Karlsruher Institut für Technologie

Risk assessment of genotoxic carcinogens in the low dose range

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Genotoxic/ carcinogenic substances: Background

In spite of manifold approaches to substitute carcinogens, there are **many carcinogens present in the environment, in food and at workplaces:**

- Combustion products
- Carcinogenic metal compounds
- Carcinogenic chemicals
- Natural bioactive food ingredients
- Substances generated during storage and preparation of food (mycotoxins, acrylamide, nitrosamines, heterocyclic aromatic amines, benzo[*a*]pyrene....)

Genotoxic/ carcinogenic substances: Background

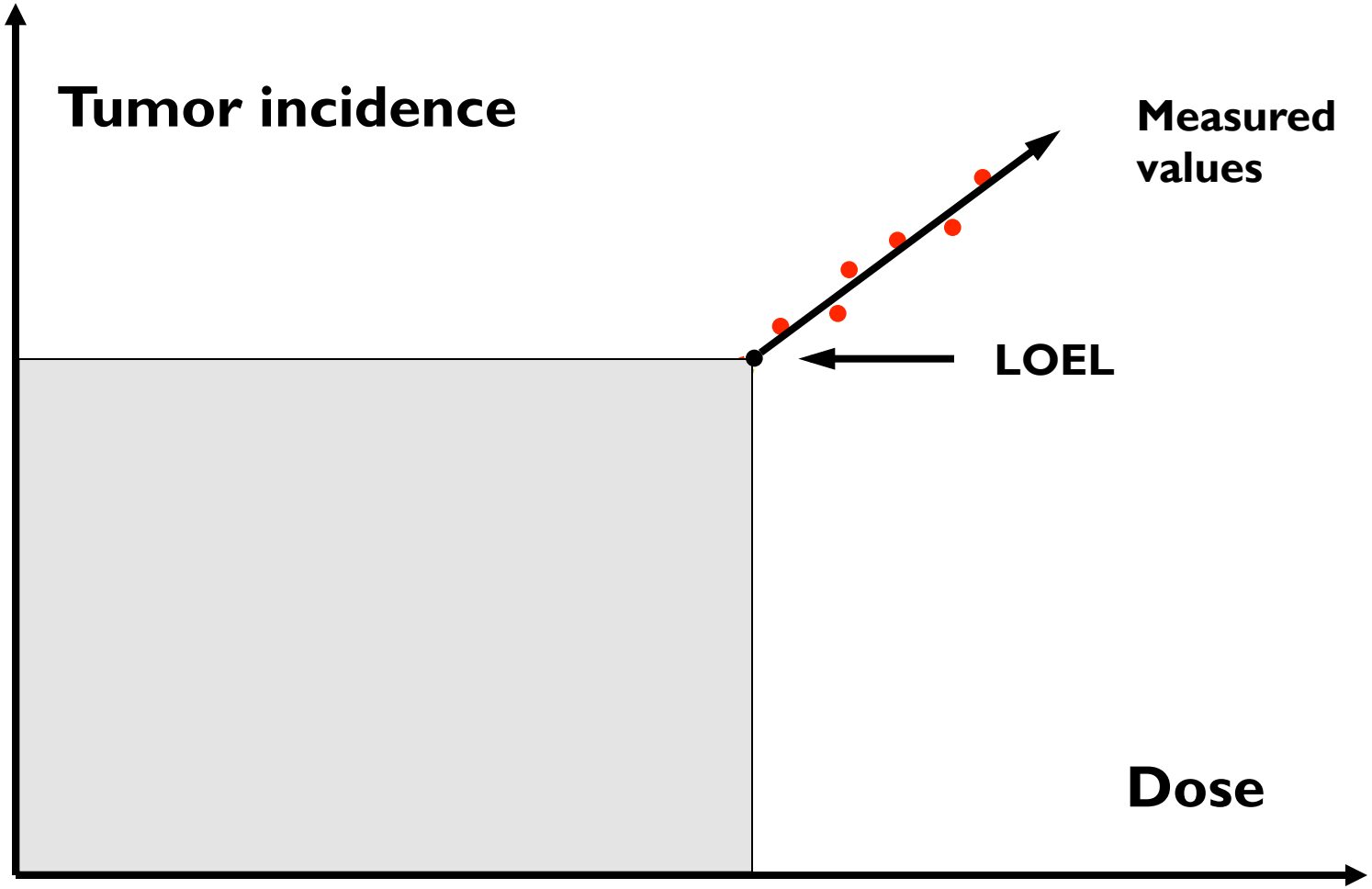
Important question:

- Is there a **carcinogenic potential relevant under realistic exposure conditions**?
- What are the **underlying mechanisms** involved?
- Is it **possible to define threshold values which protect from carcinogenicity?**

Hazard identification vs. risk estimation

Health based exposure limits for carcinogens at workplaces also required by the german legislation („Gefahrstoffverordnung“)

The problem of dose-response-relationships in case of carcinogenic compounds…



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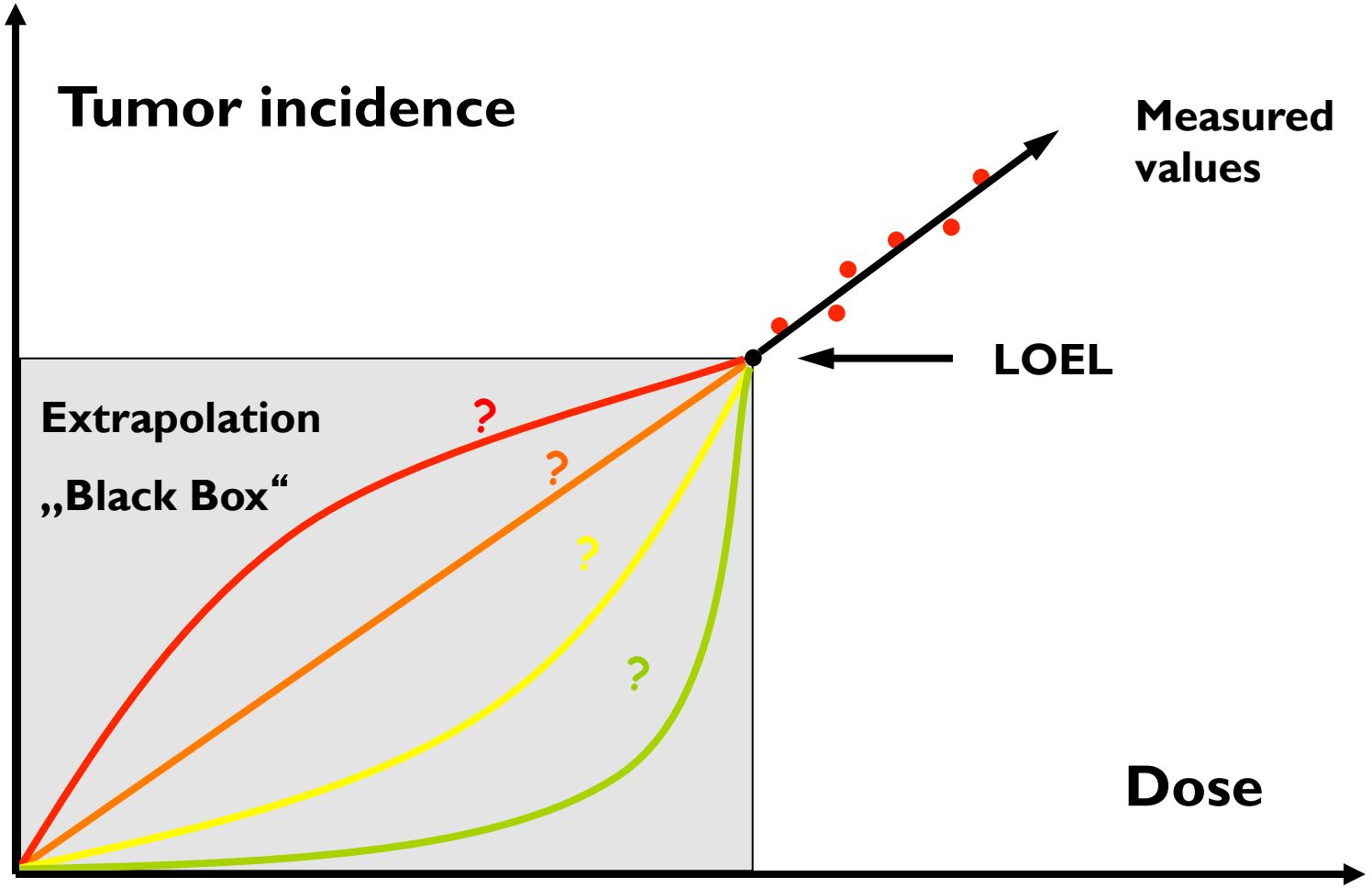

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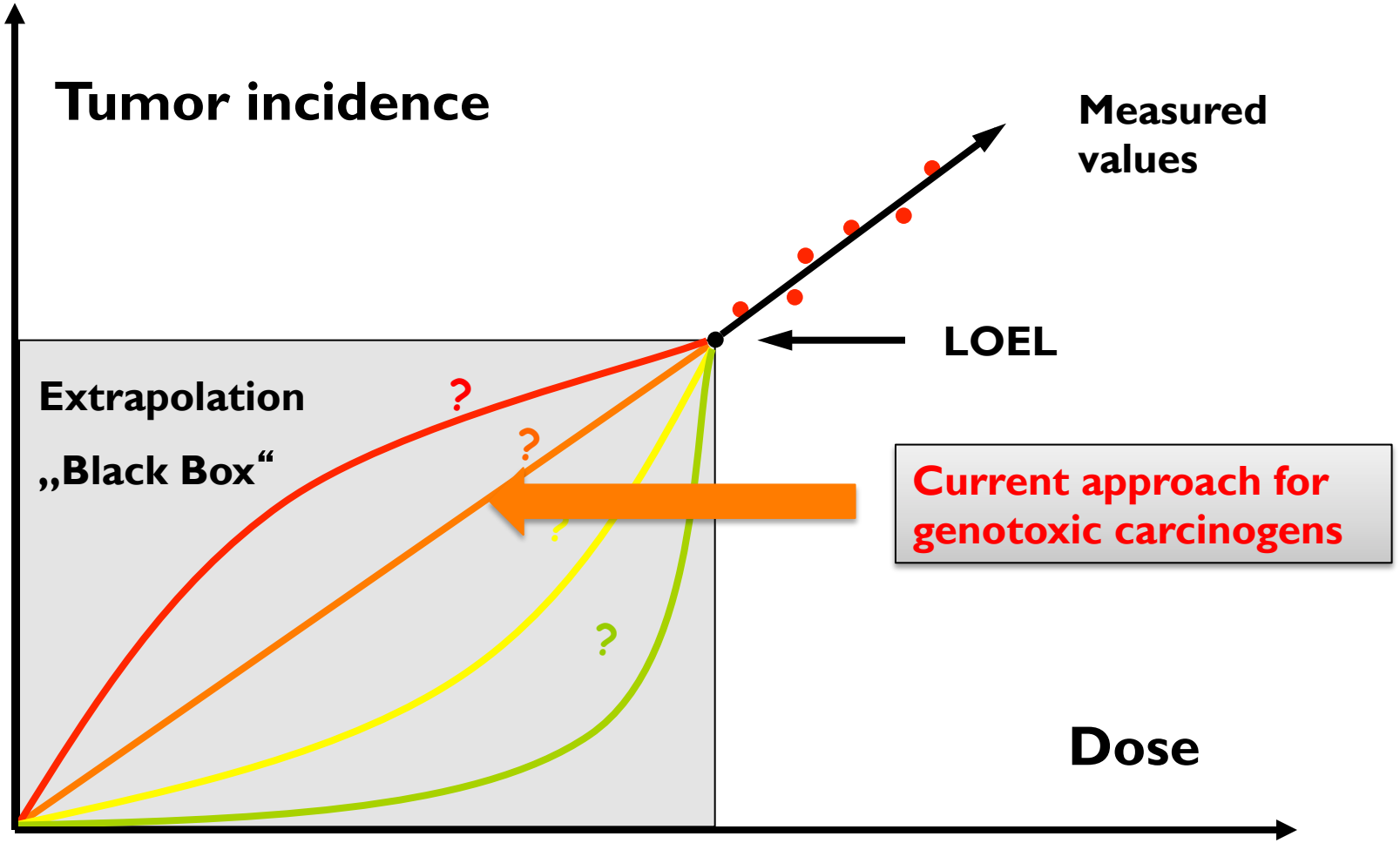


Current approaches for genotoxic carcinogens in food

Threshold of Toxicological Concern (TTC)

- Originally established for chemicals present in food, but also applied for food contact materials, food flavouring, impurities in pharmaceuticals
- Pragmatic approach for compounds of unknown toxicity
- For compounds with structural alerts for genotoxicity: TTC of 0.15 µg/person/day
- Based on rodent carcinogenicity data, for most genotoxic chemicals in food estimated risk below 1 in 1 million
- Exclusion of aflatoxin-like, azoxy or N-nitroso-compounds (higher risk at this concentration) as well as metals and some other classes of compounds

Current approaches for genotoxic carcinogens in food

ALARA („As Low As Reasonably Achievable“)

- Intends to keep the exposure to carcinogenic substances at the lowest achievable level according to technological or economical considerations
- Based on hazard identification

However, this approach does not take into account

- Carcinogenic potency
- Mode of action
- Exposure levels

→ Not useful for risk comparison

→ Not useful for priority setting

Current approaches for genotoxic carcinogens in food

Margin of Exposure (MOE)

- Ratio between the dose leading to tumor formation in humans or experimantal animals and the measured or estimated human exposure („point of departure": T_{25} or $BMDL_{10}$ from animal studies)
- MOE of 10.000 and above based on $BMDL_{10}$ would indicate low concern and thus low priority for risk management

→ Pragmatic approach for priority setting

→ Does not take into account „mode of action"

→ MOE of several food carcinogens are well below 10,000 (e.g., acrylamaide, Aflatoxin B1)

Past and current approaches for workplace carcinogens

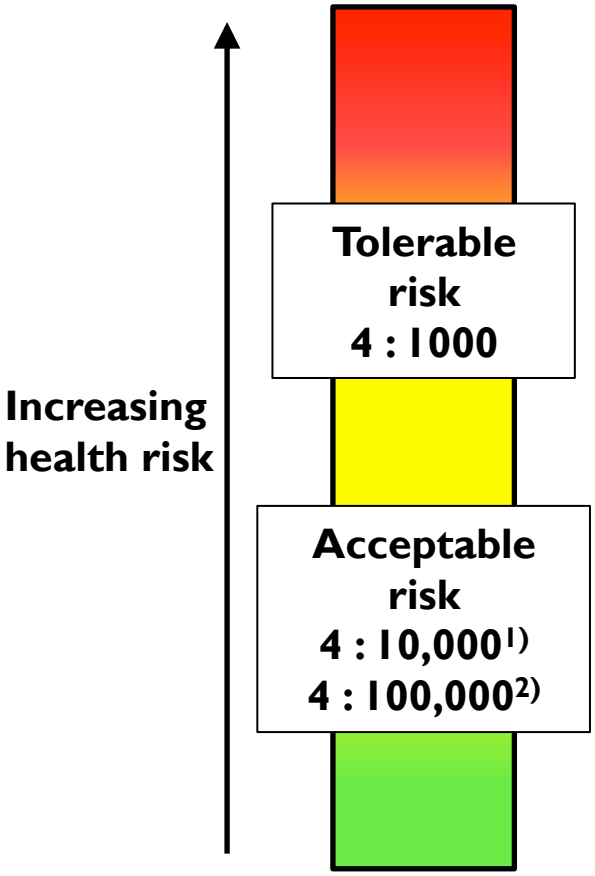
TRK-Values (technical guidance concentration)

- Strictly based on technological considerations; valid until 2005
- Since 2005: Requirement for setting health-based exposure limits also for carcinogens

In Germany two different approaches:

- **Exposure-Risk-Relationships** (Expositions-Risiko-Beziehungen; ERB) established by Ausschuss für Gefahrstoffe (AGS):
- Tolerated or accepted risks:
  - 4:1,000 (tolerated risk); 4:10,000 (accepted risk 2013); 4:100,000; accepted risk 2018 at the latest)
- **MAK categories 4 and 5**
  (since 1998; similar approach by SCOEL since 2008)

The risk-based concept for carcinogenic substances applied by the Ausschuss für Gefahrstoffe (AGS)



Red: Stop! (Hazard area)

Health risk not tolerable – prohibition of use unless exposure is reduced significantly

Yellow: Attention! (Area of concern)

Unwanted risk – provide for active risk

Green: Go! (Area of basic safety precautions)

Health risk acceptable – duty of care remains

1) until 2013
2) until 2018

BAuA (2013) The risk-based concept for carcinogenic substances developed by the Committee for Hazardous Substances, From limit-value orientation to an action-oriented approach

MAK categories for genotoxic/ carcinogenic substances

1. Substances that **cause cancer in humans** and can be **assumed to contribute to cancer risk** (adaquate epidemiological evidence or limited epidemiological evidence and mode of action relevant to humans)

→ **No MAK or BAT value established**

2. Substances that are **considered to be carcinogenic in humans** based on **sufficient data from long-term animal studies** or limited evidence from animal studies, substantiated by evidence from epidemiological studies and/or **supported by mode of action** (in vitro tests, short-term animal studies)

→ **No MAK or BAT value established**

MAK categories for genotoxic/ carcinogenic substances

3. Substances that cause **concern** that they could be carcinogenic to humans but **cannot be assessed conclusively because of lack of data.** The classification in Category 3 is provisional.

a. Substances for which the **criteria for classification in category 4 or 5 are fulfilled** but for which the **database is insufficient** for the establishment of a MAK or BAT value.

b. Substances for which in vitro or animal studies have yielded **evidence of carcinogenic effects, but not sufficient for classification** of the substance **in one of the other categories** (further studies are required). A MAK or BAT value can be established in the absence of genotoxicity.

MAK categories for genotoxic/ carcinogenic substances

4. Substances with **carcinogenic potential for which a non-genotoxic mode of action is of prime importance**; **no contribution to human cancer risk is expected** at exposure observing **MAK and BAT values** (**mode of action well understood,** related for example to increases in cellular proliferation, inhibition of apoptosis or disturbances in cellular differentiation)

Example:

- **Granular biopersistant dust** (GBD or GBS)
  (inert dust without additional specific toxicity)
- Induces **chronic inflammation in the lung on conditions of overload and diminished clearance**

Carcinogenic at high concentrations; MAK value protects from chronic inflammation

MAK categories for genotoxic/ carcinogenic substances

5. Substances with **carcinogenic and genotoxic effects**, which are **considered to contribute very slightly to cancer risk**, provided the MAK and BAT values are observed (must be supported by information on the **mode of action**, dose-dependence and toxicokinetic data pertinent to species comparison)

Up to now only five substances listed:

- Acetaldehyde
- Ethanol
- Isoprene
- Styrene
- Dichoro methane

Currently

Working group on **„Genotoxic carcinogens“ (MAK/SKLM)**

Aim:

- development of **concepts for integrating the manifold mechanisms of carcinogenicity** including current knowledge of cell biology **into risk assessment and classification** of carcinogens

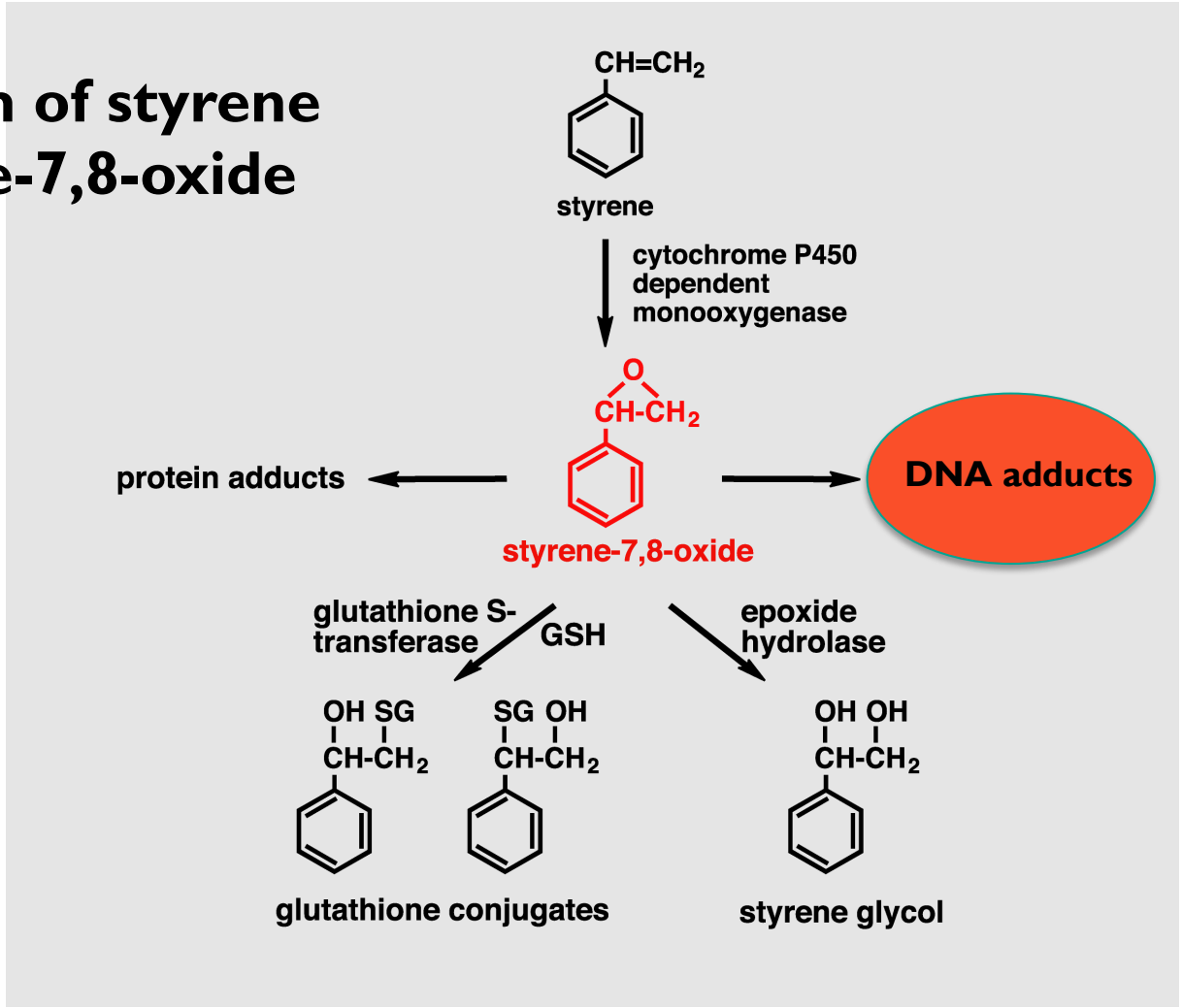
Current discussion

Science-based threshold values also for genotoxic carcinogens?

- **Must be evaluated on a case-by-case basis**
- **Required information:**
  - All **types of DNA lesions** induced
  - **Dose-response-relationship in the low dose range** for DNA lesions
  - **Cellular consequences** of respective DNA lesions
  - **Endogenous „background“ frequency of the same or similar genotoxic compound or metabolite and/or DNA lesions**
  - **Toxicokinetic data/ Modelling**

Example Styrene (MAK Category 5)

Metabolism of styrene and styrene-7,8-oxide



Metabolism of styrene and styrene-7,8-oxide

- **Critical metabolite** formed by mouse, rat and man
- Extent assessed by biochemical marker Hb-adducts:

  Mouse 1 → Rat 1/2 - 1/3 → Man 1/20 - 1/50

- **Carcinogenic risk calculation for systemic styrene exposure**
- Based on the positive oral studies in mice (lung tumors) and oral study with styrene oxide in rats (maximal statistic tumor incidence of 3 %)
- Exposure at the workplace (40 years, 8 hrs per day, 5 days per week, 48 weeks per year) at

20 ppm results in an estimated risk of about 1 : 20 000, which is well below the risk of endogeneous epoxides (for example, ethylene oxide)

Category 5, MAK 20 ppm

Significance of DNA adducts for mutagenicity and carcinogenicity?

- **New analytical methods** enable measurement of some **DNA adducts also as background frequencies** and in the low dose range
- **Frequently linear increase of DNA adducts** with dose within the low dose range
- However**, in some cases increase in mutation frequencies non-linear dose-response curve**

Significance of DNA adducts for mutagenicity and carcinogenicity?

Significance of DNA adducts for mutagenicity and carcinogenicity?

Factors important for further fate of DNA adducts with respect to mutagenicity and carcinogenicity:

- DNA repair?
- Mutagenic potential of the respective lesions?
- Other additional effects required, like elevated cell division, to convert premutagenic DNA lesions in mutations?

Conclusions and perspectives

- More science-based threshold values desirable for (genotoxic) carcinogens
- **Establishment of mode of action for genotoxic carcinogens**, considering:
  - Identification of DNA adducts
  - Dose-response relationships
  - DNA repair
  - Mutagenic potential
- **Establishment of more sensitive mutagenicity assays, especially** also for ***in vivo*** mutagenicity assessment (PigA)?
- **Inclusion of toxicogenomic data** for assessment of mode of action in the low dose range
- Establishment of suitable **biomarkers** for exposed humans

Thank you very much for your attention!