

Does interdisciplinary work between toxicologists and statisticians improve the understanding of the comet assay?

Timur Tug^{1,*}, Julia C. Duda¹, Max Menssen², Shannon Wilson Bruce³, Frank Bringezu⁴, Martina Dammann⁵, Roland Frötschl⁶, Volker Harm⁷, Janina Höfert⁸, Katja Ickstadt¹, Bernd-Wolfgang Igl⁹, Marco Jarzombek¹⁰, Rupert Kellner⁸, Jasmin Lott⁹, Stefan Pfuhler¹¹, Ulla Plappert-Helbig¹², Jörg Rahnenführer¹, Markus Schulz¹³, Lea Vaas⁷, Marie Vasquez¹⁴, Verena Ziegler¹⁵ and Christina Ziemann⁸

¹Department of Statistics, TU Dortmund University, Dortmund, Germany, ²Institute of Cell Biology and Biophysics, Department of Biostatistics, Leibniz University Hannover, ³Inotiv, Rockville, MD, USA, ⁴Merck Healthcare KGaA, Chemical and Preclinical Safety, Darmstadt, Germany, ⁵BASF SE, Ludwigshafen am Rhein, Germany, ⁶Federal Institute for Drugs and Medical Devices (BfArM), Bonn, Germany, ⁷Bayer AG, Berlin, Germany, ⁸Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany, ⁹Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany, ¹⁰NUVISAN ICB GmbH, Preclinical Compound Profiling, Germany, Germany ¹¹Procter & Gamble, Cincinnati, Ohio, USA, ¹²Lörrach, Germany, ¹³ICCR-Roßdorf GmbH, Rossdorf, Germany, ¹⁴Helix3 Inc, Morrisville, NC, USA, ¹⁵Bayer AG, Wuppertal, Germany.

*: presenting author _____

Short Bio:

2011 – 2018	Bachelor and Master's degree in statistics, TU Dortmund
Since 06/2018	Research Assistant and PhD candidate at the Chair of Mathematical Statistics and Biometric Applications (Prof. Dr. Katja Ickstadt), TU Dortmund, and a member of the research training group 2624 (Prof. Dr. Jörg Rahnenführer)
01/2023 – 05/2023	Short – Term Scholar, Emory University, Atlanta, USA

Short Summary:

The alkaline comet assay (CA) is a fast and sensitive method to identify genotoxic agents in exposed cells and tissues on a single-cell level. Based on electrophoretic mobility of DNA fragments in agarose gels on slides, the assay can detect DNA-strand breaks, DNA-DNA and DNA-protein cross-links, alkali-labile sites, and aspects of DNA-repair. The *in vivo* CA is now widely used and represents an important test in several regulatory frameworks. Therefore, the current goal of the interdisciplinary working group "Statistics" within the German Society for Environmental Mutation Research (GUM) is to evaluate and to update statistical approaches for the *in vivo* CA (OECD 489; Tug and Duda, et. al, 2024).

A large laboratory effect was found with the aid of variance component analysis, questioning the use of standard ranges for labs and for different organs. Analysis systems, such as Comet Assay III, Comet Assay IV, and Metafer System, played a significant role in the inter-laboratory variance. Results varied depending on variables like software, camera, microscope, and light source type and condition, which makes it almost impossible to directly compare data from different laboratories and to define corresponding standard reference ranges.

The lively exchange within the interdisciplinary working group improves the understanding and identification of various factors influencing Comet data and enables the development of a proper statistical analysis strategy. This will be supported by the development of a corresponding R-Shiny app.