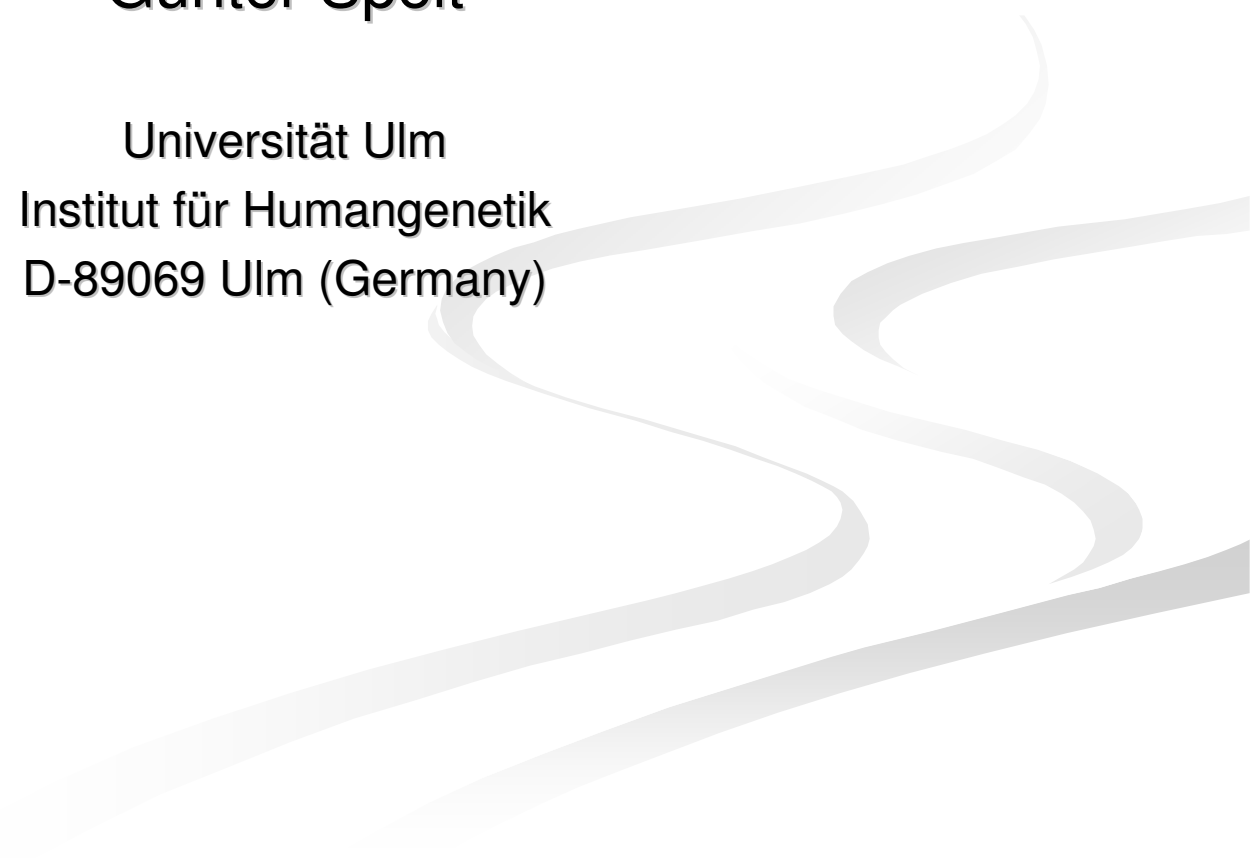


Classification of Carcinogens: Aspects of the „Mode of Action“ for genotoxic substances

Günter Speit

Universität Ulm
Institut für Humangenetik
D-89069 Ulm (Germany)

A decorative graphic consisting of several overlapping, wavy, light gray lines that flow from the right side of the slide towards the left, positioned below the contact information.

“Genotoxic carcinogens” are mutagenic carcinogens

- Mutations are the critical key events for the induction of cancer by “genotoxic carcinogens”.
- Mutations are the critical biomarkers for cancer risk assessment for “genotoxic carcinogens”.
- Further events are required beyond the induction of mutations in cancer-related genes for the evolution of cancer.

Use of genotoxicity data for the classification of carcinogens

Integration of all available information

into a weight of evidence (WoE) analysis to conclude:

- I. Is the substance a mutagen?
- II. Is carcinogenesis mediated via a mutagenic mode of action (MoA)?

Evaluation of mutagens: present situation

- Results from **various tests** have to be considered.
- **Quality of the studies** and plausibility of the results have to be assessed.
- **Hazard identification** is mainly based on animal tests.
- Quantitative aspects are usually not considered.

Evaluation of carcinogens: MAK

- Categories 1 and 2: Substances which are carcinogenic in man or in experimental animals (cause cancer by a MoA that is relevant to man).
 - Category 3: Suspected carcinogens; MAK or BAT values only if **not genotoxic**.
 - Category 4: Substances with **non-genotoxic mechanisms**.
 - Category 5: Genotoxic carcinogens of **weak potency**.
- Specific “genotoxic MoAs“ are not considered.

Evaluation of mutagens: “Irrelevant positive“ *in vitro* test results

**An *in vitro* effect may not occur in humans,
because of:**

- induction of damage by a process that is specific to *in vitro* conditions;
- induction of primary damage to a non-DNA target, critical target dose is not reached *in vivo*;
- induction of direct DNA damage by a mechanism with a threshold.

Ref.: Kirkland et al., Mutagenesis 22,161-175 (2007)

Genotoxic MoAs in vitro due to overload of normal cellular physiology

Table I. Summary of indirect, non-relevant or threshold mechanisms of genotoxicity

MoA	Description	<i>In vitro</i> systems affected
<i>In vitro</i> specific	Rat S9-specific or enhanced effects ^b	All (except primary hepatocytes)
	Feeding effects (e.g. histidine) ^b	Bacteria
	Bacterial-specific metabolism (e.g. sodium azide) ^b	Bacteria
Direct DNA effect but with a threshold ^a	Cell-specific metabolism ^c	Mammalian cells
	Inadequate detoxification ^b	All
	DNA repair deficiency ^c	All
Non-DNA ^a	Metabolic overload (including production of ROS, lipid peroxidation, sulphhydryl depletion) ^b	Mammalian cells
	Azo- and nitroreduction ^b	Bacteria
	Inhibition of DNA polymerases, topoisomerases or kinases ^b	Mammalian cells
	Imbalance of DNA precursors ^b	Mammalian cells
	Energy depletion ^c	Mammalian cells
	Nuclease release from lysosomes ^c	Mammalian cells
	Inhibition of protein synthesis ^c	Mammalian cells
	Protein denaturation ^c	Mammalian cells
	Aneuploidy ^b	Mammalian cells
High cytotoxicity ^b	Mammalian cells	

^aThe majority of mechanisms shown in this category can be encompassed by the description 'overload of normal cellular physiology'.

^bPublished data suggest reasonable opportunity to obtain experimental evidence to support mechanism.

^cPublished data suggest difficult prospect to obtain experimental evidence to support mechanism.

Ref.: Kirkland et al., *Mutagenesis* 22,161-175 (2007)

Evaluation of mutagens: dose-response

- The basic assumption is that mutagens have a non-threshold mode of action.
- Thresholds may arise from the type of reaction with the genetic material or by toxicokinetic properties.
- When a threshold mode of action is plausible, safe levels may be derived.

Ref.: Toxicology and Risk Assessment, H. Greim & R. Snyder (eds.)
Wiley, 2008

Plausibility of a threshold mode of action

- **Threshold mode of action** for a chemical is plausible when a substance with a known mutagenic potential does not induce mutations at low concentrations due to a specific type of reaction with the genetic material and / or physiological protective mechanisms.

→ **Information on dose-response and MoA is required!**

Protective mechanisms leading to a threshold MoA for mutagens

Exposure of cells / tissues



Induction of **DNA damage**

↓ Replication

Fixation of **mutations**

pre-lesion protection by:

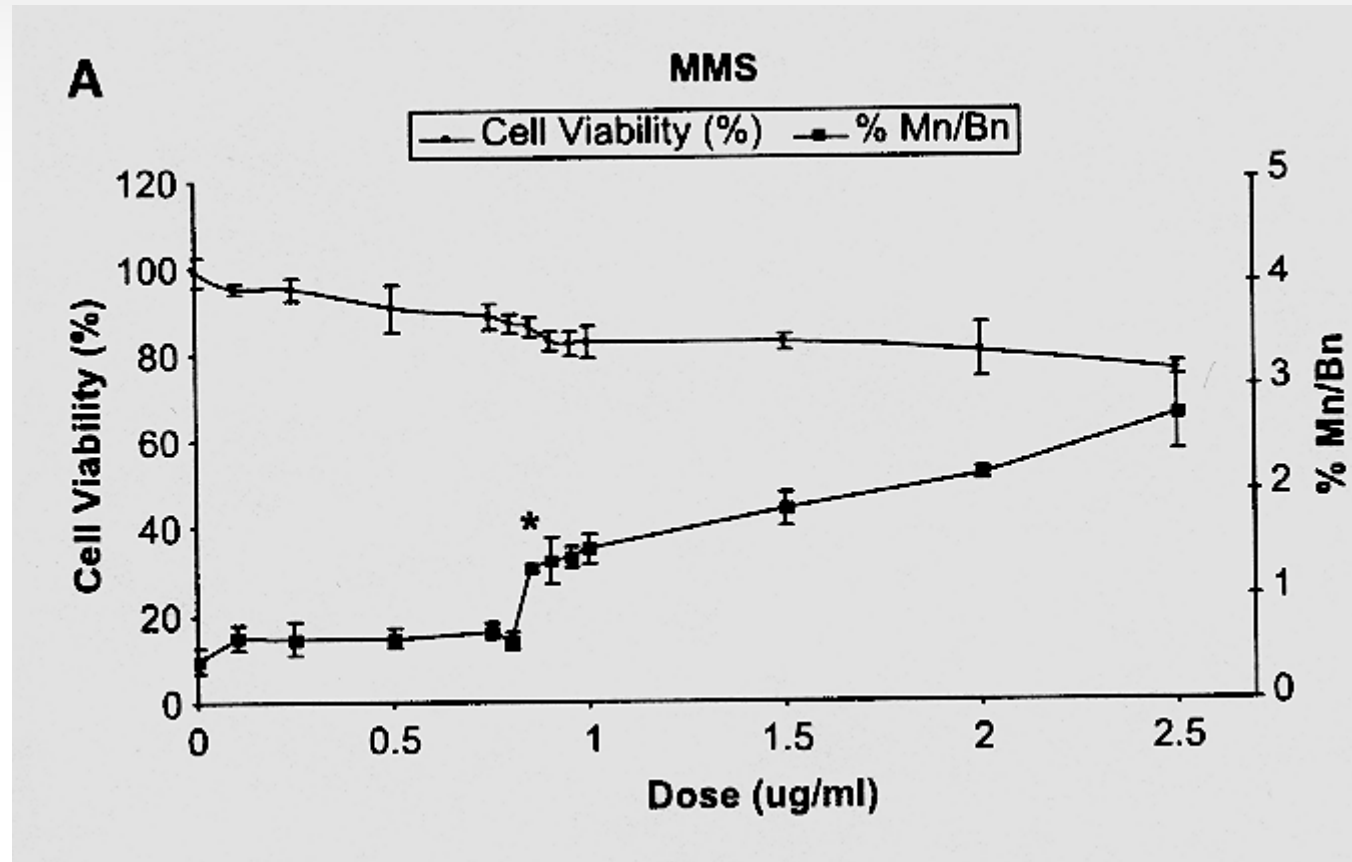
- Proteins, membranes
- Metabolic inactivation

post-lesion protection by:

- DNA repair

Ref.: Speit et al., Mutat. Res. 464,149 (2000)

Induction of micronuclei in AHH-1 cells by MMS: LOEL due to homeostatic maintenance by DNA repair?



Doak et al., Cancer Res. 67,3904 (2007)

Different dose-response relationships for DNA adducts and mutations

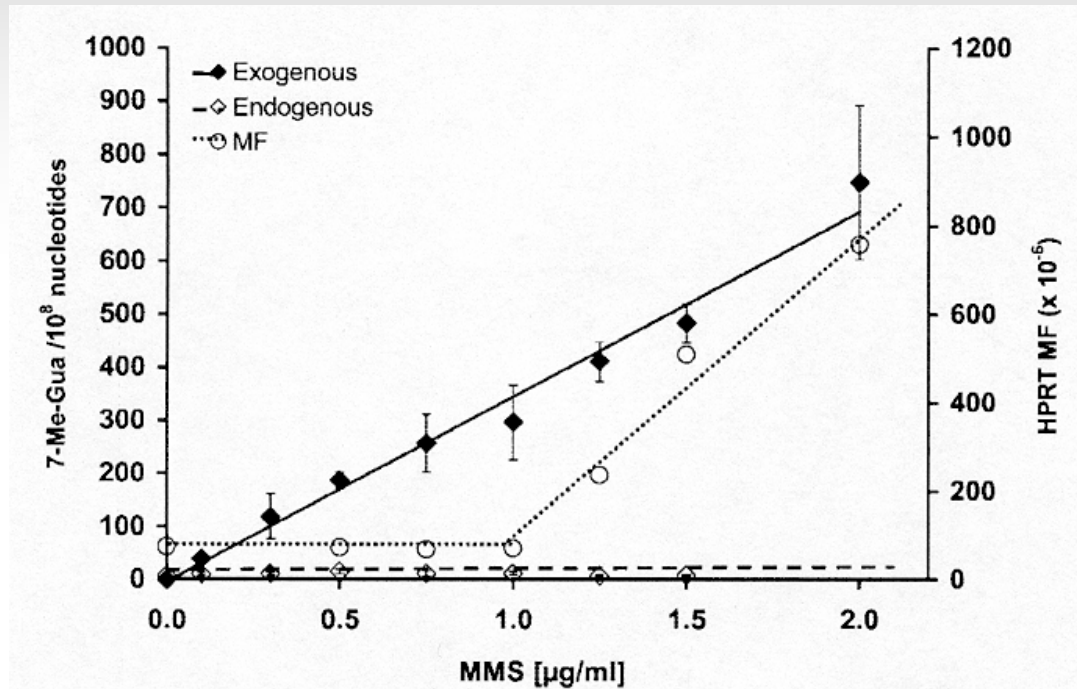


Figure 8. Comparison of N7-methyl guanine DNA adducts and HPRT mutations in AHH-1 cells exposed to MMS for 24 h. The endogenous adducts are N-7Me-G (\diamond), while the exogenous adducts are [$^{13}\text{C}^2\text{H}_3$]-7Me-G (\blacklozenge). The Hprt mutant frequency is shown as \circ (110).

Swenberg et al., Chem. Res. Toxicol. 21,253 (2008)

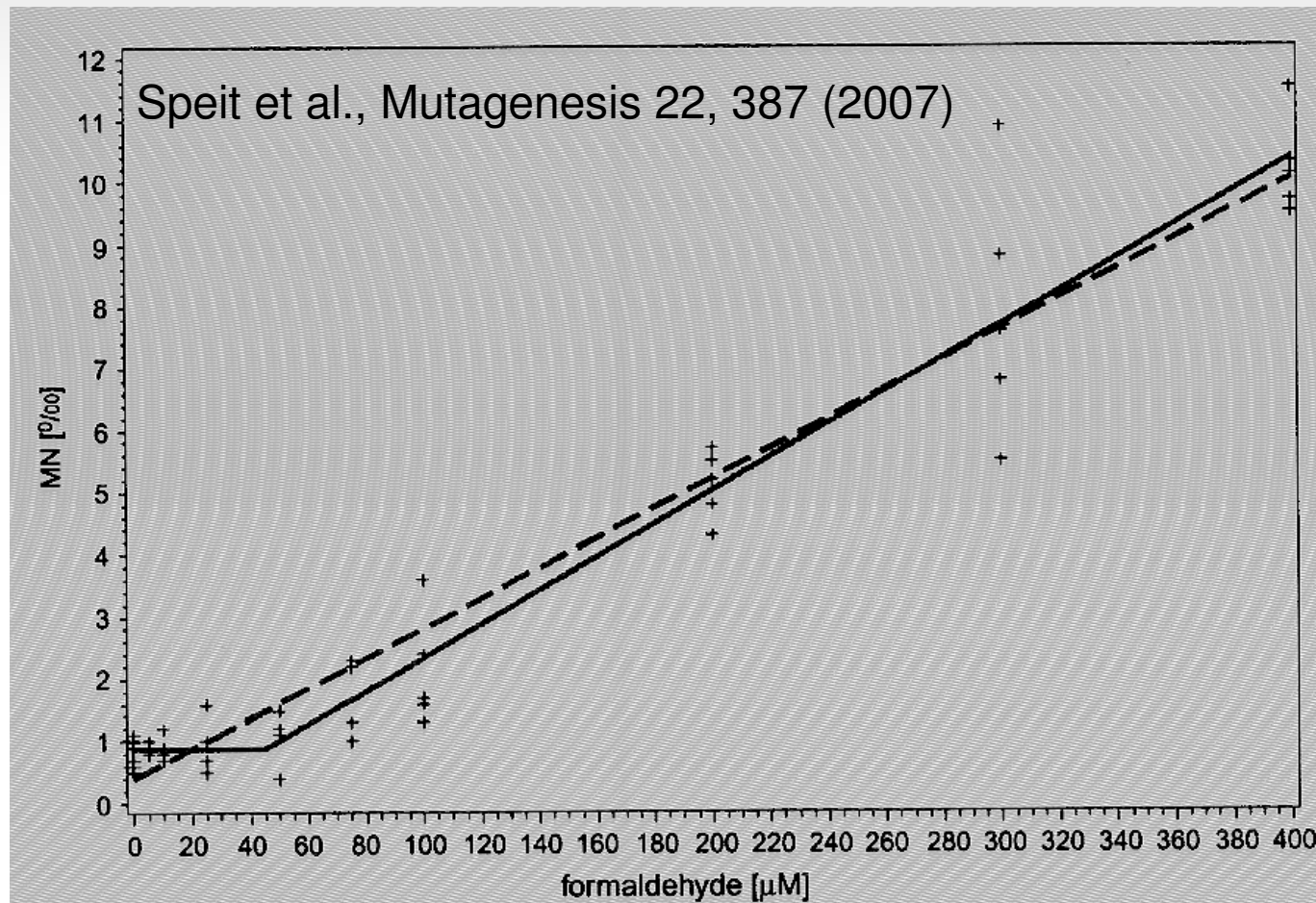
Formaldehyde: Induction of genotoxic and mutagenic effects in cultured mammalian cells:

- DNA-Protein-Crosslinks (DPX)
- Sister Chromatid Exchanges (SCE)
- Micronuclei (MN)
- Chromosome Aberrations (CAb)
- “Gene Mutations“ (MLA; small colonies)
- ➔ **Predominantly clastogenic mode of action**

Why is formaldehyde a good candidate for a mutagen with a threshold MoA?

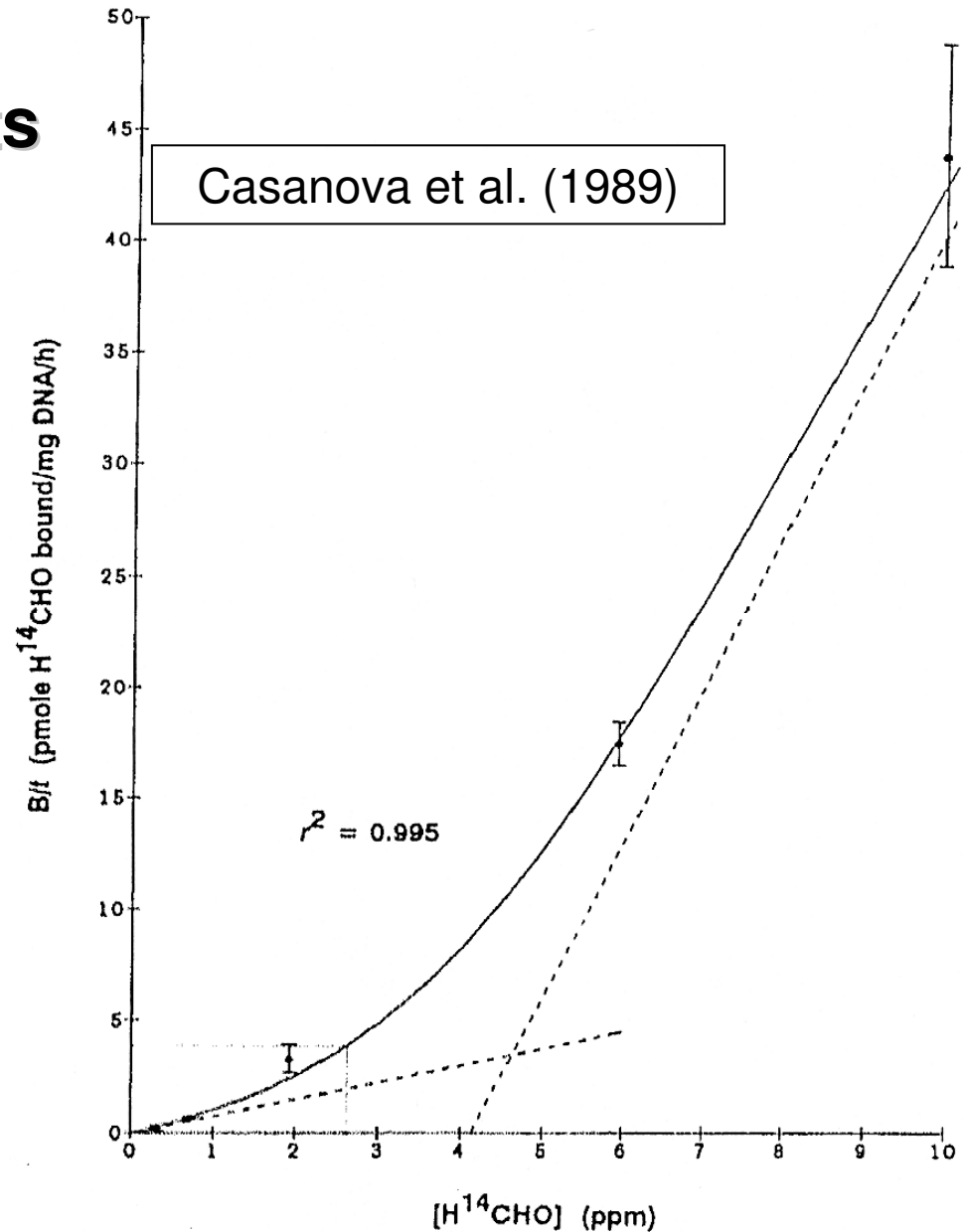
- Naturally occurring substance
(high endogenous FA levels;
background levels of DPX)
- High reactivity
- Rapid metabolic inactivation
- Efficient repair of primary DNA damage

Induction of micronuclei in V79 cells by formaldehyde: A two-phase regression model gives “the best fit”.



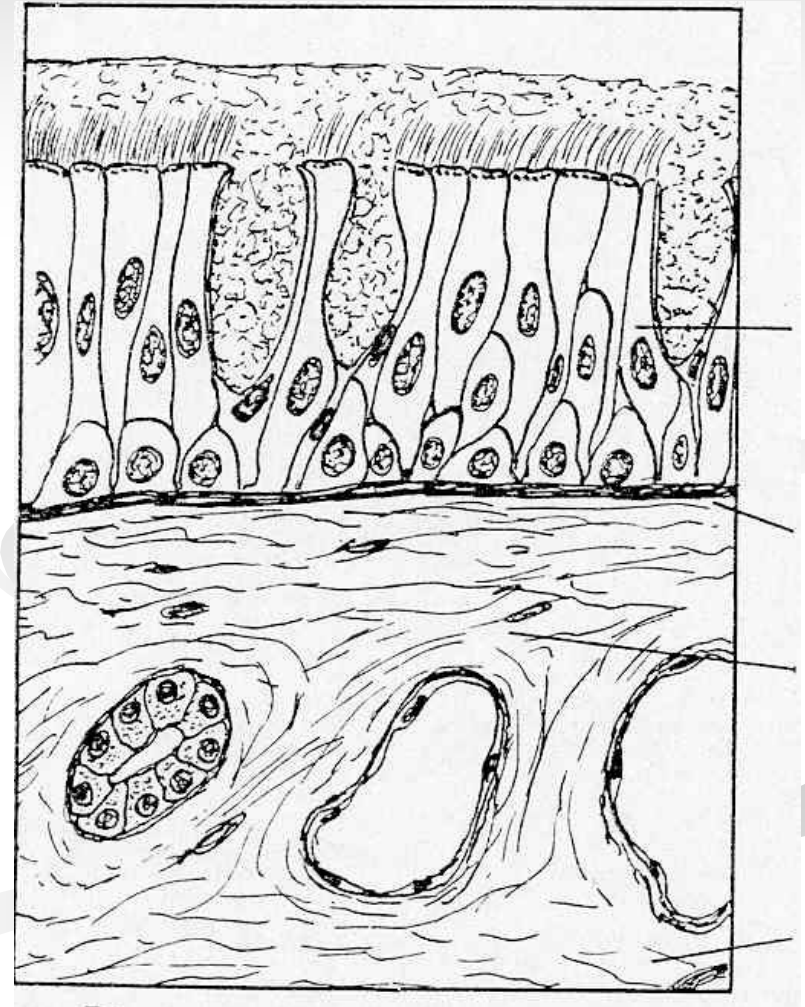
Induction of DPX by FA in the nasal mucosa of rats

- DPX are induced at all concentrations (0.3 – 10 ppm).
- The concentration-response curve is bi-phasic.
- No indication of a threshold for the genotoxic action of FA in vivo!



Does formaldehyde induce mutations in tumor relevant tissues / cells?

- **DPX** are induced in all cell types of the nasal mucosa.
- **Mutations may be induced:**
 - when DPX are induced in basal cells
 - when DPX escape repair
 - when damaged basal cells proliferate
- ➔ Different dose-response for the induction of DPX and mutations *in vivo*?
- ➔ No data is available for the induction of mutations!



Transcellular transmission of FA: Co-cultivation experiments

A549 human lung cells were exposed to FA for 1h and then co-cultivated with V79 cells in the presence of BrdU

- a) Without change of medium
- b) With change of medium

→ Evaluation of SCEs in V79 cells

Neuss & Speit, Mutagenesis 23,355-357 (2008)



Co-cultivation experiments indicate that FA is not passed on from one cell to another

Without change of medium

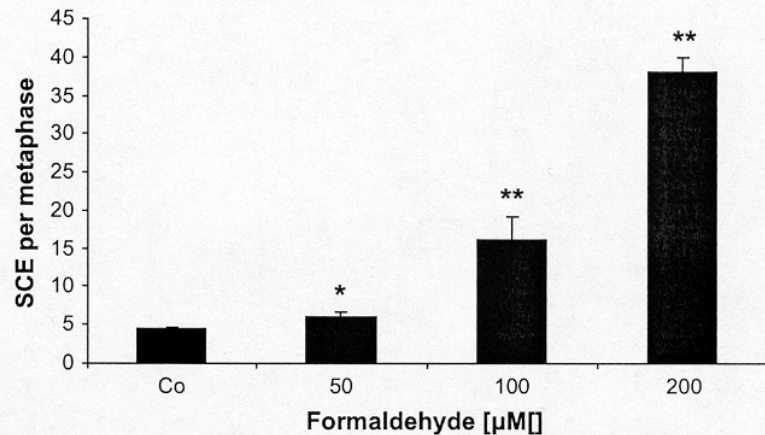


Fig. 3. Induction of SCE in V79 cells after co-cultivation with A549 cells which were treated with FA for 1 h. V79 cells and BrdUrd were added to the treated A549 cells and co-cultivated for two cell cycles. Mean \pm standard deviation of three independent tests; * $P < 0.05$ and ** $P < 0.01$.

With change of medium

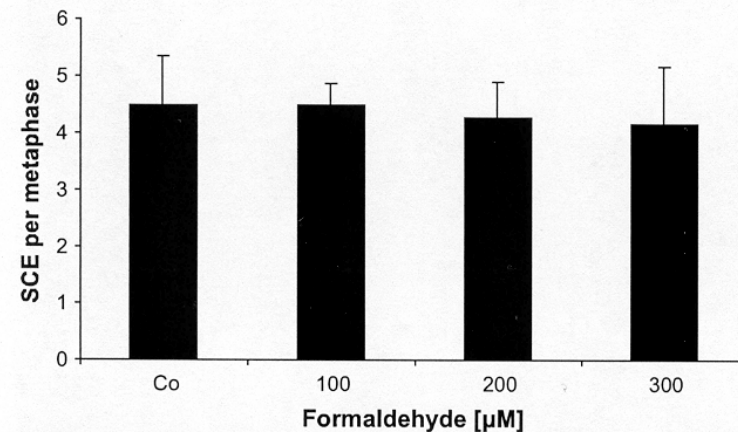


Fig. 4. Induction of SCE in V79 cells after co-cultivation with A549 cells which were treated with FA for 1 h with FA. V79 cells and BrdUrd-containing medium were added to the treated A549 cells after washing the exposed cells and co-cultivated for two cell cycles. Mean \pm standard deviation of three independent tests.

Neuss & Speit, Mutagenesis 23,355-357 (2008)

**Local mutagenicity of FA in humans:
Micronucleus test in buccal cells of volunteers
exposed to FA under strictly controlled conditions**

- study performed under GLP-like conditions
- healthy non-smokers (defined exclusion criteria)
- defined exposure over 10 consecutive working days
- 4 h exposure with peak levels up to 1 ppm
- bicycle exercises during exposure (3 x 15 min)
- several sampling times (up to 3 weeks after exposure)

Time course of exposure towards formaldehyde of the five study groups

Table 2
Time course of exposure towards formaldehyde (ppm) of the five study groups

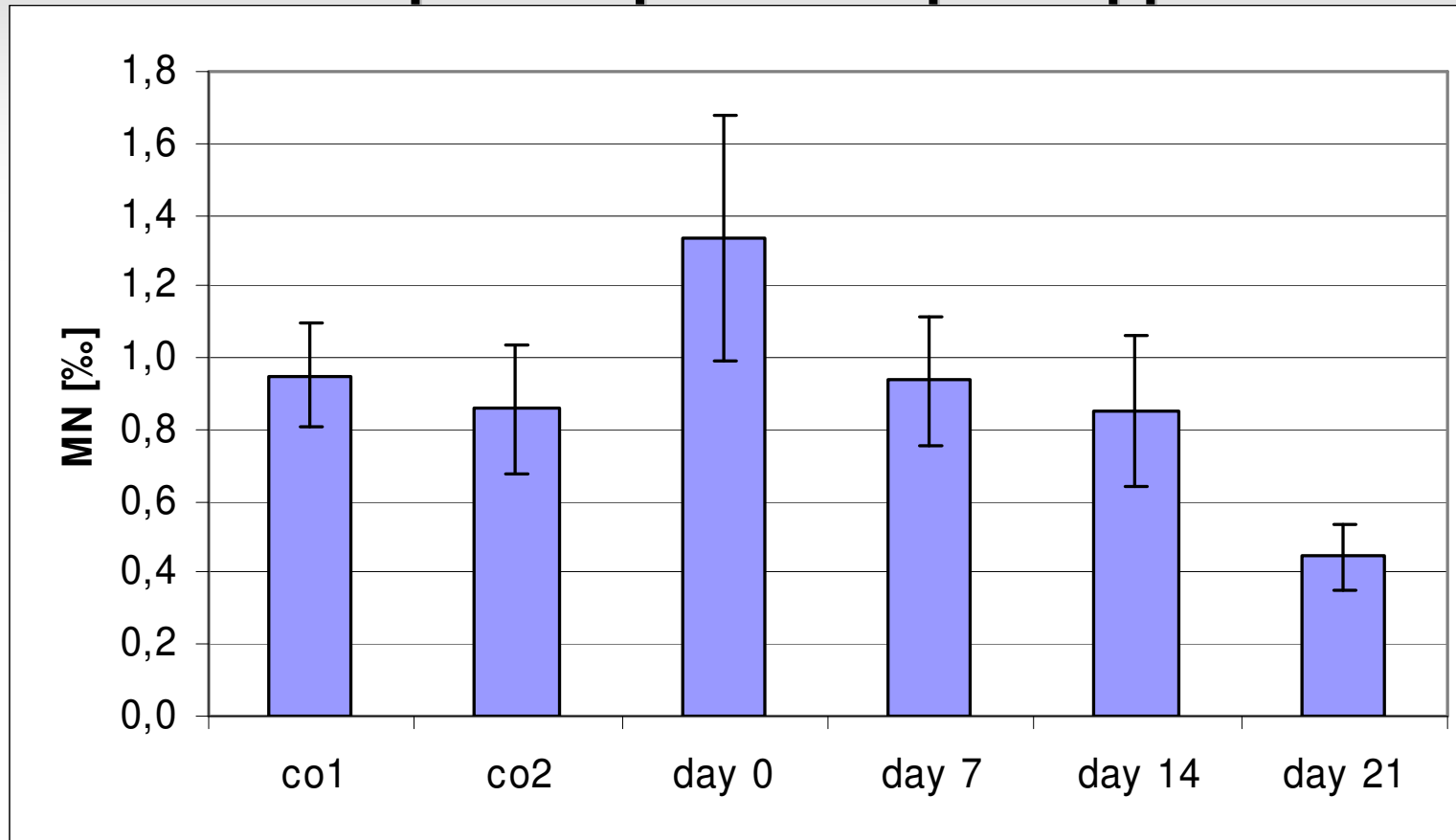
	Group 1	Group 2	Group 3	Group 4	Group 5
Day 1	0.5	0.15	0	0.3	0.15
Day 2	0.3	0	0.3+4 × 0.6	0+EA	0.3+EA
Day 3	0.15	0.3+4 × 0.6	0.3	0.15	0.3+4 × 0.6
Day 4	0.3+EA ^a	0.5	0.5+4 × 1.0	0.5+4 × 1.0	0+EA
Day 5	0.5+4 × 1.0 ^b +EA	0+EA	0.5	0.5+EA	0.3
Day 6	0	0.5+4 × 1.0+EA	0.3+EA	0.5+4 × 1.0+EA	0
Day 7	0.5+EA	0.3	0.5+4 × 1.0	0.5	0.5+EA
Day 8	0+EA	0.5+4 × 1.0	0+EA	0.3+4 × 0.6	0.5+4 × 1.0+EA
Day 9	0.5+4 × 1.0	0.5+EA	0.5+EA	0	0.5+4 × 1.0
Day 10	0.3+4 × 0.6 ^c	0.3+EA	0.15	0.3+EA	0.5

^a Co-exposure to ethylacetate (10–20 ppm).

^b Four peaks of 1.0 ppm for 15 min each.

^c Four peaks of 0.6 ppm for 15 min each.

**Formaldehyde does not induce micronuclei
in buccal mucosa cells after exposure for 10 days
with peak exposures up to 1 ppm.**



Ref.: Speit et al., Mutation Res. 627, 129-135 (2007)

Summary

Genotoxic MoAs and classification of carcinogens

The following question should be considered:

- Does the substance have a clear mutagenic potential *in vivo*?
 - relevant endpoint(s) measured?
 - relevant target cells investigated?
 - effects related to toxicity / cytotoxicity?
- Is there sufficient evidence for a mechanism with a threshold?
 - type of reaction with the genetic material?
 - toxicokinetics?
 - dose-response relationship?
- Is there sufficient evidence that other mechanisms are more important than genotoxicity/mutagenicity?

Thank you!

**Vielen Dank
für Ihre Aufmerksamkeit!**

The bottom right portion of the slide features several thick, light gray wavy lines that curve and flow across the page, adding a decorative touch to the design.