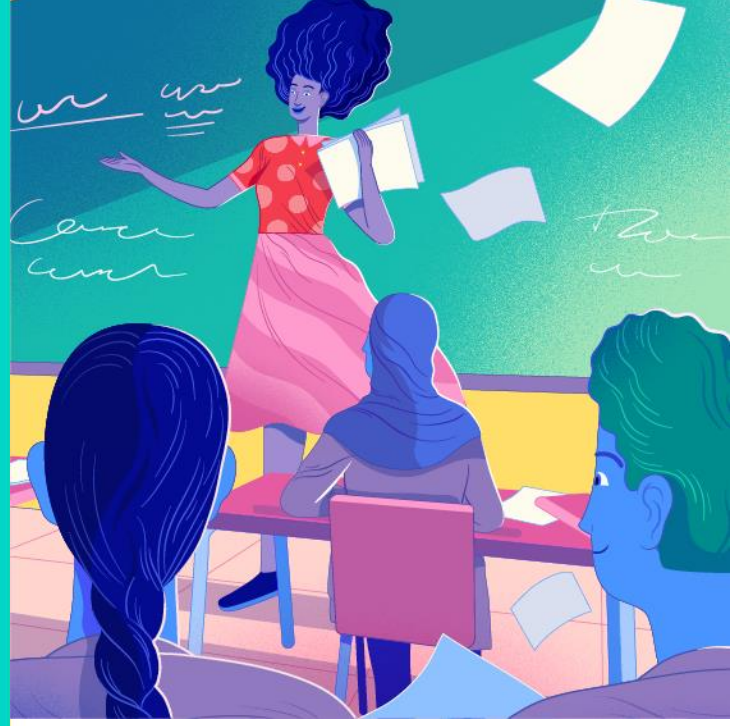
# Application of Data Analysis Based Expert Knowledge Elicitation in Deriving In Vitro Point of Departure of DNA Damage from Three In Vitro Assays

Dawei Tang, Andrew White, Andrew Scott, Mona Delagrange



Unilever

# Outline

- Introduction
- In Vitro Assays
- Expert Knowledge Elicitation
- Conclusion

# Introduction

- DNA Damage can be caused by exposure to certain genotoxic chemicals via environmental occurrence

- In this presentation:
  - Exposure of a p53-positive cell line (HT1080) to quercetin in 3 in vitro assays

  - Dose Response data of the assays were analysed using a Bayesian method to infer a PoD[1] for each assay

  - Expert Knowledge Elicitation was conducted based on data analysis to derive an in vitro PoD for DNA Damage[2]

[1] PoD: Point of Departure

[2] PoD for DNA damage - the Lowest Observed Effective Level to cause saturation of endogenous DNA repair capacity in HT1080 cell line

# In Vitro Assays – Mechanism

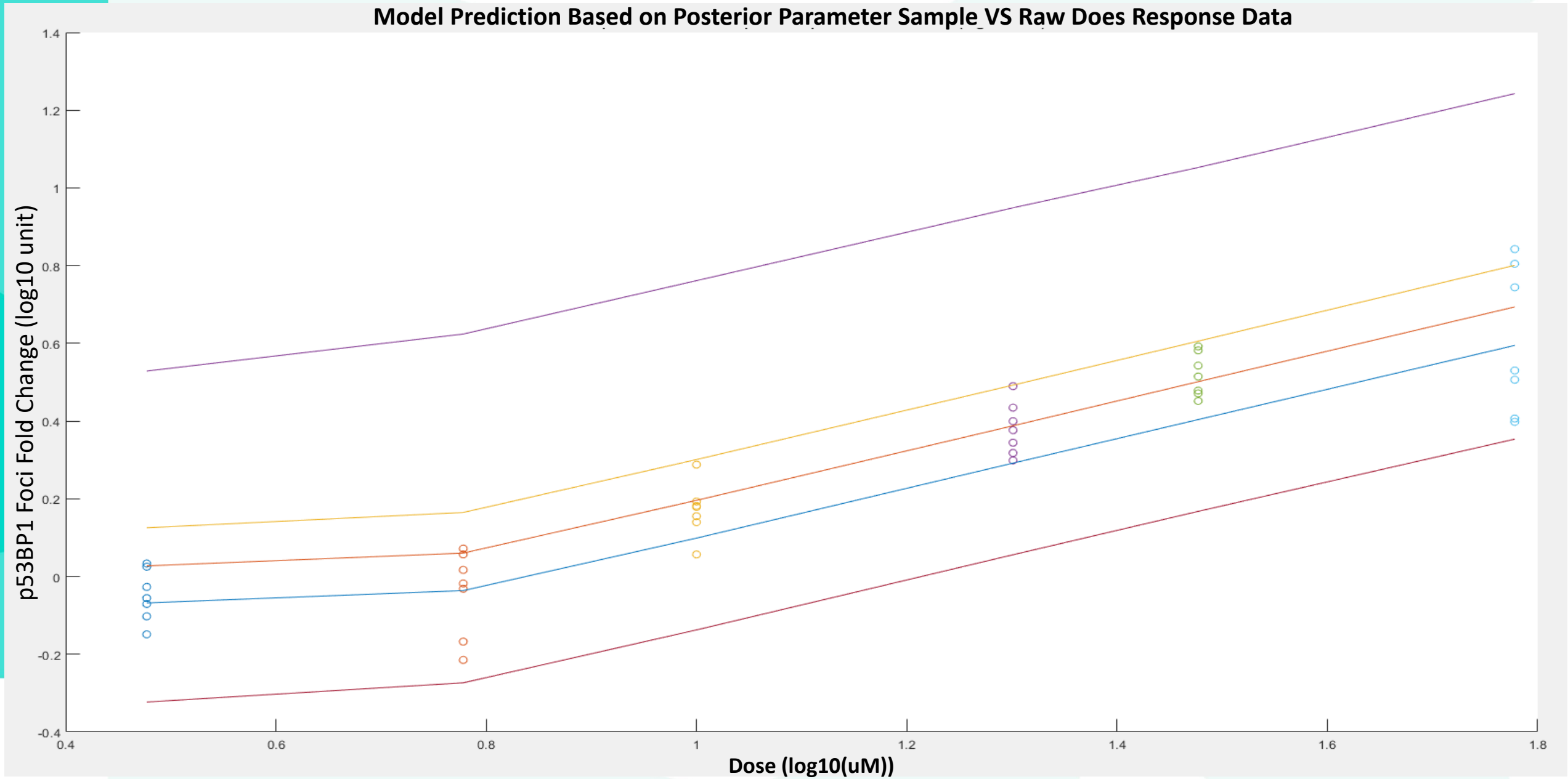
- When DNA strand breaks are sensed following chemical exposure, DNA Repair Centres (RC) at the sites of DNA strand breakage will accumulate to repair DNA damage.

- When the exposure increases, further responses will lead to transcriptional activation, regulating key DNA damage response proteins.

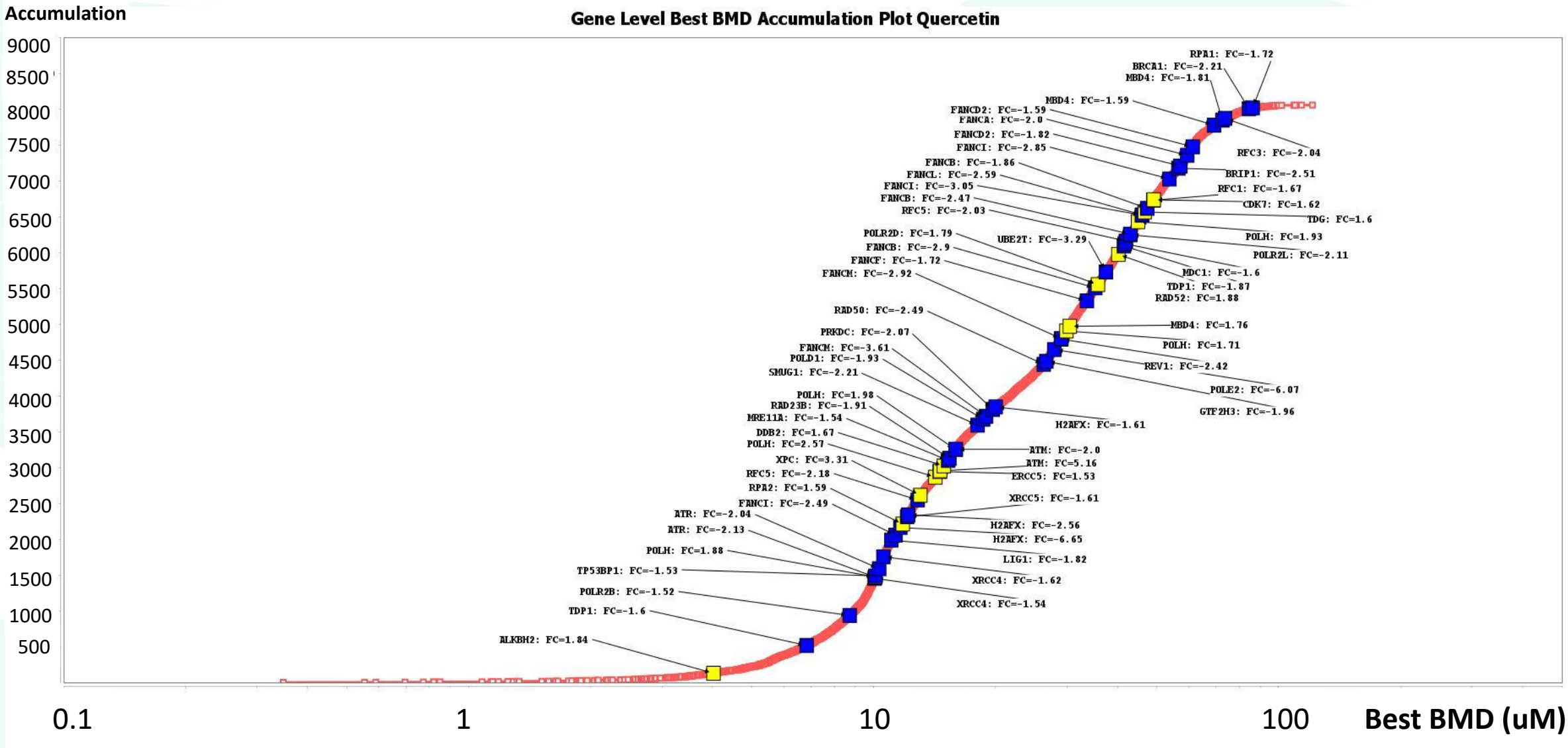
- When incidence of unrepaired double strand breaks increases, Micronuclei (MN) are formed, as incomplete or stalled repair will lead to lagging strands of chromatin at cell division.

Unilever

# In Vitro Assays – DNA Repair Centre (RC) Assay

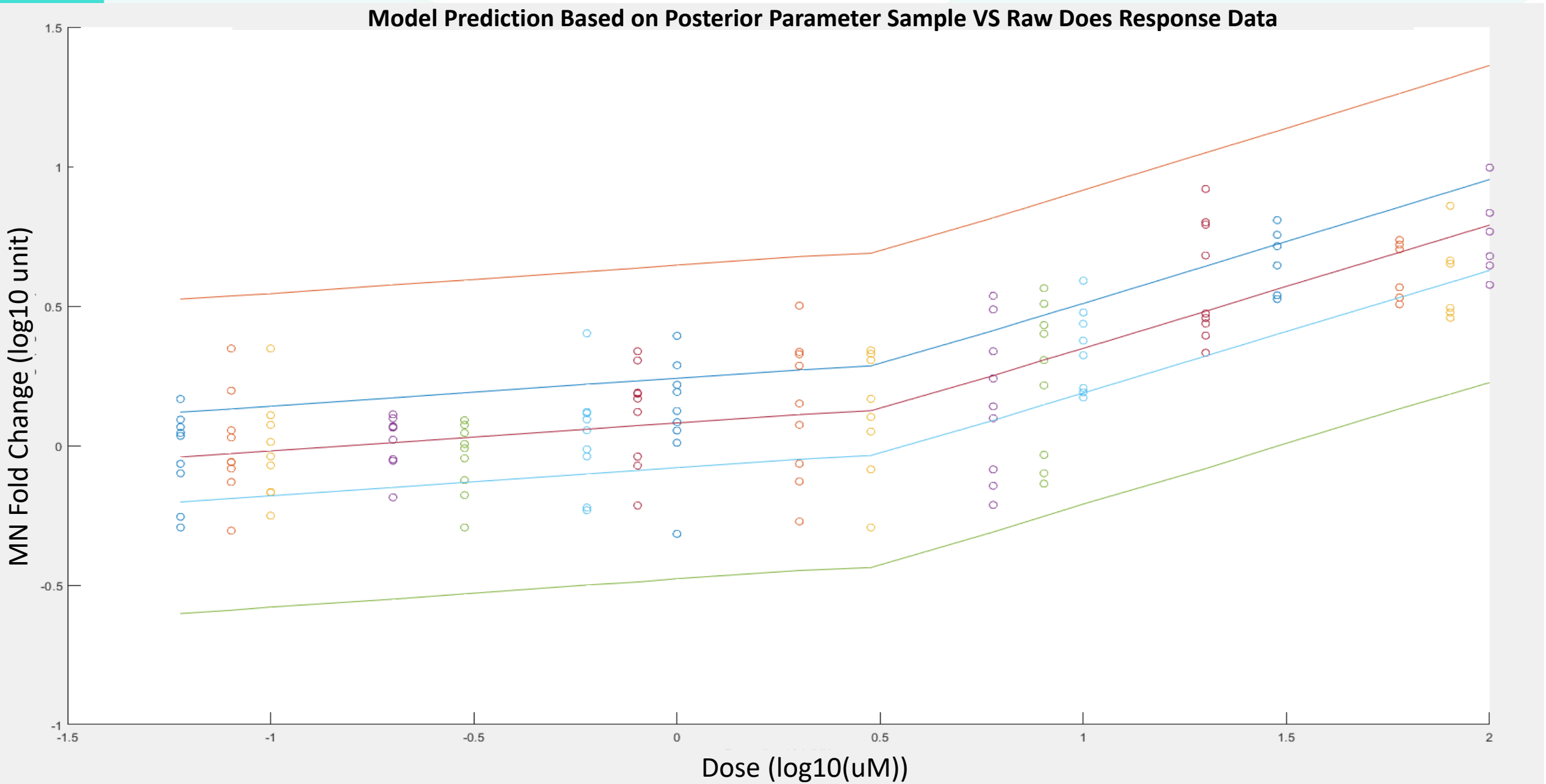


# In Vitro Assays – Microarray Assay




- 0.3uM: no significant change in levels of DNA damage-related gene transcription below this dose
- 1uM: the dose response profiles for gene transcriptional changes are more robust (consistent) above this dose
- 11.9uM: the lowest dose that P53-associated activation happened

# In Vitro Assays – Micronucleus Assay



# Expert Knowledge Elicitation

Why do we need EKE[1]:

- Induction of DDR[2] differential gene expression, formation of DNA RCs and MNs are closely related to DNA damage
- None of them directly measures the PoD of DNA Damage
- Knowledge exists on the relationship between assay outcomes and DNA damage

How did we do the Expert Knowledge Elicitation: SHELF

Expert invited: 3 toxicologist in Unilever invited, expertise in genotoxicity and computational toxicologist

[1] EKE: Expert Knowledge Elicitation
[2] DDR: DNA Damage Response

# EKE - Process

SHELF method:

- Experts to estimate the lower bound (L), upper bound (U) and median (M) of the variable under concern.
- Probability distributions are then fitted for each expert's judgment.
- The experts then discuss the rationale of their judgments based on the implications of the fitted distribution. They can modify their judgments after the discussion.
- A consensus distribution is agreed based on the modified individual judgments.

Informativeness in understanding PoD of DNA Damage:

- PoD from the MN assay < PoD from the MA assay < PoD from the DNA RC assay

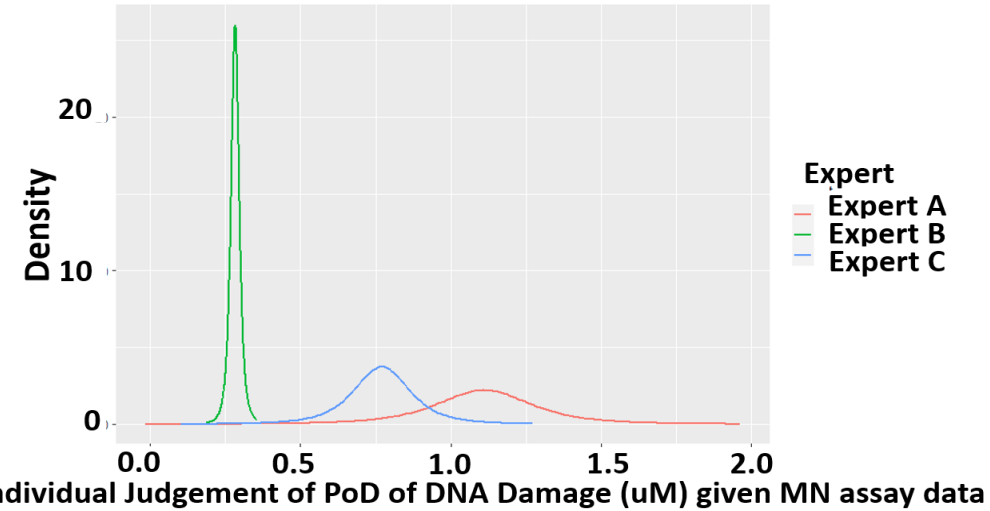
Sequence of providing assay outcome to expert:

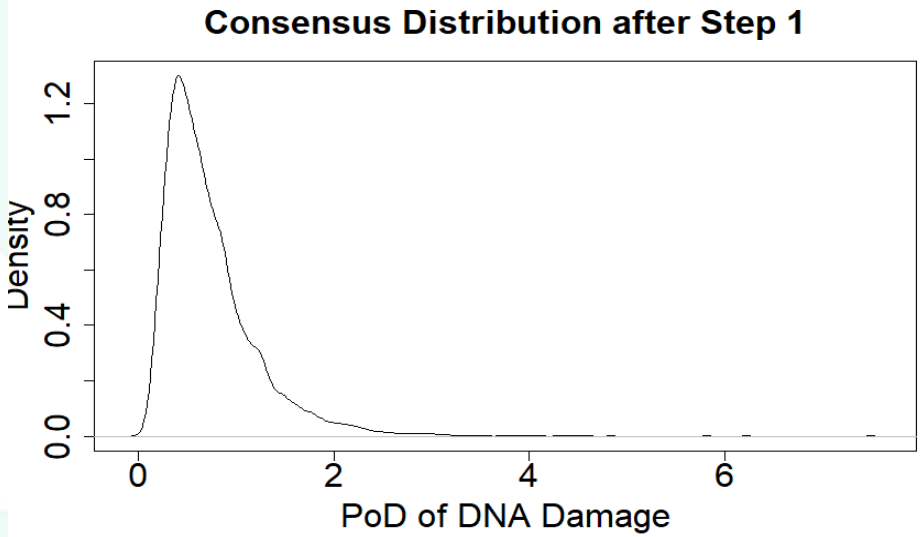
- MN → MA→ DNA RC

# EKE – MN

| | 2.5th Percentile | 25th Percentile | 50th Percentile | 75th Percentile | 97.5th Percentile |
|---|---|---|---|---|---|
| $\theta$ (log scale) | 0.34 | 0.45 | 0.50 | 0.56 | 0.67 |
| $\theta$ (natural scale) | 2.21 | 2.79 | 3.16 | 3.61 | 4.62 |

| | L | M | U |
|---|---|---|---|
| Expert A | 0.15 | 1.5 | 5 |
| Expert B | 0.2 | 0.316 | 3.16 |
| Expert C | 0.2 | 1 | 3.16 |




| DNA DSB PoD Range based on MN Assay | Probability |
|---|---|
| < 0.13 uM | 0.5% |
| < 0.48 uM | 25% (1 out of 4) |
| < 0.76 uM (or > 0.76 uM) | 50% |
| > 1.22 uM | 25% (1 out of 4) |
| > 4.51 uM | 0.5% |

# EKE – MA

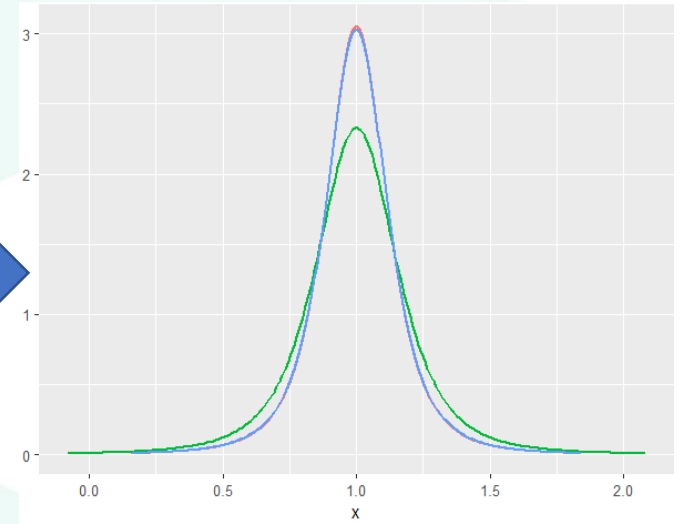
PoD (uM) of DNA DSB based on PoD of MN

| 2.5th Percentile | 25th Percentile | 50th Percentile | 75th Percentile | 97.5th Percentile |
|---|---|---|---|---|
| 0.13 | 0.48 | 0.76 | 1.22 | 4.51 |

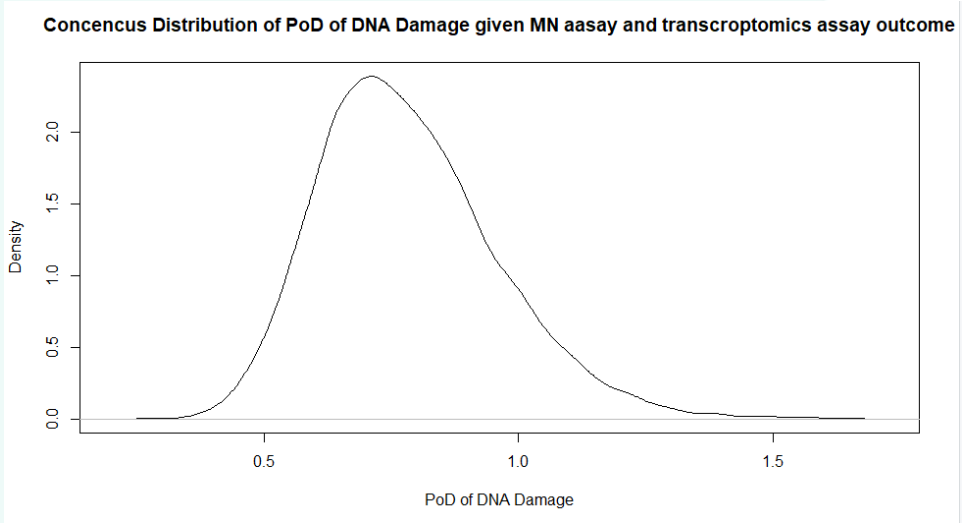
0.3uM: no significant change in levels of DNA damage-related gene transcription below this dose
1uM: the dose response profiles for gene transcriptional changes are more robust above this dose than below this dose

11.9uM: the lowest dose that P53-associated activation happened

| | Step 2 | | |
|---|---|---|---|
| | L | M | U |
| Expert A | 0.3 | 1 | 4.62 |
| Expert B | 0.1 | 1 | 3.16 |
| Expert C | 0.3 | 1 | 3.16 |




| DNA DSB PoD Range based on MN and MA Assay | Probability |
|---|---|
| < 0.51 uM | 0.5% |
| < 0.82 uM | 25% (1 out of 4) |
| < 0.98 uM (or > 0.98 uM) | 50% |
| > 1.16 uM | 25% (1 out of 4) |
| > 1.87 uM | 0.5% |



# EKE - DRC

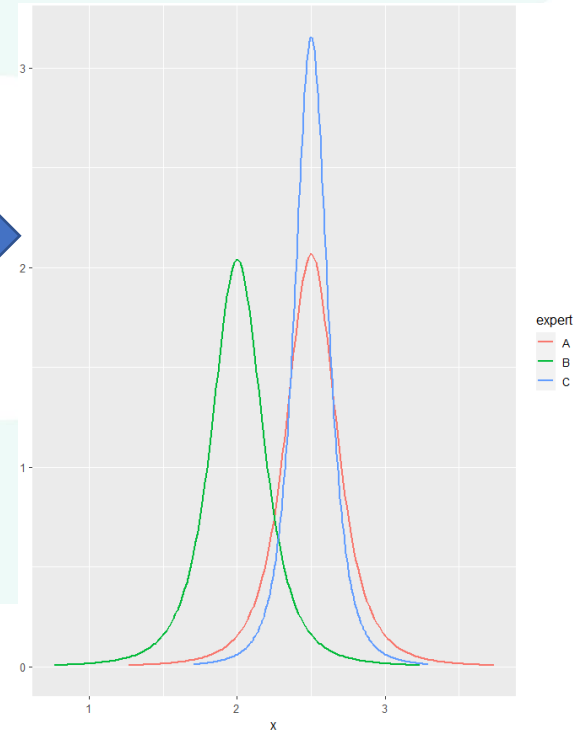
PoD of DNA DSB based on MN assay and gene transcriptomics

| 2.5th Percentile | 25th Percentile | 50th Percentile | 75th Percentile | 97.5th Percentile |
|---|---|---|---|---|
| 0.51 | 0.82 | 0.98 | 1.16 | 1.87 |

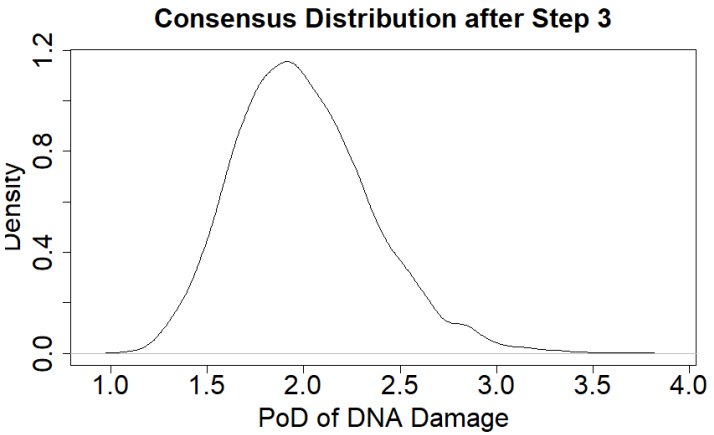
PoD of DNA DRC:

| 2.5th percentile | 25th percentile | 50th percentile | 75th percentile | 99.5th percentile |
|---|---|---|---|---|
| 1.77 | 4.77 | 5.43 | 5.97 | 7.07 |

| | Step 3 | | |
|---|---|---|---|
| | L | M | U |
| Expert A | 1.5 | 2.5 | 3.6 |
| Expert B | 1 | 2 | 3.16 |
| Expert C | 1 | 2.5 | 3.16 |

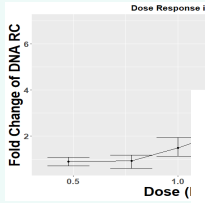


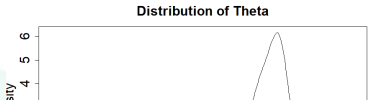

| DNA DSB PoD Range based on All Assays | Probability |
|---|---|
| < 1.26 uM | 0.5% |
| < 1.74 uM | 25% (1 out of 4) |
| < 1.96 uM and > 1.96 uM | 50% |
| > 2.21 uM | 25% (1 out of 4) |
| > 3.06 uM | 99.5% |

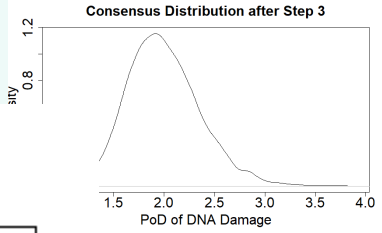


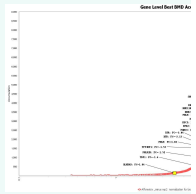

$$PoD_{in\ vitro} \sim lognormal(0.6774, 0.1723)$$

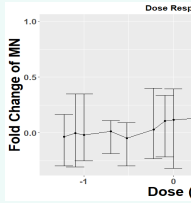
# Discussion

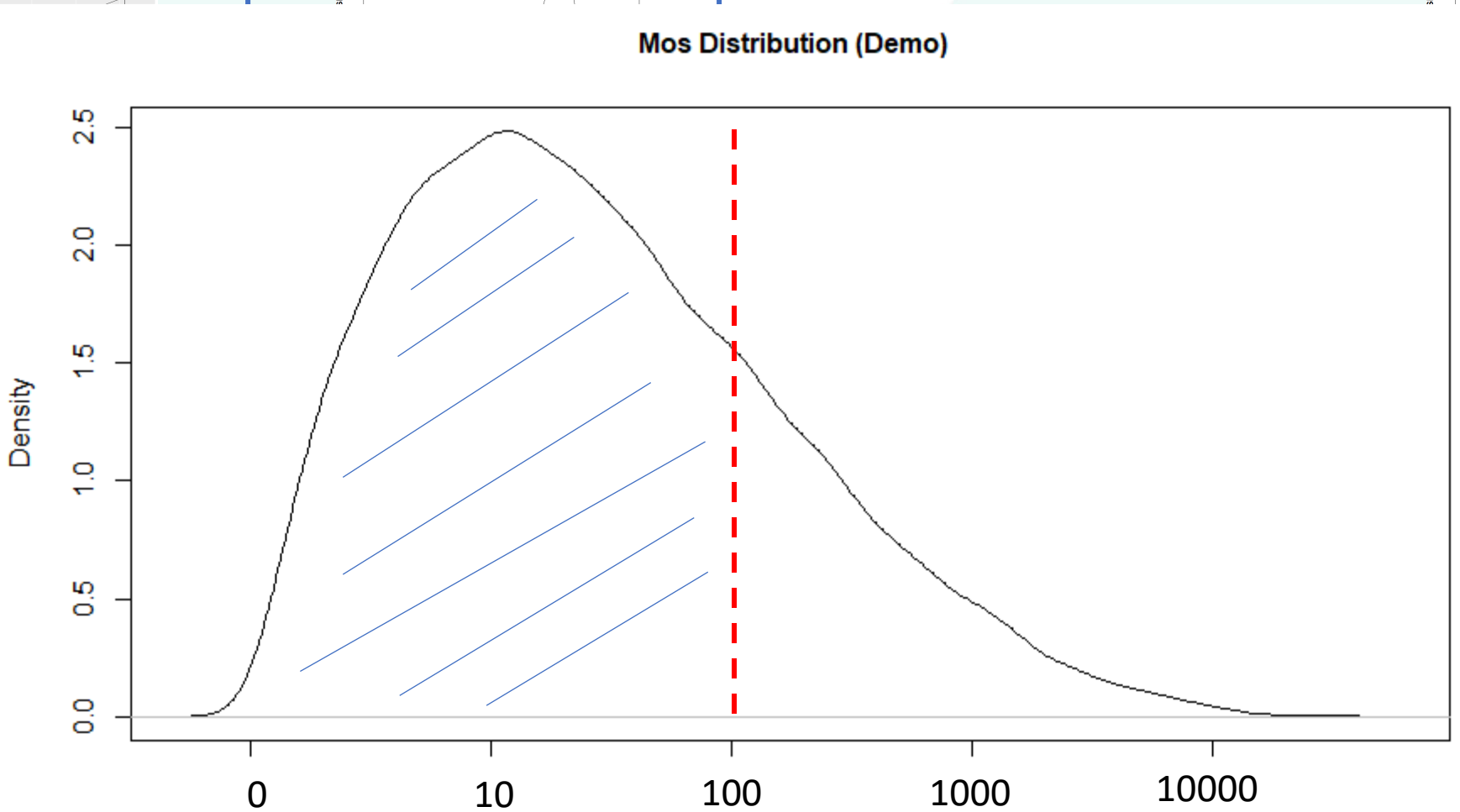





Raw
Respor

External

, 0.1723)

E[1] by EKE

MoS[2] Distribution

[1] IVIVE: In Vitro In Vivo Extrapolation
[2] MoS: Margin of Safety

# Summary

Data analysis based EKE is applied

How such process can integrate various source of information to support risk assessment decision making with explicitly characterised uncertainty is shown

The principles outlined in this work can be applied in many areas where

- data analysis could not provide a direct or adequate answer
- expert knowledge is available, and can be elicited, structured and analysed to derive a probabilistic distribution of a quantity of interest

Unilever