



Federal Institute  
for Drugs  
and Medical Devices

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*Applied Genetic Toxicity*  
*for Regulatory Decision Making:*  
*The Road Ahead*



# *Incorporating Mode of Action into Genetic Toxicity Assessment*

Dr. Roland Frötschl  
Genetic and Reproductive Toxicology



# Disclaimer

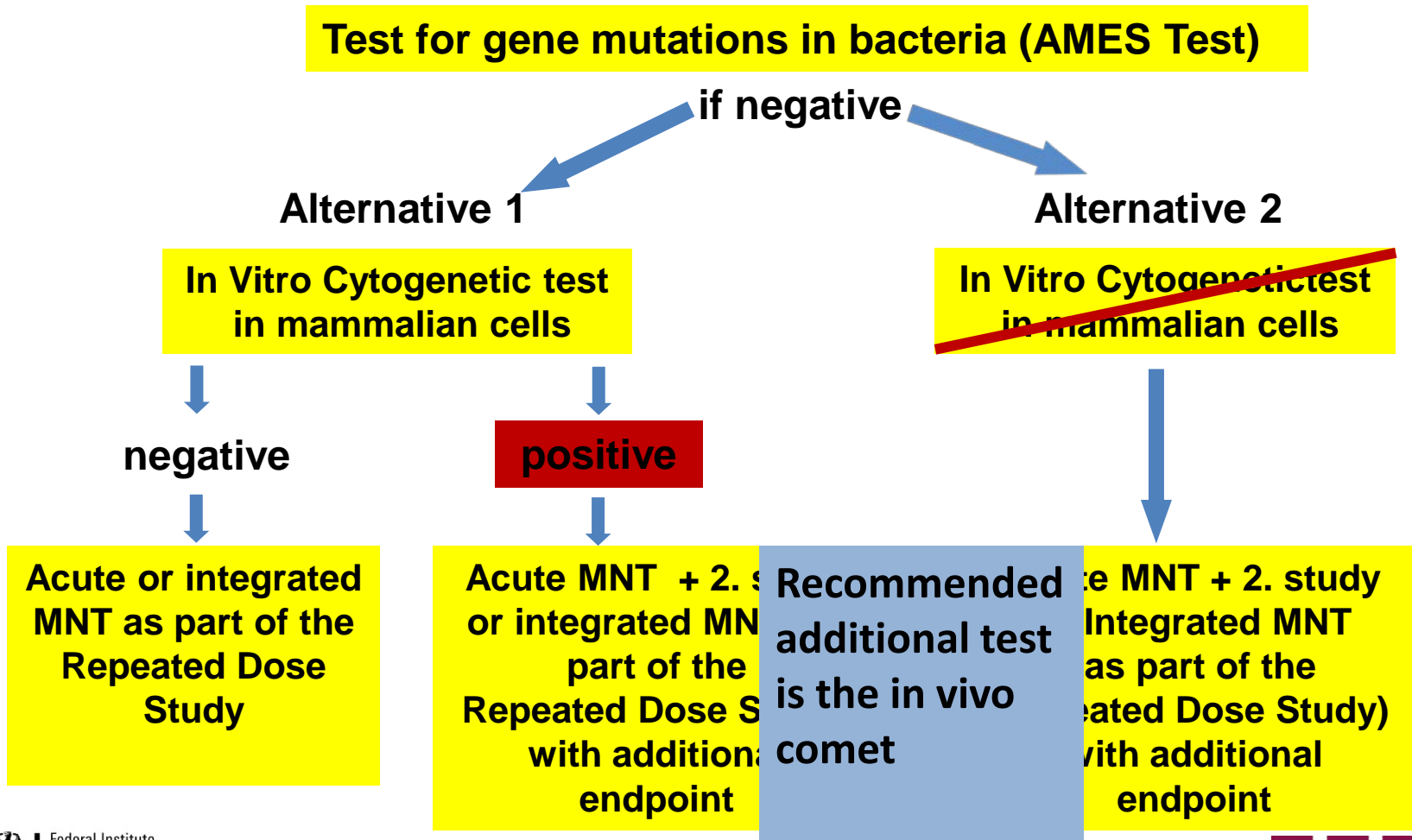
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2. Example for standard battery positive compound
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# Current Gentox Test Strategy for Pharmaceuticals

CHMP/ICH/126642/08 ICH S2 (R1)

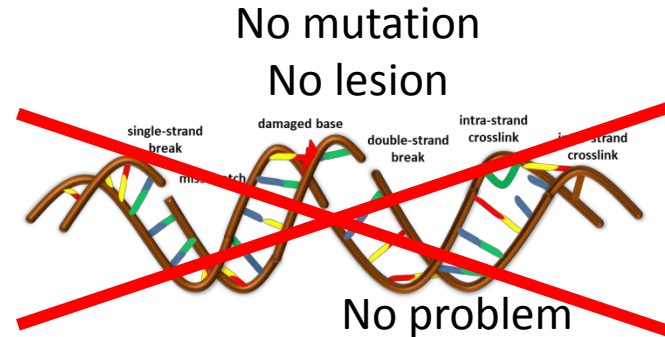


# Aim of the basic test strategy

- Identify mutagenic substances
  - Considered to be potentially carcinogenic
  - cause inheritable DNA-alteration which potentially lead to genetic diseases
- Protect consumers/patients from any unacceptable risk for
  - developing cancer or acquiring genetic diseases
- Protect populations from any unacceptable risk for
  - Increase of genetic diseases
  - Increase in predisposition for cancer
- Standard battery is focused on the detection of inheritable DNA alterations
  - Gens mutation, chromosome mutations, genome mutations

# Consequences of basic test battery results

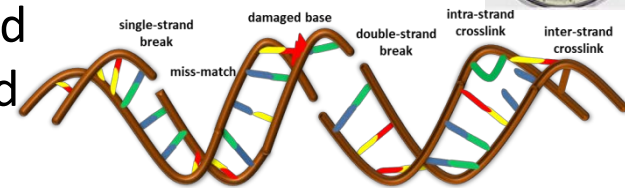
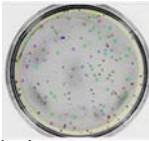
- Fine as long as test results are negative and no hazard is identified



- Problems start with positive test results and the question

- Is the hazard a risk?
- Generally additional testing for MoA are required for the risk assessment of a genotoxic compound
- Exclusion of direct reaction with the DNA and a dose response with a clear NOEL is required

MLA/Cab positive?!



What lesion, why,  
Who??????????????

# Limitations of the standard battery

- with current standard genotoxicity test battery models
  - prediction of the real risk of genotoxic compounds for mutagenicity in humans is difficult or nearly impossible, guidance for MoA clarification is very limited
- What we do is to assume a worst case and get to the safe side of it
- This is very conservative and overestimates the real risk (in most cases)

# Informed risk assessment improves safety assessment in genotoxicity

- This needs data on
  - Mode of action
  - Dose response and point of departure for relevant effects
  - Transspecies relevance
  - Exposure assessment
  - Environmental/human relevance



# Basic quantitative risk assessment for threshold (PoD) related mechanisms

- Currently a quantitative risk assessment is only accepted for compounds
  - not directly interacting with DNA (not-DNA-reactive)
- Compounds interacting directly with DNA (e.g. alkylating agents) are still in most cases regulated conservative with a yes/no risk assessment
  - Yes means there is no PoD, any dose is considered to pose a mutagenic risk
  - Acceptability is considered only for doses with a theoretical cancer risk of 1:100,000 for pharmaceuticals or 1:1,000,000 for food additives

# Determine the mode of (genotoxic)-action

- Mode of action determination needs to explain the mode of genotoxic activity
- this means practically the determination of the key activity of a molecule that leads to the final adverse outcome
- We need to know the
  - determinative molecular event (MIE molecular initiating event)
  - the dose response of the final adverse effect (gene, chromosome, genome mutations)

# MoA in risk assessment

## Fluoroquinolones

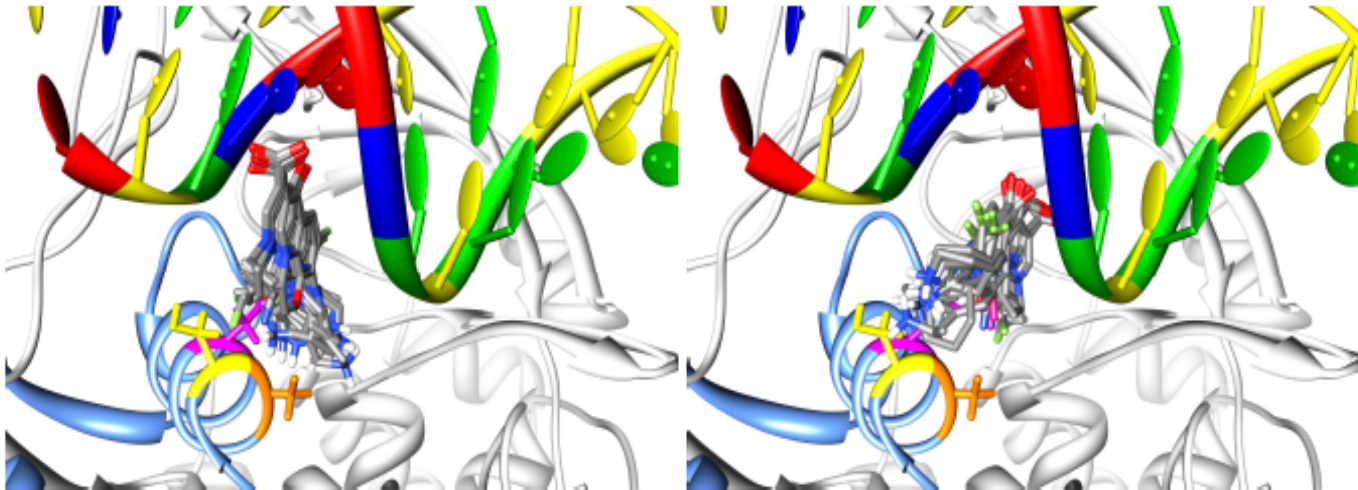


Figure 1. Complex of DNA linked to GyrA-Tyr<sub>122</sub> and docked quinolones: COOH-moieties of ligands point out of (left) and into plane (right); enzyme shown in grey, QRDR in lightblue, Ser<sub>83</sub>, Ala<sub>84</sub> and Asp<sub>87</sub> in  $\alpha_4$  helix in yellow, orange and magenta, resp.; G in green, C in yellow, T in blue and A in red.

from Lenz et al. NIC Series, Vol. 40, ISBN 978-3-9810843-6-8, pp. 289-292, 2008

# Mode of Action (MoA)

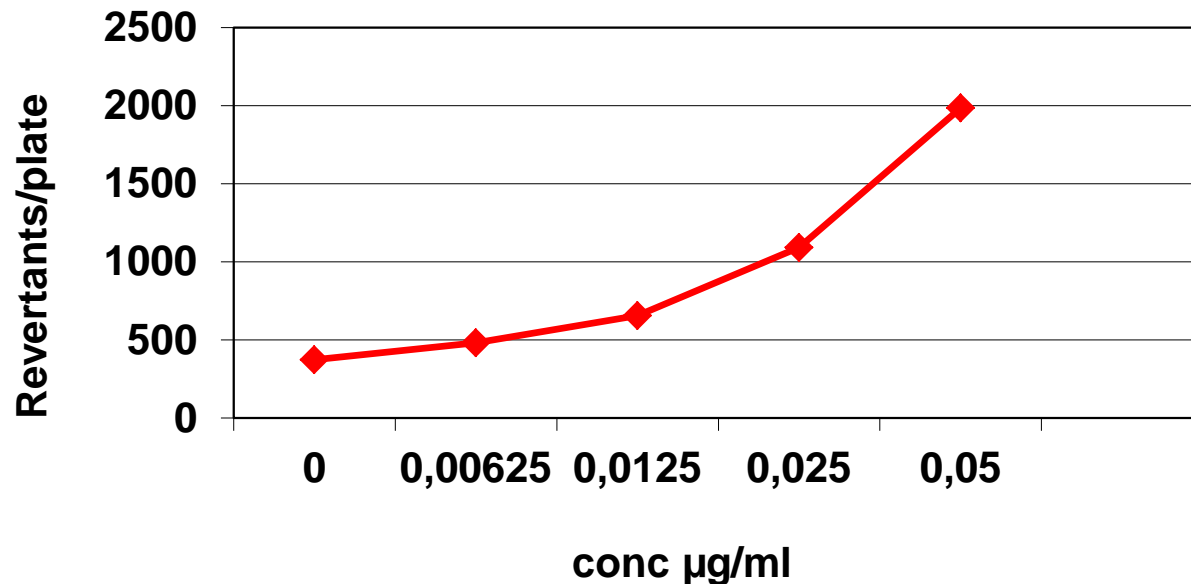
- Pharmacological (antibacterial) activity is inhibition of bacterial gyrase and topo IV inhibitor
- MoA for adverse genotoxic reactions in mammals is considered to be the inhibition of mammalian topo II

# Genetic Toxicity Assessment 1

- Standard battery test data
  - AMES
  - In vitro chromosome damage/MLA
  - In vivo chromosome damage/MN

# Mutations in *Salmonella typh* TA102 by Ciprofloxacin

## Revertants in TA 102

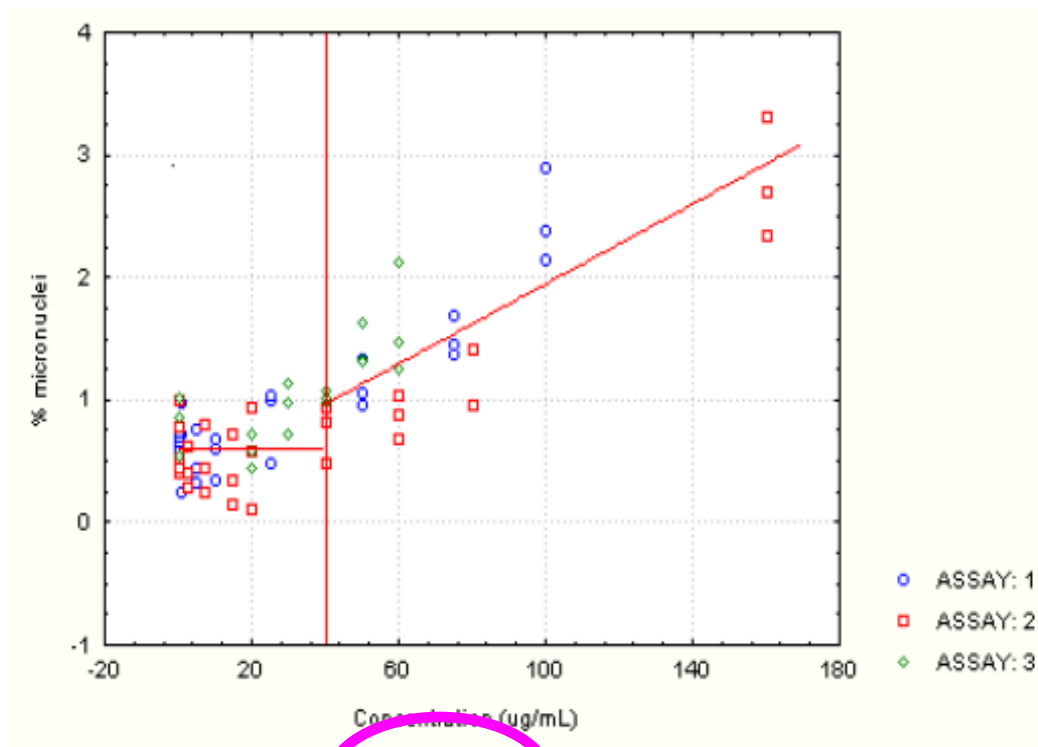


Data from Clerch et al. 1996, *Env Mol Mutag* 27:110-115

# Ciprofloxacin MN induction in vitro

Lynch et al. 2003, Mutagenesis 18:345-353

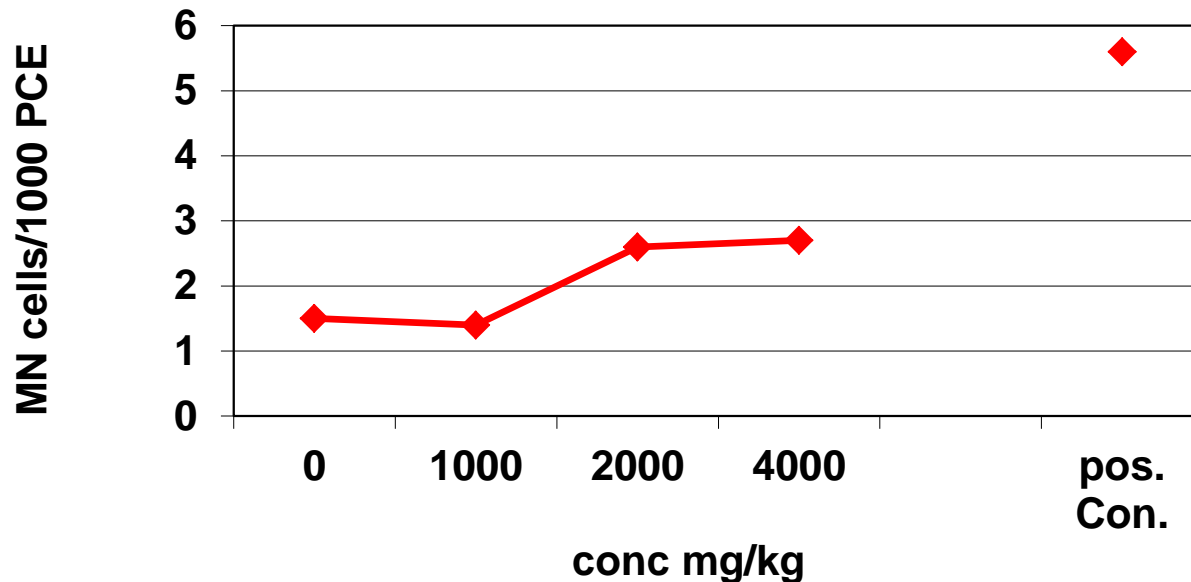
## Micronucleus Test in Mouse Lymphoma Cells



The breakpoint was identified at conc.=40 ug/mL with %MN=0.636 fitted before the breakpoint and %MN=0.392+0.016\*conc. fitted afterward.

# MN in vivo in NMRI mice in PCEs by Ciprofloxacin

## Micronuclei formation



Data from Herbolt et al. 2001, Mut Res 498:193-205



# Standard battery result for ciprofloxacin

The standard battery result

bacterial mutagenicity	positive
MN in vitro mammalian cells	positive
MN in vivo rodent BM	negative

Standard battery positive  
Knowledge on Mode of Action  
nessecary for risk assessment

# Mode of Action determination

- We need data to demonstrate
  - The lack of direct DNA reactivity
  - The mode of action leading to mutations
  - The target molecule/enzyme
  - The target molecule to be the key driver of mutation induction
  - The dose response to demonstrate a PoD (threshold)

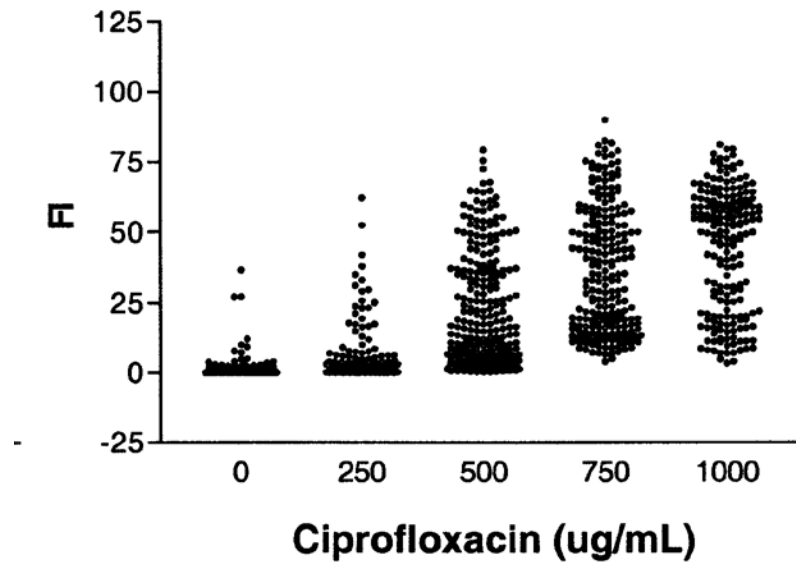
# Selectivity on target enzymes

## Minimal Concentration to induce DNA-strandbreaks (cell-free assay)

Fluoroquinolone	CC <sub>10</sub> (µg/ml)		Factor
	Gyrase	Topoisomerase II	
<b>Pradofloxacin*</b>	<b>0.005</b>	<b>4</b>	<b>800</b>
<b>Enrofloxacin*</b>	<b>0.005</b>	<b>6</b>	<b>1200</b>
<b>Marbofloxacin*</b>	<b>0.005</b>	<b>10</b>	<b>2000</b>
<b>Danofloxacin*</b>	<b>0.005</b>	<b>25</b>	<b>5000</b>
<b>Orbifloxacin*</b>	<b>0.005</b>	<b>25</b>	<b>5000</b>
<b>Ciprofloxacin*</b>	<b>0.005</b>	<b>10</b>	<b>2000</b>
<b>Moxifloxacin*</b>	<b>0.01</b>	<b>6</b>	<b>600</b>
<b>Gemifloxacin*</b>	<b>0.01</b>	<b>1</b>	<b>100</b>

\* Data from Koerber-Irrgang B, Dissertation, University Hamburg 2005

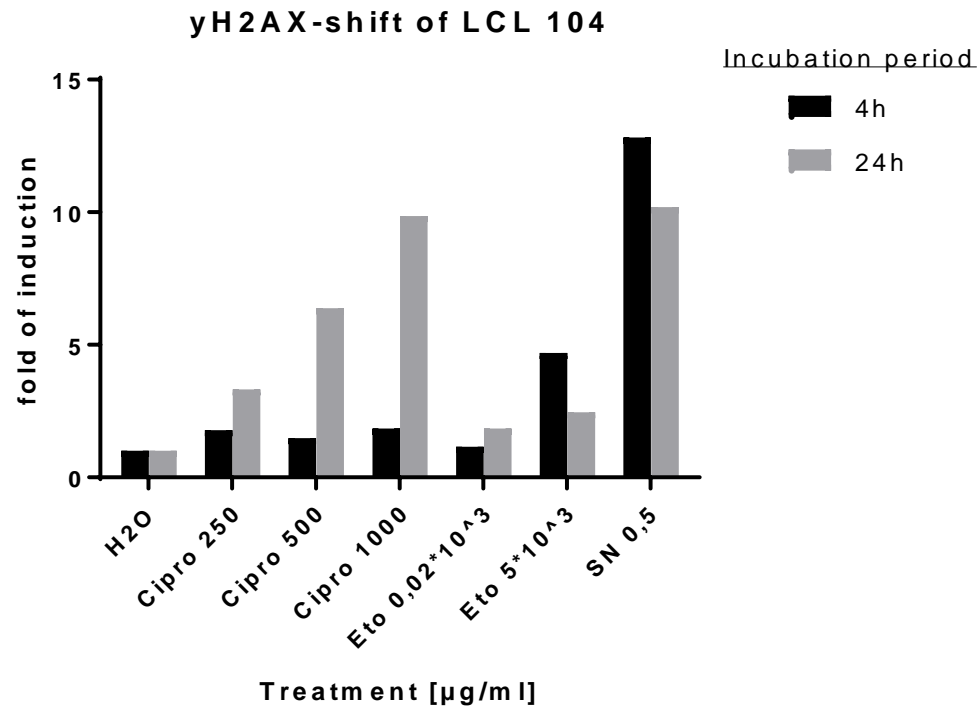
# Specific functional complex induction



**Fig. 1.** TARDIS assay results in L5178Y mouse lymphoma cells. (A) Concentration-dependent increase in SCC formation with etoposide. (B) Time-dependent reversal of SCC formation following removal of etoposide (60  $\mu\text{g}/\text{ml}$ ). (C) SCC formation by known mammalian topoisomerase II poisons [doxorubicin (3  $\mu\text{g}/\text{ml}$ ), genistein (27  $\mu\text{g}/\text{ml}$ ), mitoxantrone (2.6  $\mu\text{g}/\text{ml}$ ) and ellipticine (1.2  $\mu\text{g}/\text{ml}$ )] and two mammalian non-topoisomerase II poisons [aclerubicin (2  $\mu\text{g}/\text{ml}$ ) and

Lynch et al. 2003, *Mutagenesis* 18:345-353

# Induction of $\gamma$ H2AX as measurement for DSB

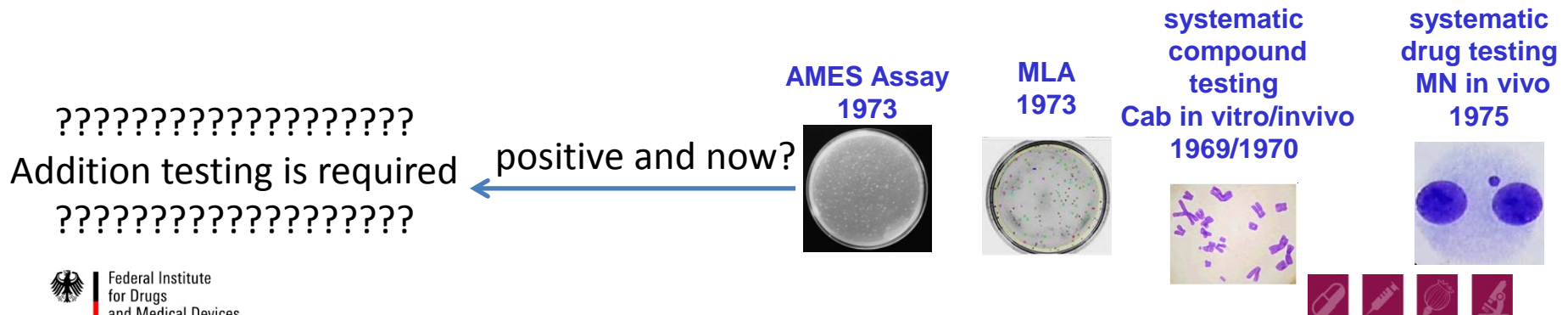


# Summary of ciprofloxacin

- Biochemical target is not DNA
- Huge difference between effect on bacterial and mammalian target enzyme
- Dose response of all assays show effects at high doses only
  
- Sufficient difference between human exposure and rodent exposure at doses with no genotoxic effect

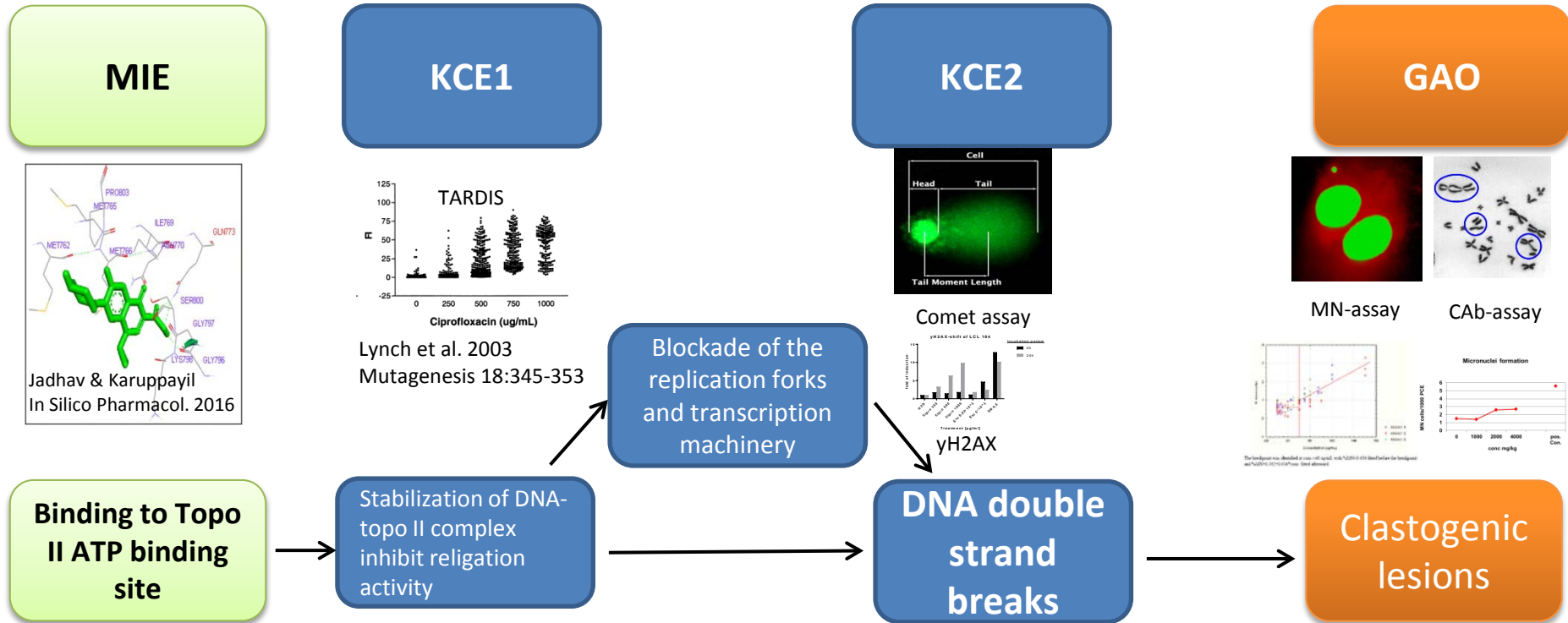
# Adverse outcome pathways – AOP – moving forward in MoA determination

- The MIE is the start of the an AOP
  - Knowledge of the dose response of the MIE
- The key events in the AOP
  - The more of the key events we know following the MIE
  - The more precise we may be to determine the complete dose response relationship between exposure and apical outcome
- This however is not possible with classical standard test systems:

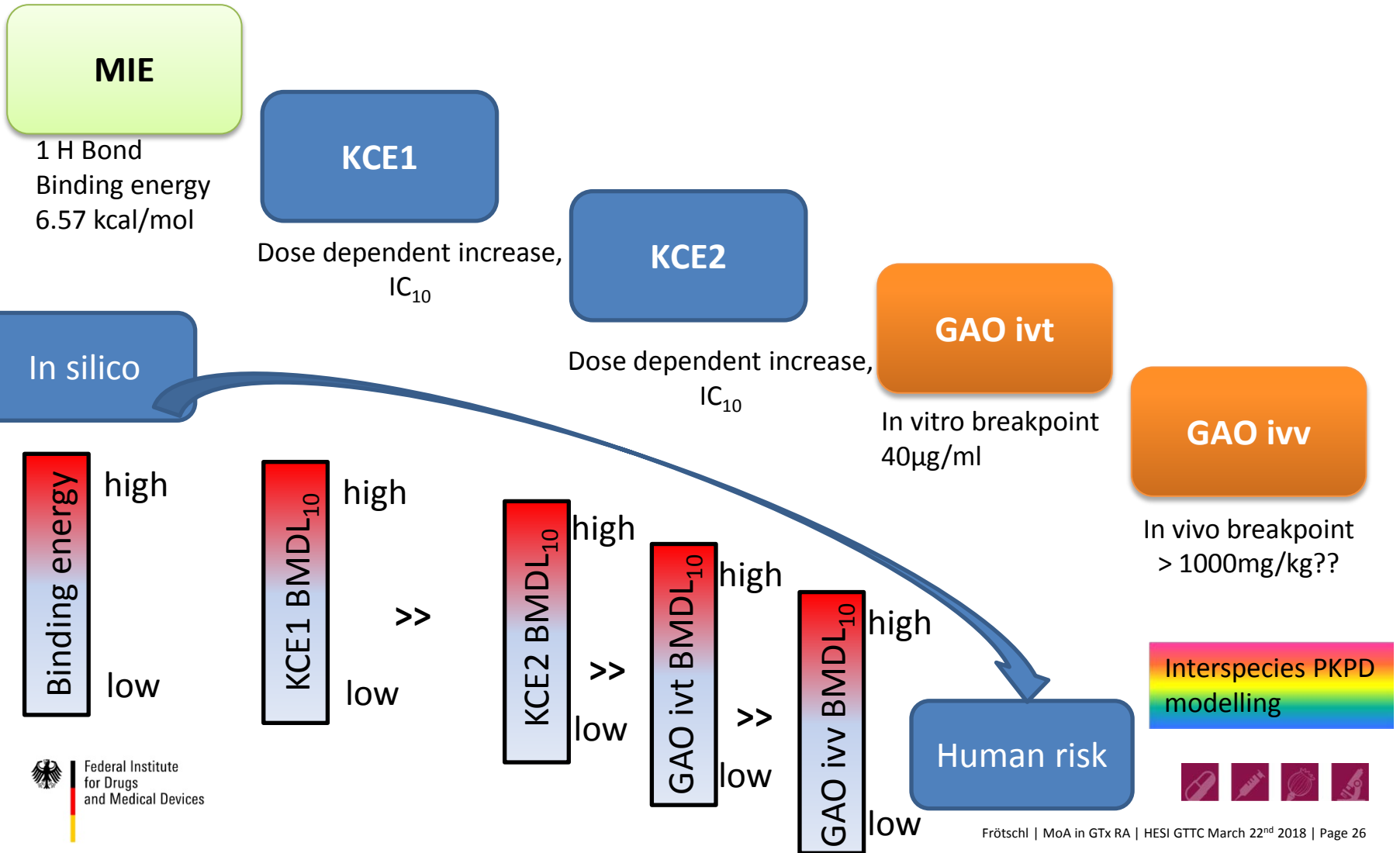




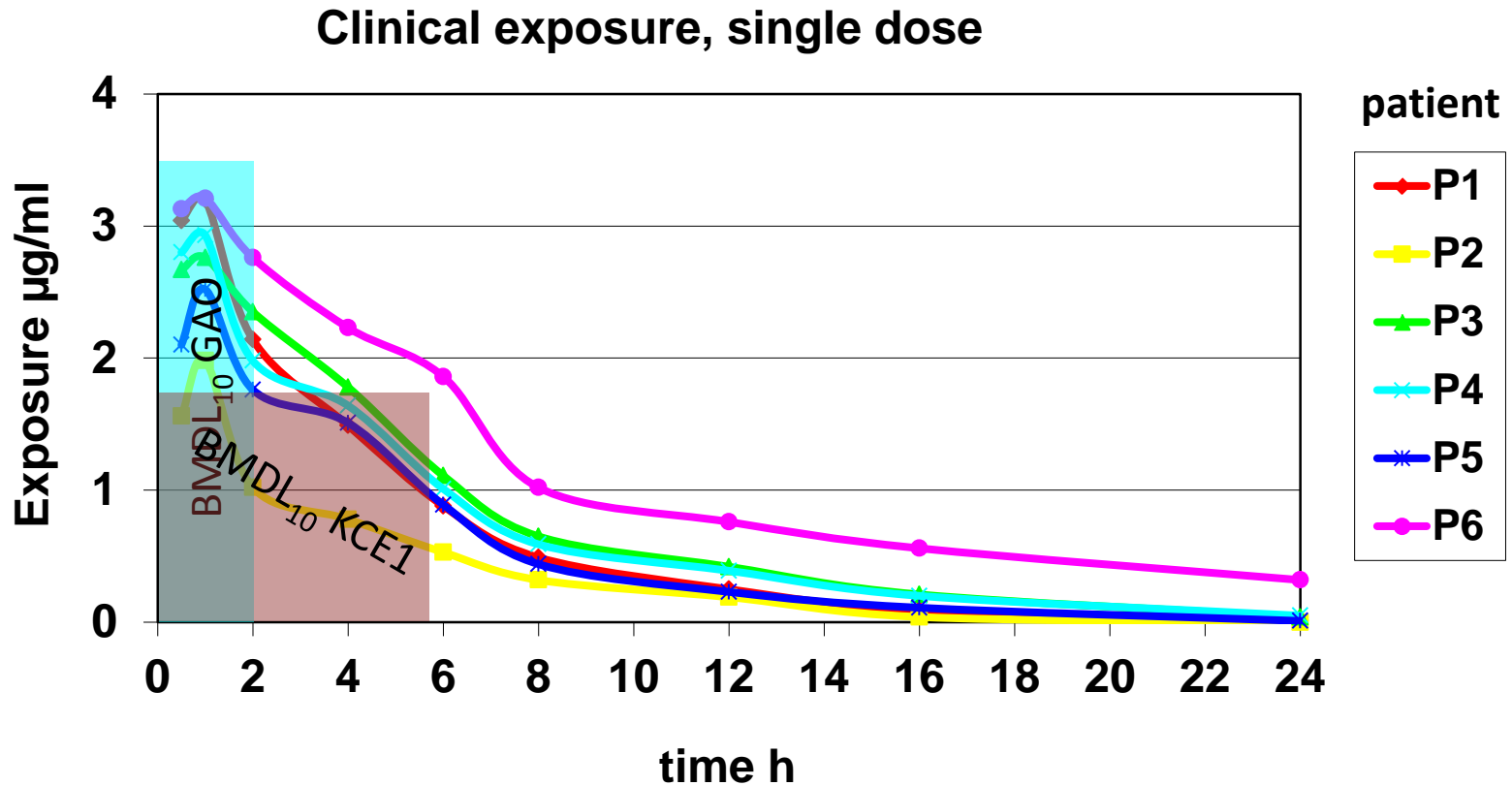
# Potential AOP for catalytic topo II inhibition



# Dose response relationship between key events



# Risk assessment using dose response of AOP



Acceptability of this will depend on the level of uncertainty in the model

# Conclusions

- Mode of action determination is crucial for genetic toxicity assessment of compounds positive in the standard battery
- Sufficient data on dose response relationship of genetic toxicity endpoints is needed for margin of exposure determination and acceptability of risk
- More informed MoA determination would potentially improve genetic toxicity assessment and also improve risk assessment
- AOPs for genetic toxicity pathways will provide guidance for MoA determination and help to improve experimental strategies in genetic toxicity testing
- Quantitative data on MIE and KCE in AOPs will support approaches in quantitative risk assessment in genetic toxicity

# Thank you very much for your attention!

## Contact

Federal Institute for Drugs and Medical Devices  
Licencing Division 2  
Genetic and Reproductive Toxicology  
Kurt-Georg-Kiesinger-Allee 3  
D-53175 Bonn

Contact person  
Dr. Roland Frötschl  
Roland.froetschl@bfarm.de  
www.bfarm.de  
Tel. +49 (0)228 99 307-3441  
Fax +49 (0)228 99 307-5599

