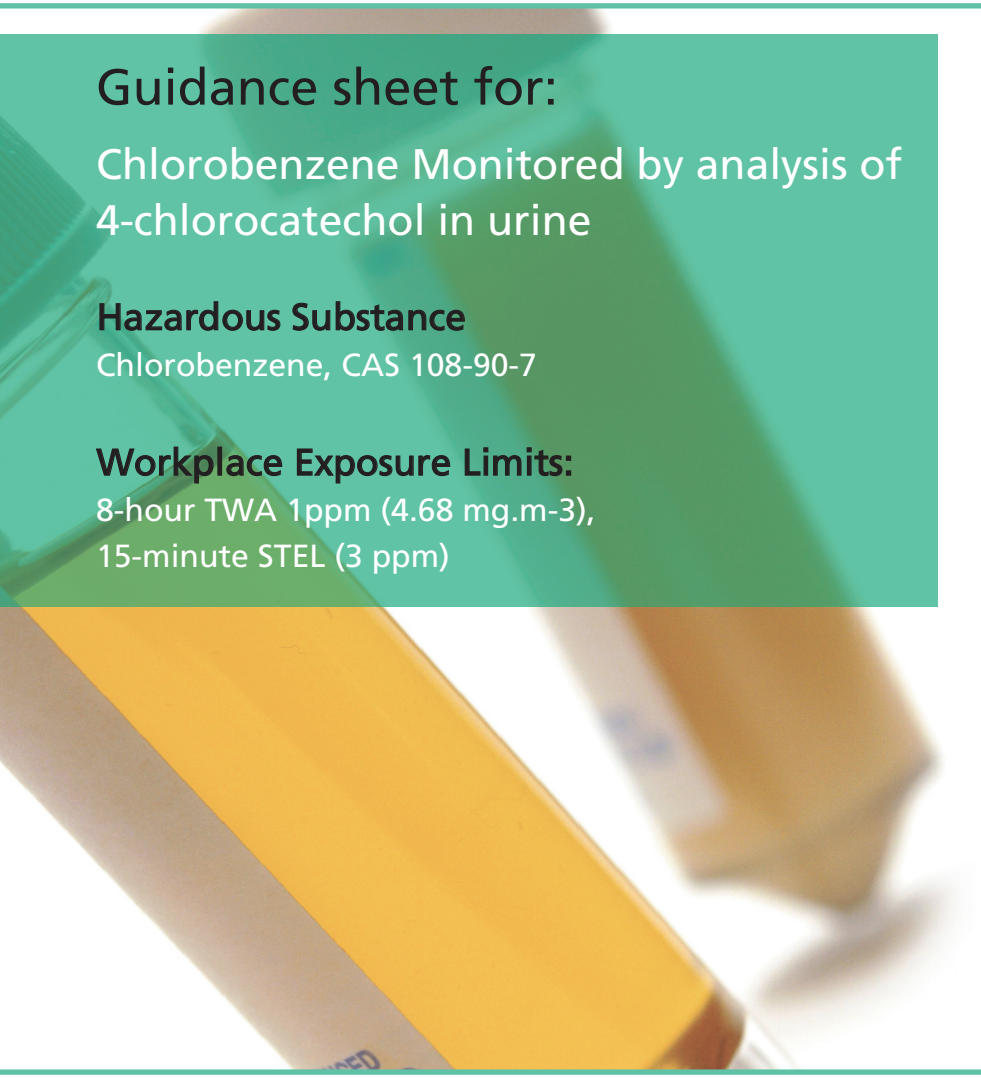
# Biological Monitoring Guidance Values

## Guidance sheet for:

### Chlorobenzene Monitored by analysis of 4-chlorocatechol in urine

**Hazardous Substance**
Chlorobenzene, CAS 108-90-7

**Workplace Exposure Limits:**
8-hour TWA 1ppm (4.68 mg.m-3),
15-minute STEL (3 ppm)



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HEALTH & SAFETY LABORATORY

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## Summary

Biological monitoring may be a useful aid to the assessment of occupational exposure to chlorobenzene. There are no data to relate metabolite concentrations to health effects but there are data to show a good correlation between inhaled chlorobenzene and levels of 4-chlorocatechol and 4-chlorophenol in urine samples collected at the end of shift.

4-Chlorocatechol is the more abundant metabolite with slightly more available data and is therefore the preferred biomarker. Based on three field studies and two volunteer studies, after exposure to chlorobenzene at 1 ppm for 8h, the average urine concentration of 4-chlorocatechol in samples collected at the end of shift would be around 5 - 10 mmol 4-chlorocatechol/mol creatinine (6 - 13 mg 4-chlorocatechol/g creatinine).

If biological monitoring results are greater than the guidance value it does not necessarily mean that ill-health will occur, but it does indicate that control of exposure may not be adequate. Under these circumstances employers will need to look at current work practices to see how they can be improved to reduce exposure.

In view of the uncertainty about the toxic effects of chlorobenzene exposure should be kept as low as reasonably practical.

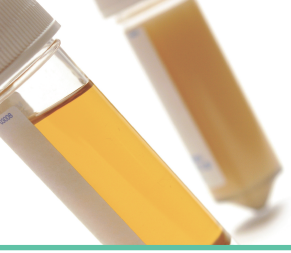
## Sample Collection

Urine samples should be collected at the end of shift into polystyrene universal containers (30ml)

## Sample Transport to Laboratory

Send samples to the laboratory by first class post (or equivalent) to arrive within 48h of collection. If any delay is anticipated, store at –20°C.

Packaging must comply with Post Office Regulations. Employers will need to look at current work practices to see how they can be improved to reduce exposure. In view of the uncertainty about the toxic effects of chlorobenzene exposure should be kept as low as reasonably practical.



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## Description of suggested method

An HPLC-UV method was developed by Heinrich-Ramm to support the DFG biological monitoring programme with an airborne chlorobenzene limit of 10 ppm and measures 4-chlorocatecol only. Briefly: the method hydrolyses 5 ml of urine, containing 3-ethylphenol internal standard, in 25% HCl at 90°C for 2 hours and, after cooling, extracts the 4-chlorocatechol into diethyl ether. The ether extract is transferred to a clean vial and the ether removed under nitrogen. The residue is redissolved in HPLC mobile phase and injected into a C18 column at 34°C. Detection is at 205 nm.

Precision: within day 2.3% RSD
Day to day 4.9% RSD

Detection limit: 0.1 mg/l (roughly 0.08 mmol 4-chlorocatechol/mol creatinine) Calibration range: linear from 0.5 mg/l to 50mg/l Sample Stability > 6 months at –20°C.

Analytical Interferences: No peaks have been found in chromatograms interfering with 4-chlorocatechol at levels greater than 0.5 mg/l (approx. 0.4 mmol 4-chlorocatechol/mol creatinine) from people not occupationally exposed to chlorobenzene.

## Alternative method

A GC-MS method developed by Knecht & Woitowitz (2000) measures 4-chlorocatechol and chlorophenols. Urine (1ml) is saturated with sodium chloride (1g) and hydrolysed with hydrochloric acid (32% at 100°C for 1.5h). 2-bromophenol and 3,4 dichlorophenol are used as internal standards. Metabolites are extracted into ether (3ml) and after removal of the solvent under nitrogen at room temperature the residue is derivatised with N,O bis(trimethylsilyl) trifluoroacetamide (100µl) in pyridine (100µl) at 90°C for 1.5 hours. Separation and detection was by gas chromatography with electron impact selected ion detection.

Precision: within day 3.7% RSD at 50 mg/l Detection limit: 1 mg/l (roughly 0.8 mmol 4-chlorocatechol/mol creatinine) Calibration range: linear from 5 mg/l to 500mg/l

## Other information

The major metabolites of chlorobenzene are 4-Chlorocatecol (74%) and o-, m-, p-chlorophenols (3,7, 12% respectively). The half-life of 4-chlorocatechol in urine is 3 – 6h. The molecular weight of 4-chlorocatecol is 144.56

## Confounding factors

Non-occupationally exposed levels < 0.5 mg/l (approx. 0.4 mmol.mol creatinine-1)

## Quality Assurance

Internal QC must be established
External QC none at present

## Toxicity of chlorobenzene

Evidence from studies in animals indicates that bone-marrow and liver are the key target organs for the toxic effects of chlorobenzene. There is an additional concern for possible genotoxic effects. Chlorobenzene also produces kidney damage but only at exposure concentrations greater than those required to cause effects on the bone-marrow and liver.

## Interpretation

Urinary 4-chlorocatechol results reflect systemic exposure to chlorobenzene that may have entered the body by inhalation or through the skin. If biological monitoring results are greater than the guidance value it does not necessarily mean that ill-health will occur, but it does indicate that control of exposure may not be adequate. Under these circumstances employers will need to look at current work practices to see how they can be improved to reduce exposure.

## Other Guidance Values

The ACGIH (2007) have a BEI and the DFG (2007) a BAT of 100 mg 4-chlorocatechol.g creatinine-1 (78 mmol/mol-1) in end of shift urine after exposure for 8h to 10ppm chlorobenzene. There is also a BEI of (20 mg p-chlorophenol/g creatinine-1).

## Conversions

1 mmol 4-chlorocatecol/mol creatinine is equivalent to 1.278 mg/g
1 mg 4-chlorocatecol/g creatinine is equivalent to 0.7824 mmol/mol.

## Links

EH40 List of Approved Workplace Exposure Limits http://www.hse.gov.uk/coshh/table1.pdf

Biological Monitoring at HSL
http://www.hsl.gov.uk/capabilities/biological.htm

## References

ACGIH (2007) TLVs and BEIs Threshold limit values for chemical substances and physical agents. Published byACGIH ISBN 978-882417-69-8 DFG (2007)Deutsche Forschungsgemienschaft (DFG) List of MAK and BAT values 2007 Wiley-VCH Verlag GmbH & Co KgaA ISBN 978-3-527-31955-8 Heinrich-Ramm R. 3,4 Dihydrochlorobenzene (4-chlorocatechol). In DFG Deutsche Forschungsgemeinschaft Analyses of Hazardous Substances in Biological Materials volume 6 page 125 – 139. Jurgen Angerer & Karl-Heinz Schaller eds published by Wiley-VCH 1999 ISSN 0179-7247

Knecht U; Woitowitz H-J: Human toxicokinetics of inhaled monochlorobenzene: latest experimental findings regarding re-evaluation of the biological tolerance value. Int Arch Occup Environ Health 73:543–554 (2000).

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