The revised OEL for benzene in the Netherlands - implications for the biological monitoring of benzene at low exposure levels.

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OEL setting by DECOS

- **DECOS** = Dutch Expert Committee on Occupational Safety - a standing committee of the Health Council of The Netherlands, which was established in 1902 as the highest advisory committee on health-related issues for the Dutch government and parliament.

- DECOS has two subcommittees:
  - Sub-committee on Classification of Carcinogenic Substances
  - Sub-committee on Classification of Reproductive Substances

- The Health Council is particularly sensitive on (perceived) conflicts of interest.
For the evaluation of benzene the President of the Health Council made a deliberate exception on the ‘general conflict-of-interest’ rules:

The preparatory committee had an equal number of persons with opposite perceived conflict-of-interest, and the same number of persons with no conflict-of-interest.
DECOS classification of benzene (1)

- DECOS first asked the Sub-committee on Classification of Carcinogenic Substances to evaluate the carcinogenic properties of benzene and in particular, its genotoxic mode of action.
- The Sub-committee evaluated the available data up to early 2013 and concludes that:
  - there is sufficient evidence for a causal relationship between benzene exposure and the haematological malignancies in humans.
  - benzene is carcinogenic to man, based on epidemiological evidence for AML and total leukemia, and supporting evidence in experimental animals.

→ Recommendation to classify in Category 1A.
DECOS classification of benzene (2)

With regard to the genotoxic mode of action, the sub-committee concludes that the weight-of-evidence points to an indirect mode of action, whereas there is no evidence to substantiate a direct genotoxic mode of action.

Overall conclusion: benzene acts by a non-stochastic genotoxic mode of action.
DECOS evaluation of benzene (1)

- DECOS concludes that benzene-induced carcinogenicity is caused by a complex mechanism (AOP) involving
  - the metabolism of benzene,
  - subsequent toxicity to blood cells and blood-forming organs (haematotoxicity),
  - genotoxicity and formation of initiated, mutated bone marrow target cells,
  - altered oncogenic signaling, and
  - clonal proliferation
DECOS evaluation of benzene (2)

- A number of potential mechanisms were identified
  - Topoisomerase II inhibition (most consistent with clinical findings; considered most likely)
  - Adduct formation of reactive metabolites (protein adducts; no DNA adducts)
  - Oxidative DNA damage (oxidative stress)
  - Error-prone DNA-repair (non-homologous end-joining DNA repair)
  - Epigenetic alterations
DECOS evaluation of benzene (3)

- Overall DECOS concludes that benzene-induced carcinogenicity is caused by a thresholded mechanism, and that benzene should be considered an indirect, non-stochastic genotoxicant.
- As a consequence, a health-based recommended occupational exposure limit (HBR-OEL) can be derived.
- The cells of the bone marrow/haematopoietic system are considered to be the most sensitive targets for benzene-induced toxicity.
- There is a range of epidemiological studies addressing the haematological effects of benzene exposure with variable results.
DECOLOR evaluation of benzene: epidemiology

- Cohort studies with (intermittent) high exposures (Pliofilm, NCI/CAPM) → positive
- Cohort studies with moderate exposures (DOW, CMA, Chinese shoe workers) → variable (positive to no effect)
- (Nested) case-control studies with low exposures (Health Watch, UK petroleum, Canadian) → variable (positive trend or no effect)
  → Pooled analysis – low exposures → no effect (AML)
- Cohort of off-shore workers → positive in the past (higher exposures) but negative now (lower exposures)
DECOS overall evaluation (1)

- Meta-analyses: variable results, but some positive
- Animal data are not particularly helpful
- Mechanistic data indicate (indirect) genotoxicity

→ Overall conclusion: benzene is carcinogenic and causes AML (ANLL)

- Inconsistencies between ‘Western’ and ‘Asian’ population with regard to AML and low exposure levels → no indications for genetic reasons to explain difference (or any other reason)
DECOS overall evaluation (2)

- Precautionary approach: take data from Asian studies as leading
- Chromosomal aberration and other gentoxicity studies are limited in design and reporting and not suitable for derivation of an OEL
- Studies on haematotoxicity include low-dose data and haematological effects seem to be the most sensitive
- Conflicting data → Weight-of-Evidence approach
- Reasonable LOAEL (first haematological effects) ~ 2 mg/m$^3$ (0.6 ppm)
- Standard assessment factor of 3 was applied without additional factors
- HBROEL of 0.7 mg/m$^3$ (0.2 ppm) advised
Human biomonitoring of benzene exposure

- Human biomonitoring (HBM) of urinary benzene metabolites is well established: phenol, t,t-muconic acid (tt-MA) and S-phenyl-mercapturic acid (S-PMA) and unchanged benzene (BB, BU)
- With increasingly lower OELs (100 ppm → 10 ppm → 1 ppm) first phenol became useless because of natural backgrounds and subsequently tt-MA (1 ppm → 0.5 ppm or lower)
- As far as we know, S-PMA has no background, but it picks up benzene exposure caused by smoking
- However, S-PMA not (yet) validated at ultra-low OELs
Proven applicability benzene biomarkers

OEL airborne benzene

- Phenol (U)
- t,t-muconic acid (U)
- S-phenylmercapturic acid (U)
- Benzene (B, U)

0.01 ppm  0.1 ppm  1.0 ppm  10 ppm  100 ppm

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# HBM of low-level benzene exposure (1)

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<thead>
<tr>
<th>Benzene in air</th>
<th>tt-Muconic acid in urine</th>
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Benzene HBM by urinary tt-MA (1)

- tt-MA doesn’t seem a useful parameter for low-level benzene exposure
- The MAK Kommission found 9 studies with low-level benzene exposure

The 95 percentile for the general population is 150 μg tt-MA/g creat.

- Dietary uptake of sorbic acid contributes to tt-MA excretion and could lead to urinary values as high as 700 μg/g creatinine.
HBM of low-level benzene exposure (2)

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* For non-smokers
Benzene HBM by urinary S-PMA (1)

- ELISA methods are excluded; only MS-methods are considered
- There are 6 studies available on low-dose benzene and S-PMA
- One study is excluded as the data do not ‘fit’ the other studies
- One study (most extensive; good quality) was excluded (reasons unclear)
- One study is confounded (smokers and non-smokers data; benzene not measured on day of urine collection), but nevertheless included
- The four included studies report median and average values
- It is unclear how the, statistically not significant (!), correlation was obtained that was used to calculate the low-level biological limit values
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Benzene HBM by urinary S-PMA (2)

- For higher airborne benzene levels, the study by Van Sittert et al., 1993 was used to calculate the EKA values (first study that validated S-PMA).
- This study used S-benzylmercapturic acid as internal standard which led to unreliable results at lower concentrations.
- Validation was repeated with [ring-\text{d}_5]-S-PMA as internal standard.
- When the regression line from this study is applied, higher biological limit values (EKA values) are obtained.
- The ‘old’ regression line would yield negative values for the low-dose EKA values; the ‘new’ regression line yields positive, but higher values.
The well validated methods to determine urinary S-PMA are not validated below 1 mg/m$^3$ (0.3 ppm)

Available studies measuring S-PMA at low level benzene exposure are highly inconsistent

Values obtained with the validated regression lines give results that are not consistent with the studies at low dose – reasons are not clear

Available data (Maestri et al., 2005) give poor correlations ($r^2 = 0.18$)

Proper validation studies of urinary S-PMA at low airborne levels of benzene are needed!
### HBM of low-level benzene exposure (3)

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0.3 (95 percentile for general population)
Benzene HBM by urinary benzene (1)

- Historically benzene in urine has been considered difficult due to the volatility of benzene (evaporation from samples; potential contamination)
- For the EKA values only 4 studies were available; one study is discarded as it doesn’t ‘fit’ the other three studies
- The remaining three studies report only mean or median values no correlations
- One study explicitly states that airborne benzene was not correlated with urinary benzene
- A highly significant correlation was obtained from 4 mean/median values of the three remaining studies → applied to 0.1 and 0.2 ppm
Benzene HBM by urinary benzene (2)

- For the concentration range of benzene in air > 0.2 ppm, no studies have been reported
- The correlations between urinary benzene and both urinary tt-MA and S-PMA have been established
- The values differ for tt-MA and S-PMA and are 20-50% higher for S-PMA than for tt-MA
- The correlation between urinary benzene (μg/L) and S-PMA is used to calculate the urinary benzene levels at airborne levels > 0.2 ppm
- The EKA values for low and high airborne benzene are consistent
- Overall, BU seems to work, but more validation is needed.
Benzene HBM by blood benzene (BB)

- Benzene in blood (BB) was not considered by either RAC or MAK-Kommission as they do no support invasive methods.
- Volatility issues are less important for BB than BU as blood can be collected in air-tight systems.
- BB might be a useful parameter for recent exposures.
- We are currently investigating the possibilities of the suitability of micro-samples for determination of BB.
- Once the analytical methodology is established, we can start (extensive) validation in occupational settings.
Conclusions (1)

- DECOS has concluded, based on the available data, that benzene is a non-stochastic genotoxic human carcinogen, implying a true threshold.

- DECOS derived a health-based recommended OEL of 0.7 mg/m$^3$ (0.2 ppm) as 8-h TWA (2014).

- More recently (March 2018) RAC has recommended an OEL of 50 ppb (0.16 mg/m$^3$) as 8-h TWA.

- Human biomonitoring of exposure at 0.2 ppm is very well possible by S-PMA and possibly BU, but not by tt-MA.
Conclusions (2)

- Human biomonitoring at the OEL recommended by RAC (50 ppb) is quite essential as personal air monitoring at these levels is challenging
- The suggested biological exposure limit values for low-dose benzene exposures (< 0.15 ppm) seem to lack sound justifications
- There is an urgent need to properly validate S-PMA for these low levels of benzene exposure
- Benzene in blood (and maybe urine) may be a valid alternative
Thank you for your attention!

Any questions ???