3,5,6-Trichloro-2-pyridinol (TCPyr)

Application	Determination in urine
Analytical principle	Capillary gas chromatography/ mass spectrometric detection (MS)
Completed in	March 2004

Summary

The internal levels of the organophosphate pesticides Chlorpyrifos or Chlorpyrifos-methyl in persons as a result of occupational or environmental exposure to these substances can be determined by means of this method. For this purpose the specific main metabolite of this organophosphate pesticide, 3,5,6-trichloro-2-pyridinol (TCPyr), is assayed in urine by means of GC/MS-SIM.
The acidified urine samples, together with 2,6-dibromophenol, which serves as an internal standard, are hydrolysed at 80 °C and then subjected to steam distillation. This process completely hydrolyses the TCPyr conjugates and simultaneously separates them from the organic matrix. The distillate is purified over an Isolute®-101 polymer phase (polystyrenedivinylbenzene copolymer), concentrated and dried. After elution the TCPyr and the internal standard are derivatised with N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA). The resulting *tert*-butyldimethylsilyl ethers are separated by capillary gas chromatography and detected by means of mass spectrometry.
Calibration is performed using calibration standards that are prepared in pooled urine and are treated in the same manner as the samples to be analysed.

3,5,6-Trichloro-2-pyridinol (TCPyr)

Within-series imprecision:	Standard deviation (rel.)	$s_w = 4.2\%$
	Prognostic range	$u = 9.9\%$
	at a spiked concentration of 2.5 µg per litre urine and where n = 8 determinations	
Between-day imprecision:	Standard deviation (rel.)	$s_w = 2.0\%$
	Prognostic range	$u = 4.7\%$
	at a spiked concentration of 2.5 µg per litre urine and where n = 7 determinations	

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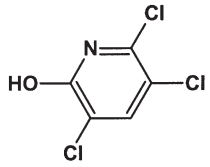
Accuracy:	Recovery rate	$r = 104\%$

Test for matrix effects using urine samples from individual test persons (creatinine 0.79 to 4.7 g/L):

	Standard deviation (rel.)	$s_w = 7.1\%$
	Prognostic range	$u = 16.7\%$
	at a spiked concentration of 1.8 µg per litre urine and where n = 5 individual urine samples	
Accuracy:	Recovery rate	$r = 94\%$ (85 to 103%)
Detection limit:	0.05 µg 3,5,6-trichloro-2-pyridinol per litre urine	

3,5,6-Trichloro-2-pyridinol (TCPyr)



3,5,6-Trichloro-2-pyridinol (CAS No. 6515-38-4, synonyms: hydroxy-3,5,6-trichloro-pyridine; 3,5,6-trichloropyridinone) is a white crystalline substance (vapour pressure: 0.00103 mmHg (25 °C), solubility in water: 80.9 mg/L (25 °C)).
It is formed during the intermediate metabolism of the organophosphate pesticides Chlorpyrifos and Chlorpyrifos-methyl (see Figure 1).

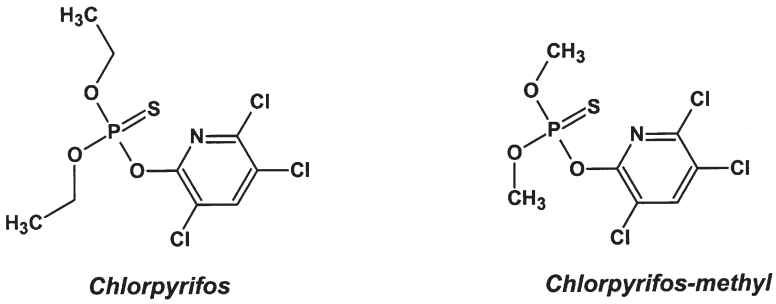


Fig. 1. Structural formulae of Chlorpyrifos and Chlorpyrifos-methyl

TCPyr is primarily excreted in urine as a glucuronide or a sulphate conjugate. Quantitative determination is possible only after hydrolysis of the conjugates. According to Nolan et al. [1] and Aprea et al. [2] TCPyr excretion is a reliable indication of exposure to Chlorpyrifos and Chlorpyrifos-methyl, and is therefore a more sensitive and more specific parameter than inhibition of the cholinesterase activity. TCPyr is a specific metabolite of the above-mentioned organophosphate pesticides Chlorpyrifos

and Chlorpyrifos-methyl. It is therefore a useful supplementary method to alkyl-(thio)phosphate analysis, which is only able to detect the cumulative exposure to a variety of organophosphate pesticides by determining the six possible alkyl-(thio)phosphate moieties of this pesticide class [3, 4].

Chlorpyrifos and Chlorpyrifos-methyl are degraded to TCPyr via the Chlorpyrifos oxone in warm-blooded organisms. This oxone is responsible for inhibition of specific and unspecific cholinesterases, which is characteristic of the toxicity of organophosphates. Figure 2 shows an overview of the metabolism of Chlorpyrifos in mammals.

70% of the orally ingested and less than 3% of the dermally applied chlorpyrifos are excreted in the urine as TCPyr with an elimination half-life of 27 h [1]. 10% of the

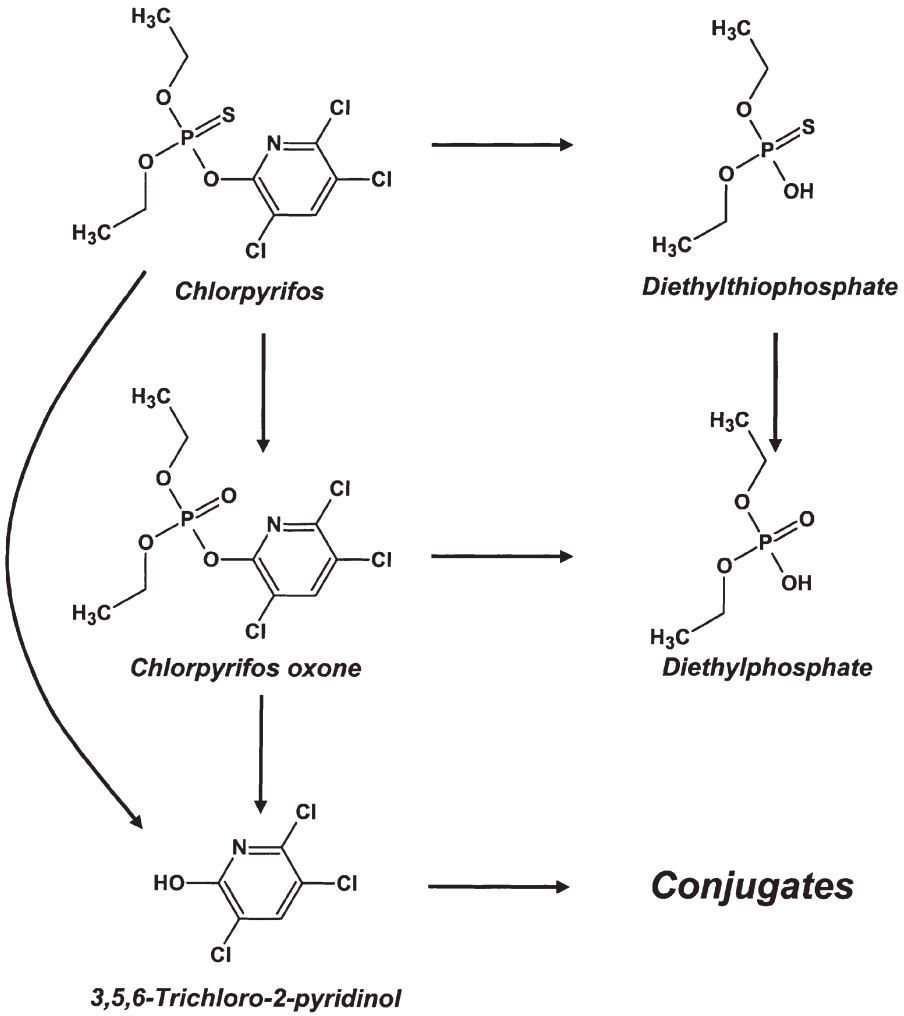


Fig. 2. Metabolism of Chlorpyrifos in mammals

Chlorpyrifos metabolites are excreted with the faeces [5]. Only a small amount of Chlorpyrifos is accumulated in the fatty tissue and it is excreted with an elimination half-life of 62 h [5]. Similar values can be expected for Chlorpyrifos-methyl.
Technical grade Chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is an amber crystalline substance, but it is a colourless crystalline solid (melting point: 42 to 43.5 °C) with a slightly sulphurous odour in its chemically pure form. At present Chlorpyrifos is used in Germany for agricultural purposes in seven commercially available products, of which Dursban® and Ridder® are the best known. It has a broad application range against pests that affect fruit and vegetables and ornamental plants. In addition, Chlorpyrifos is also contained in biocidic products (in particular for controlling ants) for household or trade use and in antiparasitic agents for pets. At present there is neither a MAK nor a BAT value for Chlorpyrifos.
Chlorpyrifos-methyl (O,O-dimethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is a colourless crystalline solid (melting point: 45.6 to 46.5 °C). Chlorpyrifos-methyl is not authorised for agricultural use in Germany, but it is employed in other European countries and worldwide, and it reaches the German market in agricultural products. An application in biocidic products cannot be ruled out in Germany either. At present neither a MAK nor a BAT value exists for Chlorpyrifos-methyl.
On account of the wide distribution of the Chlorpyrifos and Chlorpyrifos-methyl organophosphates and the numerous areas in which they are applied they may be contained as residues in many plant foodstuffs and they may even occur in the ambient air after use under certain circumstances. As a result they are taken in by the general population and are metabolised in the human body to TCPyr. The TCPyr is mainly bound to glucuronic acid and excreted in the urine. It cannot be ruled out that TCPyr itself, as a degradation product of the above-mentioned pesticides, is also partly absorbed with nutrition. However, a study showed that the TCPyr content in urine is correlated with the dietetic and non-dietetic exposure of the general population to Chlorpyrifos, and that absorbed Chlorpyrifos/Chlorpyrifos-methyl quantities and excreted amounts of TCPyr are largely concordant [6].
Table 1 shows an overview of the excretion of TCPyr in the general population.

Table 1. Concentrations (in µg/L and µg/g creatinine) of TCPyr in urine samples of persons not exposed at the workplace

	n	Group of test subjects	Median	95%	References
µg/L	993	General population, USA	3.0	13	[7]
	50	General population, Germany	1.4	11.3	[8]
µg/g creatinine	993	General population, USA	2.2	8.8	[7]
	50	General population, Germany	1.0	6.7	[8]
	42	General population, Italy	2.6	No data	[2]

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1 General principles

The acidified urine samples together with 2,6-dibromophenol, which serves as an internal standard, are hydrolysed at 80 °C and then subjected to steam distillation. This process completely hydrolyses the TCPyr conjugates and simultaneously separates them from the organic matrix. The distillate is purified over an Isolute®-101 polymer phase (polystyrenedivinylbenzene copolymer), concentrated and dried. After elution the TCPyr and the internal standard are derivatised with N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA). The resulting *tert*-butyldimethylsilyl ethers are separated by capillary gas chromatography and detected by means of mass spectrometry.
Calibration is performed using calibration standards that are prepared in pooled urine and are treated in the same manner as the samples to be analysed.

2 Equipment, chemicals and solutions

2.1 Equipment

Gas chromatograph with split/splitless injector, mass selective detector (MSD) and data processing system

Capillary gas chromatographic column:
Length: 60 m, inner diameter: 0.25 mm; stationary phase: 35% phenylmethylpolysiloxane; film thickness: 0.25 µm (e.g. from Agilent)

10 µL Syringe for gas chromatography, but the use of an autosampler is preferable

Steam distillation apparatus (e.g. Gerhardt Vapodest Vap 20)

Laboratory centrifuge

Drying cupboard

Thermostatically controlled water bath (e.g. from Gerhardt)

3 mL Solid-phase extraction column, filled with 100 mg Isolute®-101 material (from IST, UK)

Workstation for solid phase extraction (e.g. from Baker)

Device for evaporation under a stream of nitrogen

25 mL Screw-capped jars with screw caps and PTFE-coated inner septa

1.8 mL Crimp-cap vials with PTFE-coated septa and crimp caps as well as crimping tongs (e.g. from Macherey-Nagel, Düren, Germany)

Microinserts for the crimp-cap vials, usable volume 100 µL (e.g. from Macherey-Nagel, Düren, Germany)

Glass pipettes, 25 mL and 50 mL

Microlitre pipettes, variable between 100 and 1000 µL (e.g. from Eppendorf)

Finn pipette 1–5 mL

Volumetric flasks: 50, 100, 200, 250, 500 and 1000 mL

2.2 Chemicals

25% Hydrochloric acid p.a. (e.g. from Merck)

Acetonitrile SeccoSolv® (Merck)

Glacial acetic acid p.a. (e.g. from Merck)

Toluene SupraSolv® (Merck)

Methanol p.a. (e.g. from Merck)

Bidistilled water

3,5,6-Trichloro-2-pyridinol (e.g. 10 ng/mL in acetonitrile; Dr. Ehrenstorfer, Augsburg, Germany)

2,6-Dibromophenol p.a. (e.g. from Aldrich)

N-*tert*-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) (e.g. from Fluka)

Helium 5.0 (e.g. from Linde)

Nitrogen 4.6 (e.g. from Linde)

2.3 Solutions

0.1 M Hydrochloric acid:
12.8 mL hydrochloric acid (25%) are pipetted into a 1000 mL volumetric flask, into which approx. 500 mL bidistilled water have been previously placed. The flask is subsequently filled to its nominal volume with bidistilled water.

Solution of the internal standard

Starting solution:
Approximately 25 mg 2,6-dibromophenol are weighed exactly into a 50 mL volumetric flask. The flask is then filled to its nominal volume with acetonitrile (500 mg/L).

Stock solution:
5 ml of the starting solution of the internal standard are pipetted into a 50 mL volumetric flask. The flask is then filled to its nominal volume with acetonitrile (50 mg/L).

Working solution:
2 mL of the stock solution are pipetted into a 100 mL volumetric flask. The flask is subsequently filled to its nominal volume with bidistilled water (1 mg/L).

2.4 Calibration standards

Starting solution for the calibration standards:
The certified TCPyr solution from Dr. Ehrenstorfer (10 mg/L in acetonitrile) serves as the starting solution.
The calibration standard solutions are prepared in pooled urine from non-exposed test persons. For the purpose of preparing pooled urine, spontaneous urine samples are collected from the test persons in a suitable vessel, thoroughly mixed and stored at –18 °C until the standards and the control material are prepared. If necessary, the TCPyr content of the individual urine samples used to prepare the pooled urine is checked.

Stock solution:
2.5 mL of the starting solution of the calibration standard are pipetted into a 500 mL volumetric flask. The flask is subsequently filled to its nominal volume with filtered pooled urine (50 µg/L).
Calibration standards in concentrations ranging from 0.125 to 15 µg TCPyr per litre urine are prepared from this stock solution by dilution with pooled urine. It is important to include the pooled urine used for dilution as a blank value of the calibration in order to take any possible background content of TCPyr into account.
Table 2 shows the pipetting procedure to be followed.

Table 2. Pipetting scheme for the preparation of the calibration standards

Volume of the stock solution [mL]	Pooled urine approx. [mL]	Final volume of the calibration standard [mL]	Concentration of the calibration standard [µg/L]
250	0	250	50
75	175	250	15
25	225	250	5
5	245	250	1
2	248	250	0.4
0.5	199.5	200	0.125

The calibration standards are divided into aliquots in suitable vessels and can be stored in the deep-freezer at –18 °C without loss of TCPyr for at least six months.

2.5 Conditioning of the Isolute®-101 columns

The Isolute®-101 columns are washed with 2 mL methanol, 2 mL bidistilled water and 2 mL 0.1 M hydrochloric acid in that sequence.

3 Specimen collection and sample preparation

The urine is collected in sealable polyethylene bottles and stored in the deep-freezer at approx. –18 °C until processing. The urine can be stored in this manner for at least six months.

3.1 Sample preparation

The urine is allowed to thaw and is brought to room temperature. After the urine has been shaken intensively, aliquots of 10 mL are transferred to 25 mL screw-capped jars. Then 1 mL of 25% HCl and 250 µL of the working solution of the internal standard are added to each aliquot using pipettes, the jar is sealed and the mixture is hydrolysed at 80 °C in the drying cupboard for 1.5 hours. After cooling, the hydrolysed samples are subjected to steam distillation (e.g. Gerhardt Vapodest Vap-20 automatic distillation device, vapour pressure 50%, distillation time approx. 250 seconds). Traditional steam distillation apparatus may be used as an alternative to automatic steam distillation. Approx. 50 mL of the distillate are trapped in a 50 mL volumetric flask into which 1 mL of 25% HCl has been previously placed.
The distillate is drawn through the conditioned Isolute®-101 solid phase column (approx. 5 mL/min). It is practical to use a workstation for solid phase extraction in order to process samples by means of liquid-solid extraction.
The Isolute®-101 material that absorbs the TCPyr and the internal standard is washed with 5 mL of 0.1 M HCl and then with 4 mL bidistilled water. The columns are pre-dried by briefly applying vacuum at the workstation, and then further dried by subsequent centrifugation (5 min, 4500 g). The columns are again mounted at the workstation under vacuum and sucked completely dry (approx. 45 minutes).
The cartridges are taken from the workstation and placed directly on the 1.8 mL crimp-capped vials for the purpose of elution. Elution is carried out three times under normal pressure using 0.5 mL acetonitrile in each case. After adding 75 µL toluene as a keeper, the eluate is carefully evaporated to 150 µL in a stream of nitrogen. Then 25 µL MTBSTFA are added. The solution is transferred to a glass insert, sealed and derivatised for 45 min at 70 °C in the drying cupboard.

4 Operational parameters

4.1 Operational parameters for gas chromatography and mass spectrometry

Capillary column:	Material:	Fused silica
	Stationary phase:	HP-35
	Length:	60 m
	Inner diameter:	0.25 mm
	Film thickness:	0.25 µm
Detector:	Mass selective detector (MSD)	
Temperatures:	Column:	Initial temperature 90 °C, 1 minute isothermal, then increase at a rate of 25 °C/min to 125 °C, 1 minute isothermal, then increase at a rate of 6 °C/min to 230 °C, 2 minutes isothermal; then increase at a rate of 30 °C/min until 290 °C, 12 min at the final temperature
	Injector:	280 °C
	Transfer line:	280 °C
Carrier gas:	Helium 5.0 with the following pressure program:	
		5 minutes at 1.45 bar; then increase of 0.0035 bar per minute until 2.0 bar; then increase of 0.07 bar per minute to the final pressure of 2.25 bar
Split:	Splitless, split on after 55 s	
Sample volume:	1 µL	
Ionisation type:	Electron impact ionisation (EI)	
Ionisation energy:	70 eV	
Dwell time:	50 ms	
Electron multiplier:	1600 V + 600 V rel.	

All other parameters must be optimised in accordance with the manufacturer's instructions.

5 Analytical determination

The urine samples processed as described in Section 3.1 are analysed by injecting 1 µL of each derivatised extract into the gas chromatograph. The temporal profiles of the ion traces shown in Table 3 are recorded in the SIM mode:

Table. 3. Retention times and recorded masses

Compound	Retention time [min]	Masses
TCPyr	18.52	254 256* 258
2,6-Dibromophenol (IS)	21.55	307 309* 311

*The masses marked * are used for quantitative evaluation.*

The retention times given in Table 3 serve only as a guide. Users of the method must satisfy themselves of the separation power of the capillary column used and the resulting retention behaviour of the substances. The chromatogram of a native urine sample from the general population (2.42 µg/L TCPyr) is shown in Figure 3.
If the measured values are above the linear range of the calibration graphs (> 50 µg/L), the urine samples are diluted with ultrapure water in the ratio of 1 : 10, processed anew and measured.
A quality control sample and a reagent blank value consisting of ultrapure water are analysed in each analytical series.

6 Calibration

The calibration standards (Section 2.4) are processed in the same manner as the urine samples (Section 3.1) and analysed by gas chromatography/mass spectrometry as described in Sections 4 and 5. Calibration graphs are obtained by plotting the quotients of the peak areas of the TCPyr and that of the internal standard as a function of the concentrations used. It is unnecessary to plot a complete calibration graph for every analytical series. It is sufficient to analyse one calibration standard for every analytical series. The ratio of the result obtained for this standard and the result for the equivalent standard in the complete calibration graph is calculated. Using this quotient, each result read off the calibration graph is corrected.
New calibration graphs should be plotted if the quality control results indicate systematic deviation.
The calibration graph is linear between the detection limit and 50 µg TCPyr per litre urine.

7 Calculation of the analytical result

Quotients are calculated by dividing the peak areas of the analyte by that of the internal standard. These quotients are used to read off the pertinent concentration of TCPyr in µg per litre urine from the relevant calibration graph. If the pooled urine used to prepare the calibration standards exhibits a background signal, which is to be expected, the resulting calibration graph must be shifted in parallel so that it passes through the zero point of the coordinates. The concentrations of the background exposure can be read off from the point where the graph intercepts the axis before parallel shifting in each case. No reagent blank value has been ascertained during analysis to date.

8 Standardisation and quality control

Quality control of the analytical results is carried out as stipulated in the guidelines of the Bundesärztekammer (German Medical Association) [9] and in the special preliminary remarks to this series. In order to determine the precision of the method a control urine sample containing a constant concentration of TCPyr is analysed. As material for quality control is not commercially available, it must be prepared in the laboratory. For this purpose, pooled urine is spiked with a defined quantity of TCPyr. A six-month supply of this control material is prepared, divided into aliquots in suitable vessels and stored in the deep-freezer. The concentration of this control material should be in the concentration range of the background exposure of the general population (see Table 1). The expected value and the tolerance range of this quality control material are ascertained in a pre-analytical period (one analysis of the control material on each of 20 different days) [10–12].

9 Evaluation of the method

9.1 Precision

Pooled urine is used to determine the precision in the series. This urine contained TCPyr with a concentration of 0.99 µg per litre urine. In addition, the urine was spiked with 2.5 µg/L to give a final concentration of 3.49 µg/L. Eight analyses of this urine sample yielded the precision in the series shown in Table 4.
In addition, the precision from day to day was determined. Pooled and spiked urine was also used for this purpose. This urine was processed and analysed on 7 different days.
Furthermore, the precision was investigated using five urine samples from individual test persons to obtain further information on the occurrence of matrix effects. The samples were spiked with 1.8 µg/L TCPyr and were analysed as spiked and non-spiked samples. The creatinine content of the individual urine samples was in the range of 0.79 to 4.7 µg/L. The precision results are also shown in Table 4.

Table 4. Precision for the determination of TCPyr

	n	Spiked concentration [µg/L]	Standard deviation (rel.) [%]	Prognostic range [%]
In the series	8	2.5	4.2	9.99
From day to day	7	2.5	2.0	4.72
In urine samples from individuals	5	1.8	7.1	16.7

9.2 Accuracy

Recovery experiments were performed to check the accuracy of the method. For this purpose pooled urine with and without the addition of a defined amount of TCPyr and urine samples from individual test persons with and without the addition of a defined quantity of TCPyr were analysed. The spiked quantity of TCPyr was 2.5 µg/L in the case of the pooled urine. The relative recovery rate was 104% in this case.
As part of the investigation of matrix effects the relative recovery was also measured in urine samples with a creatinine content between 0.79 g/L and 4.7 g/L from individual test persons. The urine samples of individuals were spiked with a quantity of 1.8 µg/L of TCPyr. The mean relative recovery rate for the urine samples of individuals was 94% with a range of 85 to 103%. No influence of the creatinine content on the relative recovery could be confirmed.

9.3 Detection limits

The detection limit for TCPyr was 0.05 µg per litre urine under the conditions for sample preparation and gas chromatographic/mass spectrometric determination given here. The detection limit was estimated as three times the signal-background ratio.

9.4 Sources of error

The described method for the determination of TCPyr in urine permits its reliable assay in the trace range. Other substances in the urine matrix are clearly separated from the analytes.
As a rule, background exposure is to be expected. This has an influence on the calibration of the pooled urine standards (see Section 7).
It is necessary to take into account and to eliminate the risk of carry-over contamination of analytes in the steam distillation apparatus. A washing step after distillation of urine is recommended, depending on the apparatus used.
A pre-condition for derivatisation using MTBSTFA is the absence of water. Therefore the solid phase must be thoroughly dried before elution. It is important to con-

sider that the duration of drying can differ from cartridge to cartridge and from workstation to workstation.
When the eluate is concentrated in a stream of nitrogen it is essential to add a keeper (toluene in this case). Moreover, the eluate must on no account be dried in this processing step. Otherwise irreproducible results due to losses during processing are to be expected.

10 Discussion of the method

The method described here permits reliable and accurate determination of TCPyr in urine, even at the concentration range due to environmental exposure. The range of relevance to occupational medicine can be determined at the same time. The reliability criteria can be regarded as very good and the examiners of the method were able to reproduce them at their first attempt.
In comparison with methods described in the literature to date [2, 7] this method is remarkable for its detection limit, which is lower by a factor of 10 and permits complete coverage of the concentration range of relevance to environmental medicine. Moreover, in contrast to other methods, no technically complicated detection instruments, such as a triple quadrupole mass spectrometer, are required [7].
Therefore this method is very suitable for measuring the background exposure in the general population. Despite the relatively laborious sample preparation by means of steam distillation, this procedure is still quite suitable for routine investigation.
Another internal standard, 3,5-dichloro-2-hydroxy-pyridine (CAS No. 5437-33-2) (Sigma-Aldrich, Cat. No. 574414), became available only after the method development had been completed. From the chemical point of view this substance is more closely related to TCPyr than the internal standard (2,6-dibromophenol) used in this method. In the opinion of both the authors and the examiners this standard would be more suitable as an internal standard for the assay of TCPyr. It was impossible to carry out a final test of this internal standard. Alternatively, the experience of the examiners shows that TCPyr can also be evaluated with good reproducibility without using an internal standard but by means of external calibration.
In addition to the solution of 3,5,6-trichloro-2-pyridinol available from Dr. Ehrenstorfer (10 mg/L in acetonitrile), it can also now be supplied as a solid by Supelco, which should facilitate the preparation of standard solutions.
Calibration using aqueous standards is also possible. The gradients obtained using aqueous calibration standards were almost identical to those resulting from calibration using pooled urine standards. This confirms that no relevant matrix effects are present. This was also shown in the tests for matrix effects, during which recovery experiments on urine samples from individual test persons with very different creatinine contents (0.79 to 4.7 g creatinine/L) were carried out. As the resulting relative recovery rate (at a spiked concentration of 1.8 µg TCPyr/L) of 94% (range: 85 to 103%) shows, an influence of the matrix on the analytical result can be almost completely ruled out. This is largely due to the effective separation of the urine matrix by means of steam distillation.

Solid phases specially optimised for phenols (e.g. ENV+® from IST or Strata X® from Phenomenex) can be used instead of the Isolute®-101 phase for the purification and concentration of the steam distillate. However, the different drying times must be checked in this case. In the experience of the examiners Strata X® cartridges, which have a structure similar to Isolute®-101, can also be used without problems.
The Isolute®-101 phase or other possible polymer phases are preferable to silica-based solid phases, as a conditioning step is unnecessary in the case of polymer phases and the column can run dry during processing without adverse effects.
The derivatisation techniques that can be used for chlorophenols via diazomethane or with chloroformic acid trichloroethyl ester as an alternative derivatisation reagent (Schotten-Baumann reaction) [13] proved to be inapplicable for TCPyr analysis. This might possibly be due to the keto-enol tautomerism of TCPyr.
The sample preparation by steam distillation is relatively laborious. In the view of the examiners steam distillation can be dispensed with if a high-resolution sector-field MS system is used. In this case the urine volume used should be decreased to 1 mL.
On principle the procedure described here can also serve to determine other chlorophenols of relevance for biological monitoring. For more information the reader should refer to the 7th volume of "Analysis of Hazardous Substances in Biological Materials" [14, 15].

Instruments used:
Gas chromatograph 5890 Series II with mass selective detector 5972, autosampler 7673 and data system from Hewlett-Packard.

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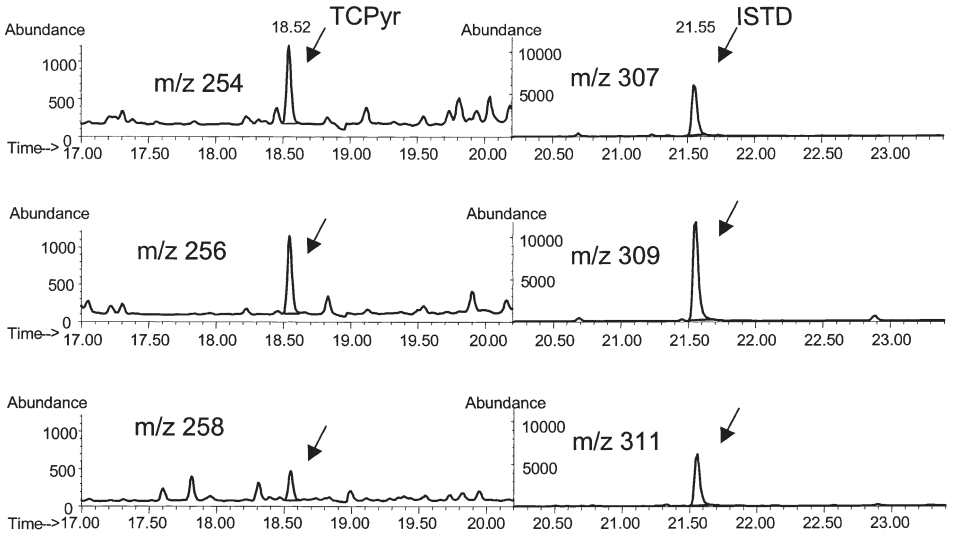

Fig. 3. Chromatogram of a worked-up native urine sample containing 2.42 µg/L of TCPyr