located in the 1 Mb loci around genome-wide significant SNPs for each chemical.

## CONCLUSIONS

- This study tested a broad range of chemicals, which are available in defined use scenarios for human risk predictions, to quantify inter-individual variability using a population-based human *in vitro* model.
- Our findings revealed that 18 chemicals exhibited concentration–response cytotoxic effects, which allowed us to derive the $TDVF_{05}$ for these chemicals.
- The median $TDVF_{05}$ was 3.8, which is in close to the default human toxicodynamic variability factor of 3.16.
- In the context of NGRA, the study confirmed that human LCLs can be used as an *in vitro* model to quantify inter-individual variability.

**These data will enhance confidence in the overall risk predictions using NAM data and will also facilitate the development of testable hypotheses regarding the mechanisms of inter-individual variability**

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