

CHLOROBENZENE : Overview of biological monitoring data available for chlorobenzene

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 10 mmol 4-chlorocatechol/mol creatinine (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.

Biological Monitoring

The IOELV and WATCH documents for chlorobenzene show the potential for dermal exposure to chlorobenzene and give a 'Skin' notation based on a prediction that it may be readily absorbed through the skin and contribute to systemic toxicity. In addition, the occupational hygiene assessment shows that occasionally control of exposure may rely on RPE. In these circumstances biological monitoring may be a useful aid to assessing exposure by all routes and the efficacy of RPE.

The metabolism of chlorobenzene is summarized in figure 1 below

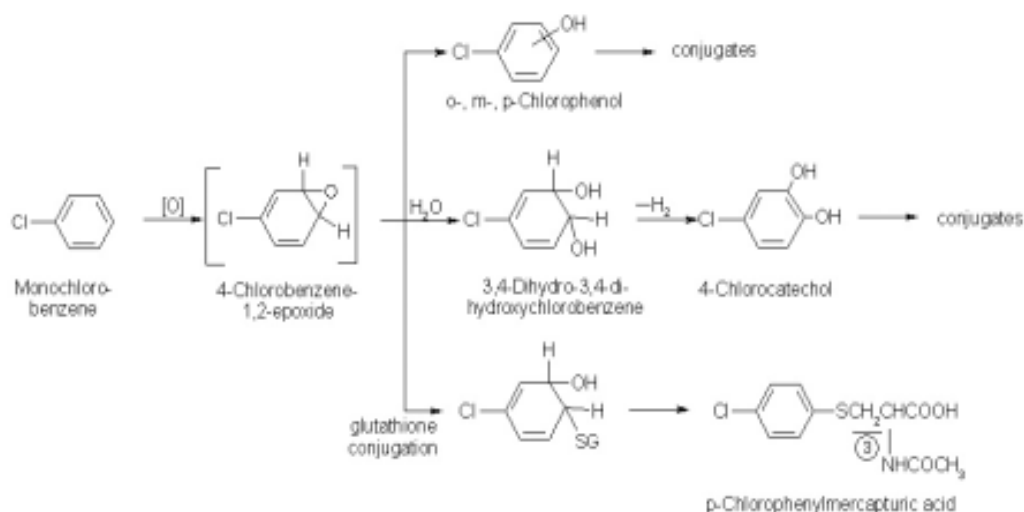


Figure 1 metabolism of chlorobenzene (adapted from Ogata and Shimada 1983 and Yoshida et al 1986).

Analysis of chlorobenzene is possible in blood (Eben 1982, Ogata 1991) but most studies have looked at its major metabolite, 4-chlorocatechol, in urine. In 1992 both the ACGIH and DFG proposed biological guidance values for chlorobenzene. The ACGIH proposed a value of 150mg 4-chlorocatechol /g creatinine (117 mmol/mol) after an 8 hour exposure to 10 ppm (ACGIH 1998). The DFG proposed a BAT value of 300 mg4-chlorocatechol/g creatinine (235 mmol/mol) with an airborne limit of 50 ppm chlorobenzene (DFG 1994). At that time both organisations considered 2 field studies (Yoshida et al 1986 and Kusters & Lauwerys 1990) and one volunteer study (Ogata et al 1991). Since then a further field study (Kumagai & Matsunaga 1994) and a volunteer study (Knecht & Woitowitz, 2000) have been published and both the ACGIH and DFG have revised their guidance values. The BAT value is now 175 mg 4-chlorocatechol /g creatinine (137 mmol/mol) and the BEI is 100 mg 4-chlorocatechol /g creatinine (78 mmol/mol - there is also a BEI of 20 mg p-chlorophenol / g creatinine). Both guidance values are for end of shift urine samples after 8h exposure to 10 ppm chlorobenzene (ACGIH 2007 & DFG 2007. The reason for the difference in values is not clear but may reflect the different approaches of the two organisations (perhaps ceiling values instead of average values).

Occupational Exposure Studies

The field study by Yoshida et al (1986) looked at 11 workers in two chemical factories using 2-3 t/day of chlorobenzene as a solvent. Organic vapour air monitoring badges were attached near the workers breathing zone and urine samples were collected at the end of shift. No details were reported of any protective equipment used or any scope for skin contact with chlorobenzene.

Composition of chlorobenzene metabolites in end of shift urine samples (Yoshida et al 1986)

Metabolite	%
4-Chlorocatechol	76.9 ± 1.6
2-Chlorophenol	3.2 ± 0.3
3-Chlorophenol	7.1 ± 0.6
4-Chlorophenol	12.4 ± 1.1
4-Chlorophenylmercapturic acid	0.4 ± 0.1
Chlorophenylmethylsulphides	not detected

Values are the mean ± SE for 11 workers exposed to a geometric mean of 3.15 ppm chlorobenzene.

Yoshida et al found a linear relationship, with a correlation coefficient 0.87, between urinary 4-catechol and the product of the airborne chlorobenzene concentration and the working time. Using their equation $y = -0.00683 + 0.0155X$ (where $y = 4$ -chlorocatechol in $\mu\text{mol}/\text{mg}$ and $x =$ the product of chlorobenzene in air [ppm] and working time [hours]) an 8hour exposure to 1 ppm would give an end of shift urine 4-chlorocatechol concentration of 17 mg 4-chlorocatechol/g creatinine (13 mmol/mol).

The field study by Kusters & Lauwerys (1990) looked at 44 workers performing maintenance work. A total of 251 whole shift personal air samples and end-of-shift urine samples were collected. The chlorobenzene TWA values were log normally distributed with a median of 1.2 ppm and a range from <0.05 to 106 ppm. After acid hydrolysis of conjugates 4-chlorocatechol and 4-chlorophenol were determined by HPLC with UV detection at 282 nm. The data are shown in figures 2 & 3.

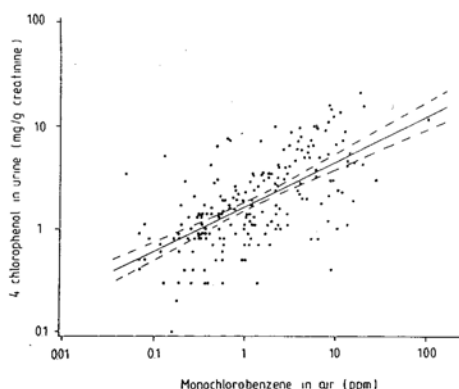


Fig. 2. Relationship between monochlorobenzene concentration (log scale) in air and 4-chlorophenol concentration (log scale) in post-shift urine samples. The interval between the broken lines represents the 95% confidence interval around the regression line (continuous line)

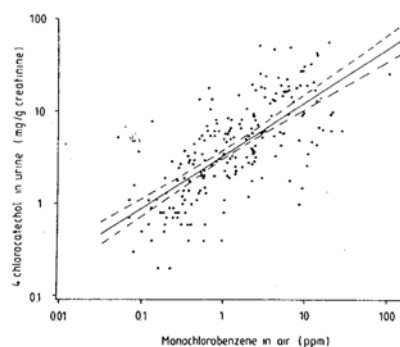


Fig. 3. Relationship between monochlorobenzene concentration (log scale) in air and 4-chlorocatechol concentration (log scale) in post-shift urine samples. The interval between the broken lines represents the 95% confidence interval around the regression line (continuous line)

Figures 2 & 3 From Kusters & Lauwerys (1990)

The Pearson's correlation coefficients between the log chlorobenzene TWA and the log concentration of 4-chlorocatechol was 0.72 ($p < 0.001$) and for 4-chlorophenol it was 0.65 ($p < 0.001$). The relationships were given by, $\log 4\text{-chlorocatechol (in mg/g)} = 0.53 + 0.58 \log \text{chlorobenzene TWA (in ppm)}$ and $\log 4\text{-chlorophenol (in mg/g)} = 0.22 + 0.43 \log \text{chlorobenzene TWA (in ppm)}$. After exposure to 1 ppm 4-chlorobenzene this would give urine concentrations of 3.4 mg 4-chlorocatechol/g creatinine (2.7 mmol/mol) and 1.7 mg 4-chlorophenol/g creatinine. When 21 workers were followed over several days there was no tendency for the metabolite concentration to increase during the week.

The Kumagai & Matsunaga (1994) study looked at 10 workers exposed to chlorobenzene and o-dichlorobenzene during synthesis of dye intermediates. Personal air samples and urine samples (before during and after work) were collected on two successive days and in two different weeks. No details of process or protective equipment were reported. The 8h TWA values of chlorobenzene ranged from 0.2 to 38.5 ppm (dichlorobenzene 0.1 to 4.5ppm) with the majority below 10 ppm. After acid hydrolysis, 4-chlorocatechol and 4-chlorophenol isomers were analysed by HPLC with UV detection at 280 nm. Linear relationships were seen between air values and urine metabolites (both 4-chlorophenol and 4-chlorocatechol) in both end of shift and mid shift samples (figure 4 & 5).

Figure 4 Relation between 8 h TWA value of monochlorobenzene and concentration of 4-chlorocatechol in urine collected during the last four hours of the workshift.

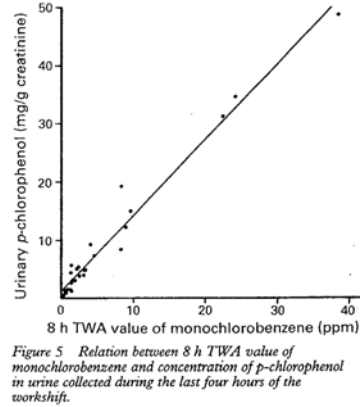
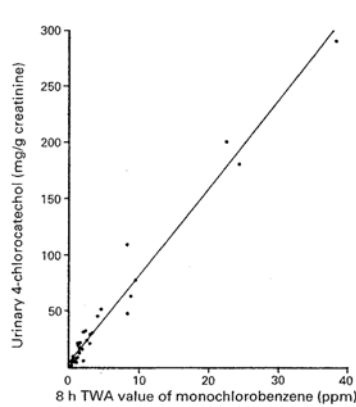
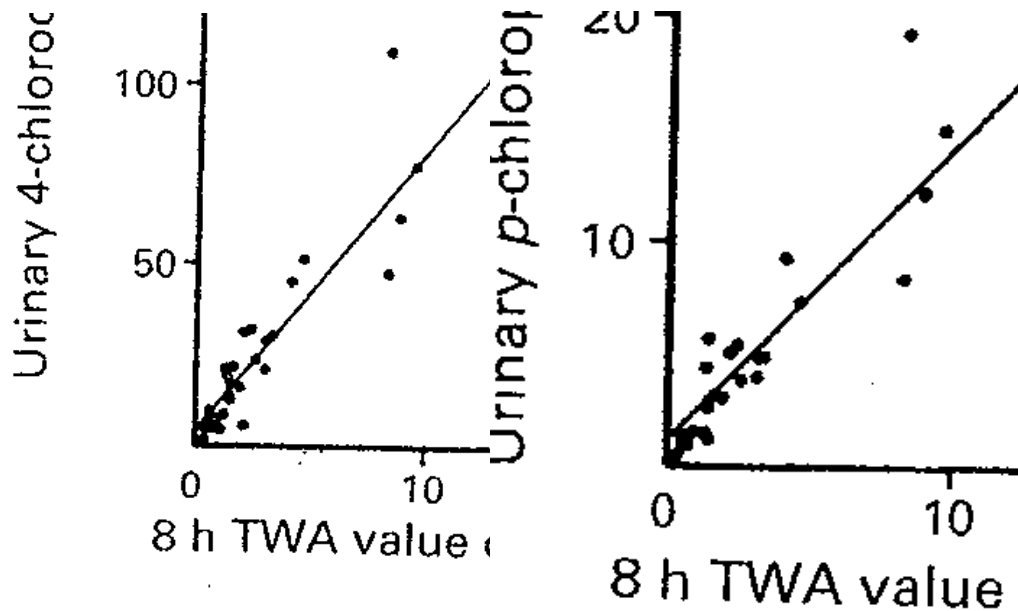


Figure 5 Relation between 8 h TWA value of monochlorobenzene and concentration of p-chlorophenol in urine collected during the last four hours of the workshift.

Figures 4 & 5 from Kumagai & Matsunaga 1994.

The correlation coefficients were highest for creatinine corrected values. In samples collected at the end of shift the relationship for 4-chlorocatechol was given by, $y = ax + b$ where $y = 4\text{-chlorocatechol in mg/g creatinine}$, $a = 7.3$, $x = 8\text{h TWA in ppm}$ and $b = 7.86$. The correlation coefficient was 0.97 ($p < 0.001$). This gives a predicted urinary concentration of 15.2 mg 4-chlorocatechol /g creatinine (11.9 mmol/mol) after 8h exposure to 1 ppm chlorobenzene. For 4-chlorophenol, $a = 1.3$ and $b = 1.93$ (correlation coefficient 0.99, $p < 0.001$). This gives a predicted urinary concentration of 3.2 mg 4-chlorophenol /g after 8h exposure to 1ppm chlorobenzene. However, examination of the data from each graph for exposures less than 10 ppm (figures 6 & 7) shows that the intercepts calculated from the regression lines may be misleading and that without exposure to chlorobenzene there is little 4-chlorocatechol or 4-chlorophenol in urine (see also Ogata et al below).



Figures 6 & 7: Expanded portions of figures 4 & 5 from Kumagai & Matsunaga 1994.

If only the slope of the regression lines are used 1 ppm of chlorobenzene results in 7mg 4-chlorocatechol /g creatinine (5.5 mmol/mol) and 1.3 mg 4-chlorophenol /g

creatinine. Alternatively, estimating from figures 6 & 7 above values of approximately 10 mg 4-chlorocatechol /g creatinine (7.8 mmol/mol) and 2 mg 4-chlorophenol /g creatinine could arise from exposure to 1 ppm chlorobenzene.

In the evaluation of the DFG BAT value (DFG 1994) the lack of agreement between the Kuster & Lauwerys (1990), Yoshida et al (1986) and Ogata (1991) studies was noted and additional, previously unpublished, data from one of the MAK Commission members was presented which agreed with the Ogata et al (1991) work. No details of the study are provided but a table shows data from 17 employees with exposures to chlorobenzene up to 50 ppm had urinary values of 300 mg 4- chlorocatechol /g creatinine (range 190-530 mg/g) and 50 mg 4-chlorophenol /g creatinine (range 10-90mg/g) in post-shift urine samples. Assuming a linear response and interpolating this to exposure at 1 ppm would give a urinary concentration of 6 mg 4-chlorocatechol/ g creatinine (4.7 mmol/mol) and a concentration of 1 mg 4-chlorophenol /g creatinine.

Volunteer studies

Ogata et al (1991) exposed 5 male volunteers (at rest) to either 11.8 ± 0.4 or 60 ± 3.9 ppm of chlorobenzene for 8 hours with a 1 hour (0 ppm) break after 4 h. Although blood and breath samples were collected the breath results were not reported. A proportional relationship was reported between chlorobenzene in air and blood. The slopes of a regression line between air and blood chlorobenzene in samples collected at the end of exposure had a mean of 4.6 ± 1.15 $\mu\text{g/l}$ for 1ppm .

The excretion of 4-chlorocatechol and 4-chlorophenol increased rapidly during exposure and reached a peak at the end of exposure. The urinary 4-chlorocatechol elimination curves showed half lives of 2.2 and 17.3 h if fitted to a two compartment exponential models and 2.9 h if fitted to a 1 compartment model. The elimination half lives of 4-chlorophenol were 3 and 12.2 h if fitted to a 2 compartment model and 7 h if fitted to a 1 compartment model. There was a linear relationship between inhaled chlorobenzene and the metabolites 4-chlorocatechol and 4-chlorophenol in urine collected at the end of exposure (figure 8).

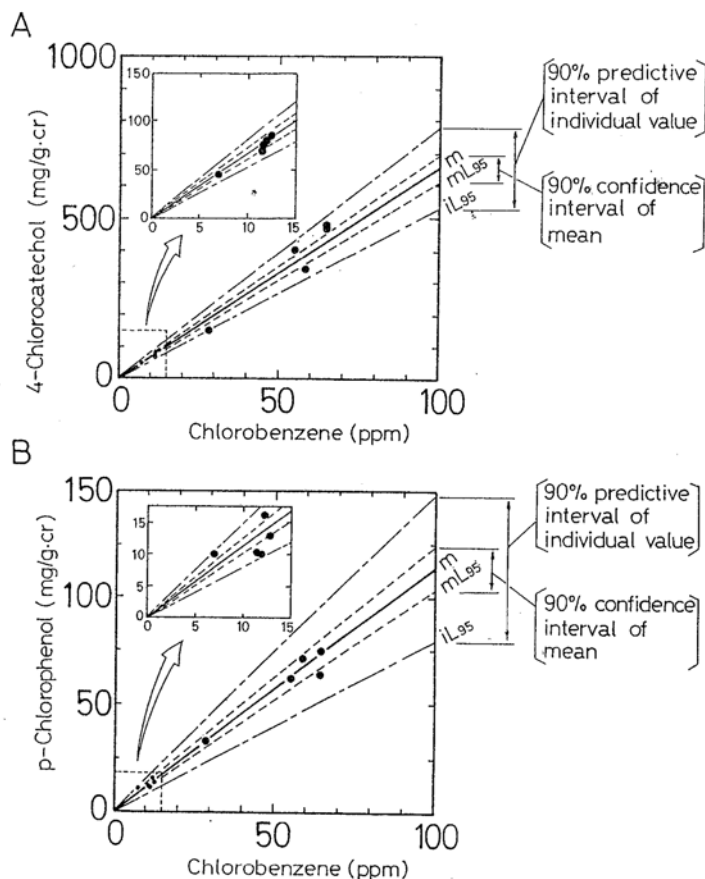


Fig. 5A, B. Relationship between the concentrations of C/Bz in air and the concentration of two metabolites [4-chlorocatechol **A** and *p*-chlorophenol **B**] in urine. Urine specimens were collected during the last 2h of exposure. **A** slopes of the mean (*m*), of the lower 95% confidence limit of the mean (*mL₉₅*), and of the lower 95% predictive limit of individual values (*iL₉₅*) were 6.56, 6.18 and 5.28 mg C/Ch/g creatinine for 1ppm C/Bz, respectively. **B** slopes of *m*, *mL₉₅* and *iL₉₅* were 1.13, 1.03 and 0.8mg *p*-C/Ph/g creatinine for 1ppm C/Bz, respectively. *cr.* Creatinine

Figure 8 Relationship between the concentrations of chlorobenzene in air and the concentrations of 4-chlorocatechol and 4-chlorophenol in urine (from Ogata et al 1991)

After exposure to 1 ppm for 8h (with a 1h break) the 4-chlorocatechol in end of exposure urine samples was predicted to be 6.6 mg 4-chlorocatechol/g creatinine (5.2 mmol/mol). The concentration of 4-chlorophenol would be 1.1 mg 4-chlorophenol/g creatinine.

Knecht & Woitowitz (2000) exposed 8 subjects for 8h to 9.6 +/- 0.4 ppm chlorobenzene over 5 successive days. One volunteer was at rest, five and two volunteers exercised on a bicycle ergometer at 75 and 50W respectively for 10 min each hour. 4-Chlorocatechol concentrations rose rapidly during exposure and reached a maximum at the end of exposure. Mean 4-chlorocatechol concentrations increase with physical exercise of 50 and 75 W by a factor of 1.2 and 2.1 respectively. No significant differences in the metabolite levels were seen day to day. The mean 4-chlorocatechol concentration at the end of 5 days exposure was 150 +/-13 mg 4-chlorocatechol/g creatinine (117 mmol/mol +/- 10) for 5 subjects exposed at 75W which decreased to 25 mg 4-chlorocatechol/g creatinine (20 mmol/mol) by the start of the next exposure. The 4-chlorophenol concentrations under the same conditions were 25 +/- 2 mg/g at the end of exposure and 9 +/-2 mg/g in pre-exposure samples. A biphasic elimination of metabolites was not seen. The elimination half-lives were 6.4 h for 4-chlorocatechol and 12.4 to 16.5 for 4-chlorophenol. Linear interpolation of these values to exposure at 1 ppm and 75W would predict concentrations of 15mg 4-chlorocatechol /g creatinine (12mmol/mol) and 2.5 mg 4-chlorophenol /g creatinine.

Summary of occupational & volunteer studies

Study	exposure ppm	N	Predicted concentrations of urinary metabolites mg/g creatinine after 8h TWA to 1ppm chlorobenzene.	
			4-chlorocatechol	4-chlorophenol
Yoshida et al 1986	3 (g.m)	11 workers	17	-
Kusters & Lauwerys 1990	1.2 (g.m)	44 workers	3.4	1.7
Ogata et al 1991	12 or 60	5 x 2 at rest	6.6	1.1
DFG 'additional data' 1992	up to 50	17 workers	6	1
Kumagai & Matsunaga 1994	0.2 - 39	10 x 4 workers	7* 10**	1.3* 2**
Knecht & Woitowitz 2000	9.6+/-0.4	1 x 5 at rest 2 x 5 50W 5 x 5 75W	7.3 8.9 15	1.3 1.7 2.5

*calculated from the slope of the regression line only

** estimated from figure 4 & 5 in Kumagai & Matsunaga 1994

The 6 reported studies show a wide range of values. There is some agreement between four with the Yoshida et al (1986) and Kusters & Lauwerys (1990) studies as outliers. The Yoshida et al (1986) study was an early field study and a possible explanation for the unusually high 4-chlorocatechol values might be dermal absorption as well as inhalation. The low urinary 4-chlorocatechol seen with the Kusters & Lauwerys study (1990) might be due to loss of metabolites caused by the perchloric acid used for hydrolysis. The Ogata et al study (1991) was a volunteer study with the volunteers at rest so it might be expected that their urinary 4-chlorocatechol values might be less than those in the Kumagai & Matsunaga study (1994) where workers were likely to be more active and breathing harder. There is a reasonable consensus in the table above with 2 volunteer studies and two field studies predicting levels of 6 – 15 mg 4-chlorocatechol /g creatinine after exposure to 1 ppm chlorobenzene for 8h.

Similarly for 4-chlorophenol after exposure to chlorobenzene at 1ppm for 8h the concentration of 4-chlorophenol in end of shift urine samples is between 1 – 2.5 mg 4-chlorophenol/g creatinine.

Biological monitoring values in EH40 are in SI units. A biological monitoring guidance value of 10 mmol 4-chlorocatechol/mol creatinine is equivalent to 13 mg 4-chlorocatechol/g creatinine and 2 mmol/mol 4-chlorophenol is equivalent to 2.2 mg/g creatinine.

Measurement of 4-Chlorocatechol and 4-chlorophenol in urine

There are several methods for the determination of 4-chlorocatechol and 4-chlorophenol in urine (Kusters & Lauwerys 1990, Ogata et al 1991, Kumagai & Matsunaga 1994 & Heinrich-Ramm 1999, Knecht & Woitowitz 2000). All are based on hydrolysis of glucuronide and sulphate conjugates of 4-chlorocatechol and either HPLC with UV detection or Gas chromatography with mass spectrometric detection. A well validated HPLC-UV method is that of Heinrich-Ramm. This method was developed and evaluated to support the DFG biological monitoring programme with an airborne chlorobenzene limit of 10 ppm and measures 4-chlorocatechol only. The most recent uses gas chromatography with selected ion monitoring of the trimethylsilyl derivatives.

Briefly: the Heinrich-Ramm 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 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. The method is linear from 0.5 mg/l to 50mg/l with a detection limit of 0.1 mg/l (roughly 0.08 mmol 4-chlorocatechol/mol creatinine) and a coefficient of variation for within-day imprecision of 2.3% and a day to day imprecision of 4.9%. No peaks have been found in chromatograms interfering with 4-chlorocatechol at levels greater than 0.5 mg/l from people not occupationally exposed to chlorobenzene.

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