Human biomonitoring of benzene. To which degree are proposed OELs a challenge for existing methodology.

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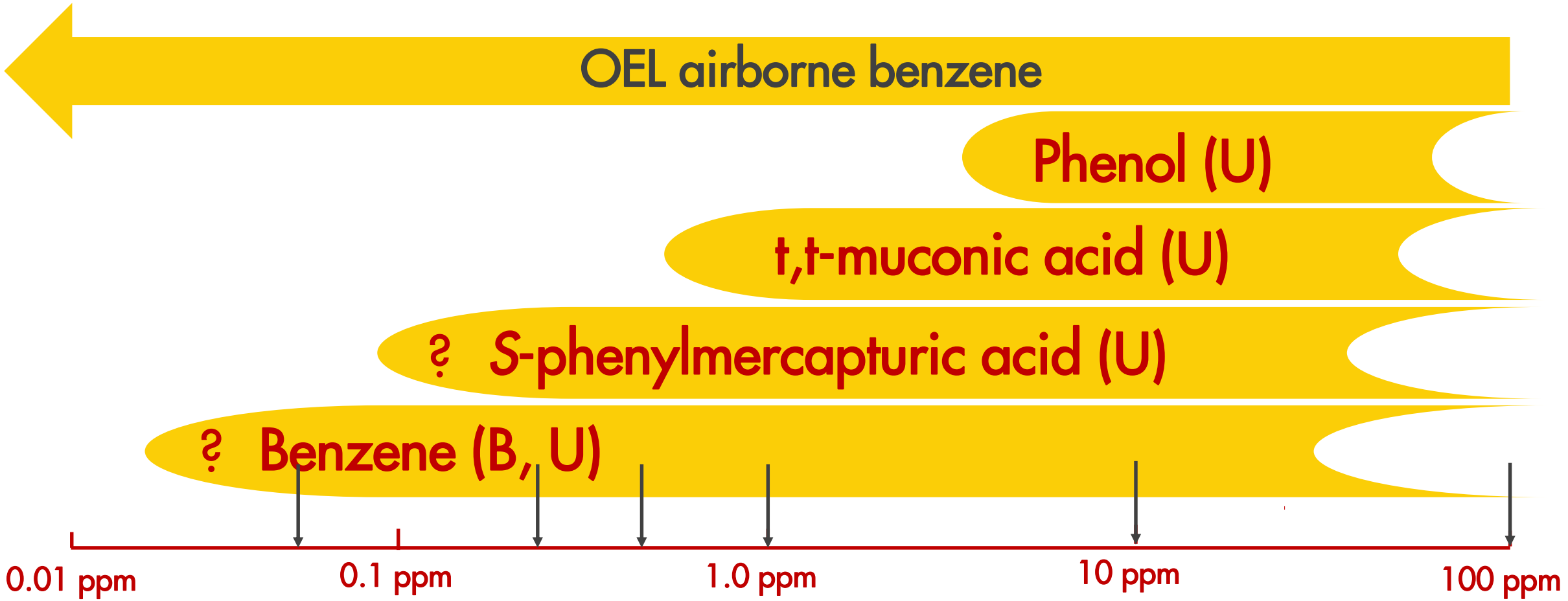
OEL values for benzene

- DECOS is of the opinion that benzene is a non-stochastic genotoxic carcinogen and derived in 2014 a health-based recommended OEL of 0.7 mg/m^3 (0.2 ppm) as 8-h TWA
- Recently (March 2018) RAC has proposed an OEL of 50 ppb (0.16 mg/m^3) as 8-h TWA
- Exposure assessment of benzene at these very low levels is challenging → personal air monitoring is difficult and expensive
- Human biomonitoring (HBM) seems a more viable option → to which extent is it possible for very low exposure levels ?

Human biomonitoring of benzene exposure

- Human biomonitoring (HBM) of urinary benzene metabolites is well established: phenol, t,t-muconic acid (tt-MA), *S*-phenyl-mercapturic acid (S-PMA), and unchanged benzene (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

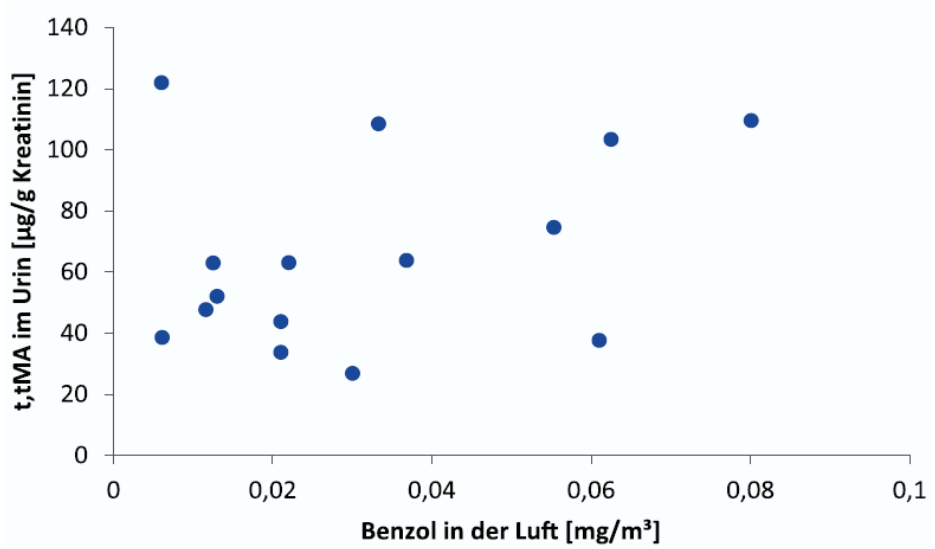


Benzene HBM by urinary tt-MA (1)

Benzene in air		tt-Muconic acid in urine
mL/m³ (ppm)	mg/m³	µg/g creat
		150 (95 percentile for general population)
0.03	0.1	-
0.06	0.2	-
0.15	0.5	-
0.3	1.0	300
0.6	2.0	500
1.0	3.3	750
2.0	6.5	1200

Benzene HBM by urinary tt-MA (2)

- 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.

Benzene HBM by urinary S-PMA (1)

Benzene in air		S-Phenylmercapturic acid (SPMA) in urine
mL/m³ (ppm)	mg/m³	µg/g creatinine
		0.5 (95 percentile for general population)
0.03	0.1	1.5*
0.06	0.2	2.5*
0.15	0.5	5
0.3	1.0	12
0.6	2.0	25
1.0	3.3	45
2.0	6.5	90

* For non-smokers

Benzene HBM by urinary S-PMA (2)

- 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
- On 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

Benzene HBM by urinary S-PMA (3)

Benzene in air		S-Phenylmercapturic acid (SPMA) in urine
mL/m³ (ppm)	mg/m³	µg/g creatinine
		0.5 (95 percentile for general population)
0.03	0.1	1.5*
0.06	0.2	2.5*
0.15	0.5	5
0.3	1.0	12
0.6	2.0	25
1.0	3.3	45
2.0	6.5	90

* For non-smokers

Benzene HBM by urinary S-PMA (4)

- 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-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

Benzene HBM by urinary S-PMA (5)

- 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 !

Benzene HBM by urinary benzene (1)

Benzene in air		Benzene in urine
mL/m³ (ppm)	mg/m³	µg/L
		0.3 (95 percentile for general population)
0.03	0.1	0.5*
0.06	0.2	0.8*
0.15	0.5	1.5
0.3	1.0	2.75
0.6	2.0	5.0
1.0	3.3	7.5
2.0	6.5	12.5

* For non-smokers

Benzene HBM by urinary benzene (2)

- 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 (3)

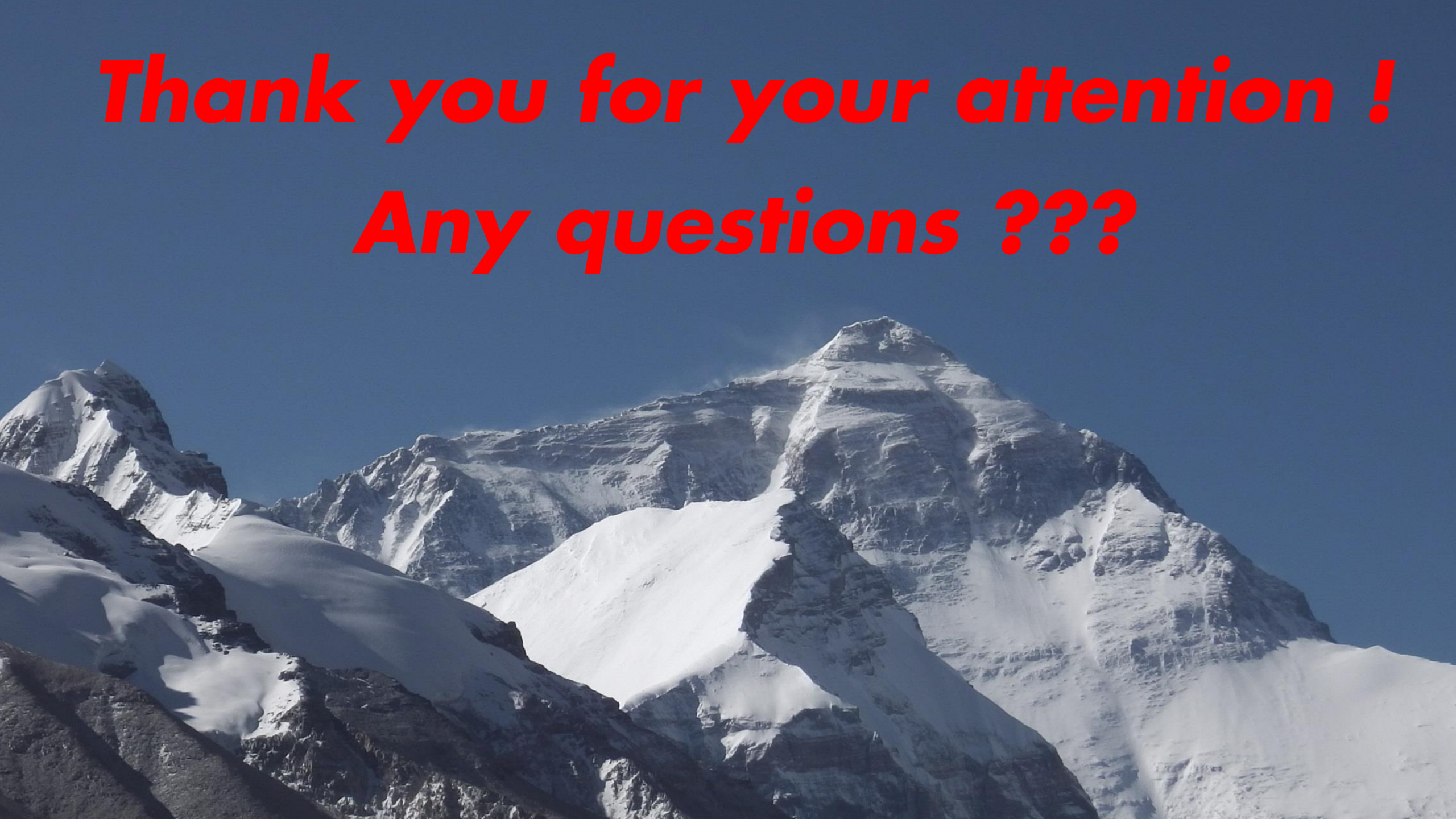
- For the concentration range of benzene in air > 0.2 ppm, no studies have been reported
- The correlations between urinary benzene and both urinary tt-MA and S-PMA have been established
- The values differ for tt-MA and S-PMA and are 20-50% higher for S-PMA than for tt-MA
- The correlation between urinary 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
- Benefit of the doubt: BU seems to work well, but 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

- DECOS derived a health-based recommended OEL of 0.7 mg/m^3 (0.2 ppm) as 8-h TWA
- RAC has proposed an OEL of 50 ppb (0.16 mg/m^3) as 8-h TWA
- Human biomonitoring (HBM) of exposure at 0.7 mg/m^3 is possible by S-PMA and BU, but not by tt-MA.
- HBM of exposure at 50 ppb may be possible by S-PMA and BU (or BB)
- The suggested biological exposure limit values for low-dose benzene exposures (RAC, MAK) seem to lack sound justifications
- There is an urgent need to properly validate S-PMA for these levels
- Benzene in blood (and maybe urine) may be a valid alternative


Thank you for your attention !
Any questions ???