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Derivation of a threshold for genotoxic carcinogens

An insight into the procedure of the MAK Commission for compounds classified in Carcinogen Category 5

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Introduction

The DFG Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) proposes maximum workplace concentrations (MAK values) for volatile chemicals and dusts and recommends further classifications. This poster illustrates the derivation of a MAK value and the allocation in the classification categories for two different compounds in order to gain an insight into the procedure of the MAK Commission. Isoprene and Dichloromethane are both classified in Carcinogen Category 5. This category contains compounds with carcinogenic properties for which the available data are sufficient for assessment of the carcinogenic potency. Here, a MAK value is defined at which no or at most a very slight contribution to the cancer risk is to be expected.

Isoprene

Most sensitive endpoint

- tumour formation

Toxicokinetic and Metabolism

- Isoprene is formed endogenously in humans.
- Isoprene is metabolized to epoxides, diepoxides and diols.

Somatic and germ cell mutagenicity

- *in vitro:* isoprene negative, metabolit (methyl-1,2:3,4-diepoxybutane) positive
- *in vivo*: positive after inhalation
  → micronucleus test (erythrocytes), SCE (bone marrow): NOAEC 70 ml/m³
- effects on sperm and/or testes in mice and rats at 700 ml/m³, but not at 70 ml/m³

Conclusion:

Isoprene is classified **in Germ Cell Mutagenicity Category 5**:

- No mutagenic effects in soma cells nor toxic effects in germ cells at 70 ml/m³.
- Germ cells could be reached and effects are suspected.

→ No genetic risk in humans is expected if the MAK value (3 ml/m³, see below) is observed.

Carcinogenicity

- No cancer studies in humans.
- studies in mice: adenomas of the Harderian glands at 70 ml/m³,
  → at ↑ concentrations: adenomas and carcinomas in liver and forestomach, adenomas in lung, haemangiosarcomas and histiocytic sarcomas after inhalation
  → Mode of action:
  activation of K-ras or H-ras →ras mutation and promoting mechanism→ tumour
  → at 10 ml/m³: no significant ↑ tumour incidence
- studies in rats: tumours in mammary gland, kidney and testes at 220 ml/m³ and ↑

Physiological toxicokinetik (PT) model and MAK value derivation

- genotoxic metabolites (e.g. diepoxides): most likely responsible for carcinogenicity however→ internal exposure to metabolites: not quantifiable
- calculation: area under the concentration/time curve in blood (AUC) for isoprene (metabolic precursor)

Determinations using the PT model (adults, 70 kg body weight):

Estimation of endogenous isoprene exposure

→ Publications of isoprene concentrations in exhaled air (a total of 337 volunteers).

- weighted mean concentration in pulmonary air: 0.0064 ± 0.049 ml/m³
- rate of formation: 13.1 ± 10.0 µmol/h
- concentration in venous blood: 5.2 ± 4.0 nmol/l
- AUC (0-80 years): 3.6 ± 2.8 mmol x h/l

Estimation of exogenous isoprene exposure

→ occupational exposure to 3 ml isoprene/m³ (8 hours/day, 5 days/week, 40 years)

- AUC (40 years): 2.8 mmol x h/l

Conclusion:

AUC (8 hours/day, 40 years) after exogenous exposure to 3 ml isoprene/m³ = AUC (lifetime exposure) at the level of the standard deviation of the mean endogenous isoprene concentration

- exposure to **3 ml isoprene /m³ → no significant contribution to the cancer risk**
- **MAK value: 3 ml/m³**

Isoprene is classified in Carcinogen Category 5

- mechanism for tumour formation: genotoxicity
- in compliance with the MAK value of 3 ml/m³:
  → no genetic risk in humans is expected
  → compared with endogenous isoprene exposure:

no significant contribution to the cancer risk

Prenatal toxicity

- exposure to concentrations at the level of the MAK value:
  → No reason to fear damage to the embryo or foetus.
  → **Pregnancy Risk Group C**

Dichloromethane

Most sensitive endpoint

- effects on the central nervous system

Mode of action

microsomal oxidative pathway (saturable at 500 ml/m³)

- conversion to carbon monoxide by CYP2E1
- CO-Hb formation by CO binding to blood hemoglobin

cytosolic GSH-dependent pathway (not saturable)

- conversion by GSH-transferases to genotoxic metabolite (S-chloromethylglutathione)
- reaction with guanosine and cytidine → adduct formation

→ Higher dichloromethane concentration: cytosolic pathway predominant

MAK value derivation

- based on: acute behavioral effects indicative for CNS depression
- NAEC: 0.85 mg dichloromethane/L blood
- exposure of 100 ml/m³ dichloromethane in resting participants
  → corresponding to blood concentration of 0.85 mg dichloromethane/L
- however: ↑ higher respiratory rates under working conditions
- extrapolation:
  - inhalation: 50 ml dichloromethane/m³
  - condition: light activity (10m³ breathing volume/8-hour shift)

  → blood level dichloromethane: 0.51 mg/L
- **MAK value: 50 ml/m³**

Somatic and germ cell mutagenicity

- *in vitro:* positive when the GSH-dependent metabolic pathway is active
- *in vivo* studies in mice (liver cells , bone marrow): positive at ↑ concentrations
- studies in germ cells are not available
- However, compared with humans, in mice
  → GSTT1 isoenzyme is five times more efficient
  → RNA-formaldehyde adducts is seven times higher
  → GSTT1 RNA expression in prostate, ovaries and placenta is higher

Conclusion: not expected to be mutagenic in germ cells in humans

Carcinogenicity

- epidemiologic studies: no evidence for carcinogenicity
- studies in mice: adenomas and carcinomas in the liver and the lung
  → in mice: rapid metabolism of dichloromethane via the GSH-dependent metabolic pathways in liver and lung

Carcinogenic potential in humans

- PBPK model in mice and humans (Marino et al. 2006, David et al. 2006)
  → unit risk for humans (liver and lung tumours; lifetime exposure of 1 µg/m³): 1.05×10^{-9} ($0\text{-}2.7 \times 10^{-9}$)
- linear extrapolation of the median unit risk by the Commission
  - 50 ml dichloromethane/m³ (MAK value)
  - working lifetime exposure (14% life time)

  → unit risk for humans (liver and lung tumours): **2.5×10^{-5} ($0\text{-}6.67 \times 10^{-5}$)**

Dichloromethane is classified in Carcinogen Category 5

- MAK value based on prevention of neurotoxicity: 50 ml/m³.
- Estimation of carcinogenic potential in humans at 50 ml/m³: 2.5×10^{-5} ($0\text{-}6.67 \times 10^{-5}$)
- Genotoxicity is of prime importance and the mode of action is well-understood:
  → genotoxic carcinogen
  → tumours in liver and lung of the mouse are due to species-specific sensitivity
  → high activity of GSTT1
- in compliance with the MAK value:

very slight contribution to human cancer risk

Prenatal toxicity

- exposure to concentrations at the level of the MAK value:
  → Damage to the embryo or foetus cannot be excluded (CO-Hb formation).
  → **Pregnancy Risk Group B**

PBPK: physiologically based pharmacokinetic

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References: www. dfg.de/en/mak

www.kit.edu