Current Approaches in Risk-Based Evaluation of Chemical Carcinogens

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

Tumor incidence

Dose

Extrapolation „Black Box“

LOEL

Measured values

? ? ? ?
The problem of dose-response-relationships in case of carcinogenic compounds...

- Tumor incidence
- Measured values
- Extrapolation
  - „Black Box“

Current approach for genotoxic carcinogens
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 experimental 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)
MAK categories for genotoxic/ carcinogenic substances

1. Substances that cause cancer in humans and can be assumed to contribute to cancer risk (adequate 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
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 four substances listed:**

- Acetaldehyde
- Ethanol
- Isoprene
- Styrene
Example Styrene (MAK Category 5)

Metabolism of styrene and styrene-7,8-oxide

[Diagram showing the metabolism of styrene and styrene-7,8-oxide]

- Metabolism via cytochrome P450 dependent monooxygenase
- Formation of styrene-7,8-oxide
- Conversion to DNA adducts
- Formation of glutathione conjugates and styrene glycol
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 one positive oral study with styrene oxide in rats
- 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
Example Ethanol (MAK Category 5)

- Human carcinogen; critical metabolite acetaldehyde
- Concentration of ethanol in blood over time determines the internal body burden of the critical metabolite acetaldehyde
- Humans are physiologically exposed to ethanol due to endogenous ethanol production
  - amount and range of internal life-time body burden are known
  - correlation of external ethanol concentration with ethanol concentration in blood is also known
  - at an exposure of 500 ml ethanol/m³ during the entire working life, the average life-time body burden of ethanol is still within the range of variation of the endogenous body burden
Example Ethanol (MAK Category 5)

From: Seitz and Stickel, Genes Nutr (2010) 5: 121 - 128
Life-time body burden of ethanol without and with occupational exposure

→ workplace exposure to concentrations up to 500 ml/m³ would contribute only little to cancer risk
Scientific Committee on Occupational Exposure Limits (SCOEL)

• Establishes occupational exposure limits (suggestions prior to final adoption)

• Follows risk-based categories for carcinogens (similar to MAK commission) (Bolt and Huigi, 2008, Arch. Toxicol., 82, 61 – 64)
SCOEL approach for chemical carcinogens, causing tumors in humans and/or experimental animals

Genotoxic

DNA reactive, causing mutations

Clearly DNA reactive and initiating

A: No threshold (LNT to apply)

Numerical risk assessment, risk management strategies

Non-genotoxic

Genotoxic only on chromosome level (e.g., spindle, topoisomerase)

Weak genotoxin, secondary mechanisms important

B: Situation not clear → LNT as default

C: Practical threshold likely

D: True/perfect threshold

Definition of NOAEL → Setting a health-based OEL

From: Bolt and Huici-Montagud, 2008, Arch Toxicol 82: 61 - 64
Example Nickel
Carcinogenicity of nickel and nickel compounds in humans

- Exposure towards nickel compounds clearly associated with increased risk of cancer (lung, nasal cavity)
- Discrimination between different nickel species:
  - Water soluble nickel compounds carcinogenic
  - Poorly water soluble nickel contributes, but extent unclear
  - No conclusive evidence for carcinogenicity of metallic nickel

Major limitations to assign the carcinogenicity to defined nickel species:
- No workplaces with exposure towards a single nickel species
- Correlations depend on exposure estimates far back in time and even comparatively small uncertainties may have a big impact on dose-response relationships
- Dramatically increased cancer incidences were seen on exposure conditions before 1930
Main mechanisms of carcinogenicity:
- Oxidative DNA damage
- Interactions with DNA repair processes
- Epigenetic alterations

Nickel species-dependent effects
- Water soluble and insoluble particulate nickel compounds carcinogenic to humans; metallic nickel no conclusive evidence

Toxic species $\text{Ni}^{2+}$
- Extent of damage depends on bioavailability and biological half live
Carcinogenicity of nickel and nickel compounds in humans

**SCOEL Carcinogen group C** (Carcinogen with a practical threshold)

**Quantitative estimates on nickel carcinogenicity:**

- Highly increased cancer incidences were seen at very high exposure levels (usually 1 to 10 mg/m³)
- 0.25 mg/m³ water soluble nickel: increased lung and nasal cancer in Finland
- Extrapolations by Grimsrud et al. for Kristiansand (Norway): significant increase in cancer risk at cumulative exposure of 1.6 mg/m³ water soluble nickel (would resemble 0.04 mg/m³ per year in case of 40 years exposure)
Low-dose non-cancer effects of nickel compounds and approach for setting an OEL by SCOEL (1)

Critical non-cancer effect:

- **Lung toxicity** (Macrophage hyperplasia, chronic inflammation, fibrosis)
- **NOAEL derived from animal inhalation studies** (2 years, NTP):
  - **Water soluble nickel**: NOAEL in rats 0.03 mg/m³
  - **Metallic nickel and poorly water soluble nickel compounds**: pronounced lung damage at 0.1 or 0.11 mg/m³, respectively; no data on lower exposure levels available

OEL should protect from cancer and non-cancer effects
Low-dose non-cancer effects of nickel compounds and approach for setting an OEL by SCOEL (2)

Proposal (SCOEL):

• **OEL of 0.005 mg/m$^3$ for all nickel compounds and metallic nickel (respirable fraction) to protect from lung toxicity**
  
  • derived from NOAEL for water soluble nickel of 0.027 mg/m$^3$
  
  • takes into account differences between rats and humans with respect to particle deposition → equivalent human concentration 0.016 mg/m$^3$ (Oller and Oberdörster, 2010)
  
  • about 20 times lower than concentrations which produce severe lung damage in case of metallic and poorly water soluble nickel compounds

• **OEL of 0.01 mg/m$^3$ for all nickel compounds except metallic nickel (inhalable fraction) to protect from lung and nasal carcinogenicity**
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
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?
Current status

• 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?
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
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!