This presentation summarises the results obtained by the participants of

Workshop 4

New approaches for the quantitative characterization of genotoxic effects based on animal and human data

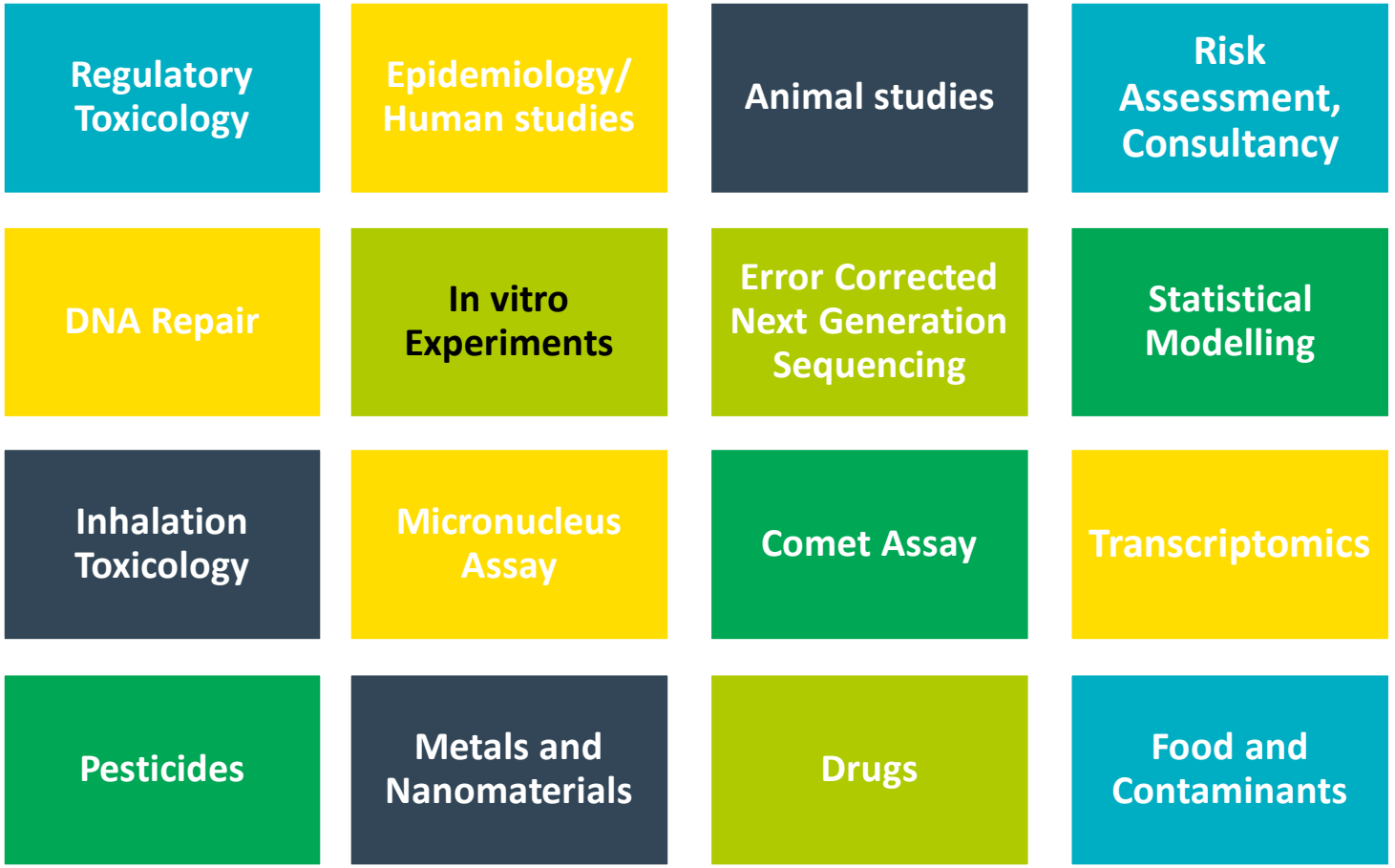
It does not necessarily reflect the opinion of the BfR.

Workshop 4:
New approaches for the quantitative characterization of genotoxic effects based on animal and human data

International Symposium: Risk Assessment of Genotoxic Compounds
26–28 February 2024, Berlin

Workshop chairs:
Helga Stopper, Julius-Maximilians University Würzburg
James Kevin Chipman, University of Birmingham

Key expertise of the WS 4 participants



Workshop 4 Agenda

Where do we want to be in 10 years time? (in a step-wise process starting today)

What is needed to achieve this goal?

- Section A: Animal studies
  - (a) Current Approaches, contribution on Benchmark Dose Modelling
  - (b) Perspectives, new technologies
- Section B: Human Studies
  - (a) Current Approaches, contribution from epidemiology
  - (b) Perspectives, new technologies

Section A:
Animal studies

Animal studies – Key questions

(a) Current Approaches

- Which *in vivo* genotoxicity test methods are currently validated and recommended?
- What is the informative value of these methods in terms of hazard identification and dose-response?
- What are the current hurdles and resulting needs?

In vivo genotoxicity test methods (OECD guidelines)

Test Method	OECD TG
In vivo Micronucleus Assay (MN)	474
In vivo chromosomal aberration test	475
In vivo comet assay	489
Transgenic Rodent Assay (TGR)	488
Pig-a gene mutation assay	470

Observations on specific test methods (in vivo Comet)

- Basis and fate of damage unclear (limited predictivity with regard to mutagenicity)
- Multiple mechanism, interpretations may diverge, requirement for further mechanistic information

- Clear value for site of contact genotoxicity, especially where bone marrow exposure not clearly demonstrated for micronucleus assay (e.g. for mixtures)

- Each lab can have its own background / baseline data, standardisation across laboratories desirable

Further observations on specific test methods

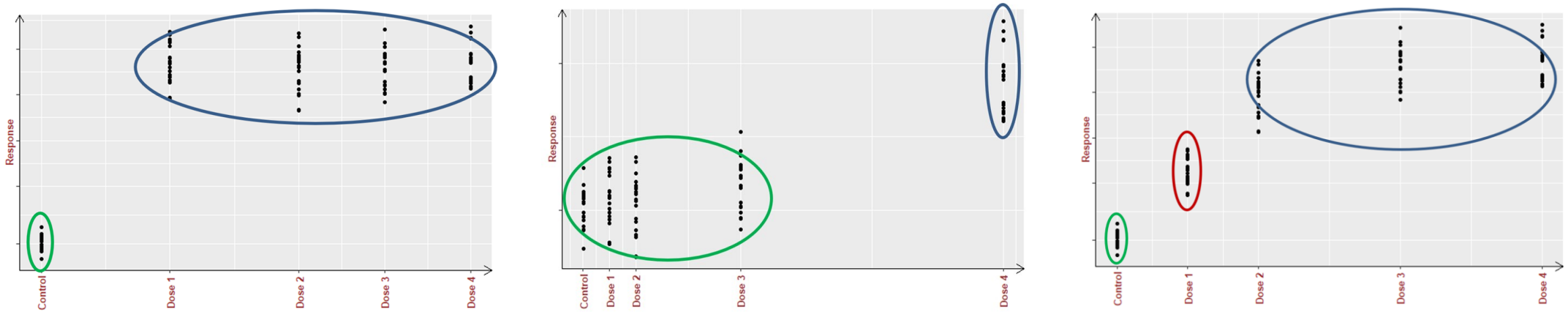
- In vivo bone marrow micronucleus assay as well as chromosomal aberration and Pig-A assay might not be predictive for other tissues
- Level of evidence for bone marrow exposure differs, discussion on what is „sufficient“ exposure
- MN assay in liver / intestine valuable, validation and guideline required

- Transgenic Rodent Assays allows to assess multiple tissues, however it employs a non-mammalian gene target

Quantitative approaches

Invited contribution: Jose Cortinez Abrahantes (EFSA)

- Benchmark dose (BMD) modelling is well established to estimate a dose associated with a certain magnitude of effect (benchmark ratio, BMR) as well as the confidence limits (BMDL / BMDU)
- Suites of models are fitted = model averaging, increasing robustness
- typically using 4 parameters (background, max response, steepness, potency)
- to be suitable for BMD modelling, experimental data should ideally represent 3 response categories (e.g. background / medium / max). This should be considered in testing protocols



What are the benefits and hurdles to BMD modelling?

- BMD is preferred, NOGEL is not suitable
- There may be cases / studies where BMD modelling is not successful
- Need for further harmonisation of BMD modelling approaches / guidance / tools
- Where to set the benchmark response (BMR)? Proposals for suitable BMRs have been published and were discussed.
- Case studies would be useful to explore BMR appropriateness for different genotoxicity endpoints. This work has started but needs to be continued.
- What then is an appropriate Margin of Exposure (MoE) where sufficient exposure data are available?
- Further comparative analyses of BMD for genotoxicity and cancer endpoints will be useful
- We concluded that an EFSA/ECHA/EMA/OECD Workshop should consider opportunities for setting an appropriate BMR and MoE for a genotoxic point of departure (in vivo)

What new opportunities may arise from the availability of error-corrected DNA sequencing technologies?

Invited contribution: George Papoutsoglou, Twinstrand Biosciences

- Enables detection of ultra-rare somatic mutations
- Allows genome-wide mutation frequency measurement
- No transgenic animals needed, can be performed with any tissue
- Can be run post-hoc on material from any in vivo study if stored properly at -80°C
- The correlation (or not) of mutation signatures with that in humans may inform about the relevance of the animal model
- A chemical causing only aneuploidy or chromosomal aberration would currently not be picked up by ec-NGS.

What new opportunities may arise from the availability of transcriptomic and proteomic markers?

- Gene expression changes can be indicative of DNA damage, e.g. induction of damage repair
- Link between transcriptomic changes, proteome and genetic signatures as well as apical effects is important
- Identification of patterns of alteration and their time-dependency for the studied effect is needed
- More standardisation of the procedures and methods recommended
- BMD analysis of transcriptome information being explored

Conclusion on animal in vivo studies

Where do we want to be in 10 yrs time? (in a step-wise process starting today)

- Integration of all relevant techniques, in particular ec-NGS and omics along with established genotoxicity endpoints assays into OECD guideline repeated dose toxicity studies
- Quantitative evaluation of the data with support of statistical tools
- For interpretation, also incorporate information from in vitro, NAM, PBTK/IVIVE and human data to make informed choices on reference point and MoE
- Beneficial outcome: reduction of animal numbers

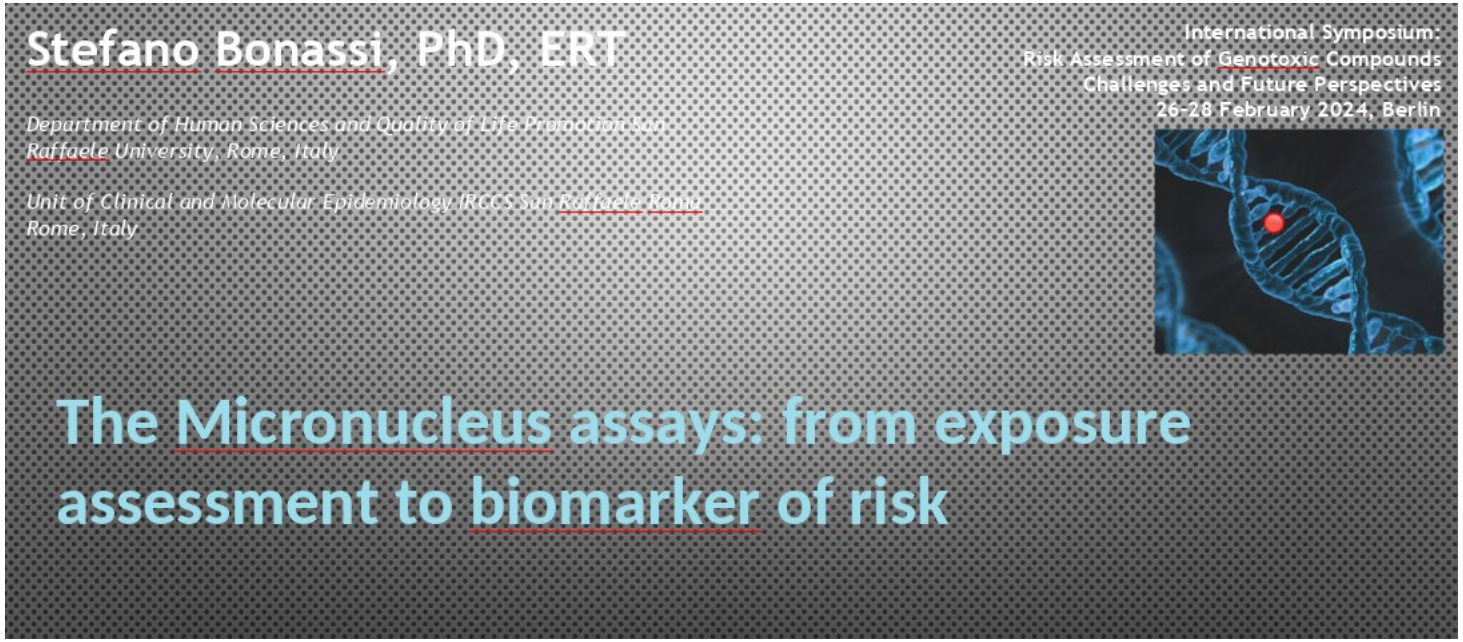
Section B:
Human Studies

Genotoxicity biomarkers available for human studies

Test Method / Biomarker
Micronuclei
Comet Assay
Gamma-H2AX
DNA adducts
ec-NGS (error corrected next generation sequencing)

What are the opportunities and limitations of dose-response assessments based on human epidemiological data?

Invited contribution: Stefano Bonassi
Professor of Hygiene and Preventive Medicine
San Raffaele University, Rome



Human studies – General comments

- General rules for evaluation of epidemiological studies should also apply here
- Confounding factors in epidemiology, biases (incl. selection bias) etc. can lead to over- or underestimation of effect size
- Distinction to be made between occupational and population studies, as well as retrospective and prospective studies (advantages of prospective studies include better defined exposure windows)
- Causality more difficult to establish than in controlled animal study

Observations on established biomarkers in human studies

- Generally much more complex situation compared to animal studies and in particular in vitro studies, making it more difficult to judge biomarker suitability and to interpret effects
- Human populations are heterogeneous. Biomarker suitability and background mutation frequency may depend on genetic background, age, etc.
- Background needs to be defined
- Comet assay limitations as discussed in the context of animal studies
- Tissues other than blood may be appropriate depending on exposure and feasibility (invasiveness, labour intensity)
- DNA adducts useful to support exposure estimation

Human studies – technical considerations

- Large sample numbers require consideration of automation and AI support
- Combination of effect and exposure biomarkers to increase confidence / strength of evidence
- Explore temporal development
- Existing biobanks may be used for some purposes but have limitations regarding suitability for different endpoints and for population coverage
- Inclusion of standards and controls to deal with (bio)analytical variability including logistics and stability strongly recommended
- Further standardization of assays, also for additional target sites / tissues

Observations on new biomarker approaches

- ec-NGS might be highly sensitive (based on animal data)
- As for established markers, temporal relationships need to be clarified and considered
- Mutation signature may inform about the causative agent
- Signature database is developing rapidly (currently 70 chemicals)

Human studies – quantitative aspects

- Potential “traffic light concept”, X-fold for yellow light and Y-fold for red light for increase in biomarker response above background
- What should be the basis for the construction of decision points X and Y?
- Dose-response in humans complex because of less info on exposure
- Lower dynamic range of endpoints than in vitro and in animals
- BMD modelling is difficult but could be used if a large exposure range is covered, e.g. in occupational settings

Human studies – Conclusion

- Established biomarkers show some limitations but can be useful to support quantitative conclusions depending on the case
- Improvements are needed regarding background characterisation, standardisation and application to various tissues
- Novel in vivo genotoxicity biomarkers based on ec-NCS and omics offer new opportunities to enhance quantitative evaluation
- Integration of epidemiological data with other streams of evidence is advocated
- Envisage a semi-quantitative approach in the next decade

Thank you to all participants of Workshop 4 !

Affiliations of the WS 4 participants

University of Potsdam	BfR	EBRC Consulting	RWTH Aachen University	Technical University Munich
TwinStrand Biosciences	Maastricht University	MPI for Human Development	Corteva Agriscience	Bayer AG
General Mills, Inc.	The Coca Cola Company	Chemsafe consulting	Deutsche Gesetzliche Unfallversicherung	BfArM Federal Institute for Drugs and Medical Devices
European Food Safety Authority	Uni Birmingham	Uni Würzburg	Maastricht University	ISS Rome Institute of Public Health