We developed an automated colorimetric method for the quantitative determination of \( p \)-aminophenol with a Cobas Mira analyzer. The procedure can be used for the biological monitoring of human exposure to aniline. An absorbed aniline dose is extensively oxidized to \( p \)-aminophenol, which is excreted in urine mainly as glucurono- and sulfo-conjugates. After enzymatic hydrolysis, we reacted the free compound with resorcinol in the presence of manganese ions to form an indophenol dye, which is measured at 550 nm. Excellent accuracy (102.8%, 103.9%, and 96.8% at 2.5, 50, and 90 mg/L, respectively) and precision (7.7%, 2.1%, and 0.8% CV for within-run and 11.1%, 4.7%, and 4.6% for total reproducibility at 2.5, 50, and 90 mg/L, respectively) were achieved over a linear concentration range of 2.0 to 100 mg/L. The detection limit was 0.9 mg/L and no significant interference (except for \( o \)-aminophenol) was found for several investigated drugs and related compounds.

The proposed method was used for a stability study and to analyze several samples from an occupational health screen.

INDEXING TERMS: 4-aminophenol • occupational medicine • aniline • stability

In toxicology and occupational health medicine, \( p \)-aminophenol is used as a biological marker to screen for human aniline exposure [1–3]. Aniline has been produced commercially since 1847 [4]; it has been classified as a possible carcinogen in humans [5] and is recognized by the National Institute for Occupational Safety and Health as a neurotoxic agent [6]. It is a component of printing inks, cloth-marking inks, paints, and paint removers. Aniline is widely used industrially for making dyes and compounds used in the vulcanization of rubber [7]. It also serves as a chemical intermediate for the synthesis of certain pharmaceuticals. Its numerous applications in various industrial processes [EC production (1991): 660 kton, US production (1992): 460 kton] makes occupational exposure its main health hazard, although contamination of the general environment has been reported to occur [4]. The 50% lethal dose (LD\(_{50}\)) for acute oral aniline toxicity is 440 mg/kg [8] and a threshold limit value of 2 ppm is generally accepted [3]. Acute aniline intoxications cause methemoglobinemia and cyanosis; chronic exposure can result in liver damage and may affect the nervous system and bone marrow [2]. Aniline vapor is absorbed mainly through the lungs, and liquid aniline readily penetrates intact skin. About 15–60% of an absorbed aniline dose is oxidized by a cytochrome P-450-dependent reaction to \( p \)-aminophenol, which is excreted in urine mainly as glucuronide and sulfate conjugates [1, 2, 9, 10]; <1% of the absorbed aniline is excreted unchanged in the urine [2]. In exposed workers, the urinary \( p \)-aminophenol concentration appears to be directly related to the blood methemoglobin concentration [2, 3]. Few other industrial chemicals (mainly nitrobenzene, acetanilide) and some pesticides (e.g., Fenuron) also result in the elimination of \( p \)-aminophenol. \( p \)-Aminophenol is also a minor metabolite of paracetamol [3], and although this implies that the presence of \( p \)-aminophenol can, in some cases, be related to exposure to chemicals other than aniline, it is nonetheless accepted as a more specific indicator than any other currently in use [3, 11].

For use as a monitoring method to detect groups of workers at risk of (excessive) exposure to aniline, the determination of total \( p \)-aminophenol in urine specimens should be fast and simple to perform. Gas chromatography has been used to identify oxidation dyes, including aminophenols, on human hair [12]. The method includes an extensive extraction procedure and involves mass spectrometry for detection. In another report, various phenols, including \( p \)-aminophenol, were extracted by supercritical fluid extraction and analyzed by using gas chromatography with atomic emission detection [13]. This
complex approach was not performed on biological samples, nor was it quantitative. Analysis of p-aminophenol by using HPLC is done for water analysis [14, 15], with direct injection or simple preconcentration, and to quantify p-aminophenol as a degradation product of paracetamol in aged pharmaceutical formulations after dissolution in methanol [16]. One group reported the urinary analysis of various phenols, including p-aminophenol, by using HPLC and solvent extraction [10]. However, the method was developed primarily for phenol and the cresols, resulting in the elution of the polar p-aminophenol in the eluent front. In real biological samples the p-aminophenol peak becomes completely obscured by endogenous urine constituents, preventing qualitative as well as quantitative interpretation. In our laboratory we were not able to reproduce this chromatographic method on real biological samples. The HPLC methods involve UV, fluorometric, and electrochemical detection. The latter principle, based on p-aminophenol being easily oxidized to p-iminoquinone, is also used in a p-aminophenol biosensor. That report involves this rather complex system to determine alkaline phosphatase activity after the catalyzed hydrolysis of p-aminophenylphosphate [17]. The most frequently used method in biological exposure monitoring is, however, based on a simple colorimetric test in which p-aminophenol, after an acid hydrolysis step, is reacted with phenol in an alkaline medium to form an indophenol dye [2, 3, 18]. The same principle was used in a procedure to measure paracetamol in serum after enzymatic hydrolysis (amidase) to an equimolar amount of p-aminophenol [19, 20]. It provided a fast, simple, and reliable method, albeit for a 100-fold higher concentration interval.

Our aim was to develop and validate a suitable method for urinary p-aminophenol based on enzymatic deconjugation and the indophenol reaction and to automate it with a clinical analyzer to provide a simple and quick screening test to be used in toxicology and occupational medicine.

Materials and Methods

Reagents

All reagents and chemicals were of analytical grade and were obtained from E. Merck (Darmstadt, Germany) unless stated otherwise. Manganese chloride tetrahydrate and β-glucuronidase/arylsulfatase (type HP-2 from Helix pomatia, 103 × 10⁶ U/L) were from Sigma (St. Louis, MO). Resorcinol was from UCB (Brussels, Belgium) and 3-cyclohexylamino-1-propanesulfonic acid (CAPS, 99%) from Aldrich (Milwaukee, MI). Doubly distilled water was used throughout the procedure.

Calibrators

A stock solution of p-aminophenol was prepared by dissolving 20 mg in 10 mL of acetonitrile. Serial dilution of this stock solution with water yielded working solutions at concentrations of 20, 50, 100, 200, 500, and 1000 mg/L. The stock solution and subsequent aqueous calibration dilutions were all made in brown glassware and on a daily basis, immediately before use, to avoid deterioration.

Samples

Urine samples were obtained from healthy adult volunteers, some after having received a 500-mg paracetamol dose, or from potentially exposed workers at the end of their workshift. The samples were kept in well-closed containers, without additives, in the dark and refrigerated. Before analysis their creatinine content was determined. All procedures and experiments were in compliance with the regulations and ethical standards of our university’s ethical committee. Subjects participating in the paracetamol experiment all gave informed consent.

Instruments and Equipment

Spectrophotometric studies were performed on a Philips Analytical PU8740 UV/Vis Scanning Spectrophotometer with printer/plotter (Cambridge, UK). A Roche Cobas Mira® (Basel, Switzerland) automated clinical analyzer was used for the analysis procedure. Dilutions of calibrator solutions were made with a Hamilton Digital Dilutor (Bonaduz, Switzerland).

Final Method

Enzymatic Hydrolysis. All urine samples were first adjusted to pH 5.0 with 2 mol/L HCl. Then, 900 μL of this urine was mixed with 100 μL of H₂O₂ or aqueous calibrator in the case of calibration samples. To 250 μL of this mixture in Roche 500-μL sample cups, 50 μL of an enzyme solution (sodium acetate buffer 1 mol/L, pH 4.5; β-glucuronidase–arylsulfatase:H₂O₂; 50:5:45) was added. The cups were closed and incubated overnight for 17–20 h at 37 °C.

Colorimetric Assay. In the final automated procedure, three pipetting steps were used. CAPS buffer (160 μL; 100 mmol/L, pH 12.0) with 10 μL of an aqueous MnCl₂ solution (1.0 mmol/L) and 2.5 μL of H₂O₂ (step 1) were mixed consecutively with 10 μL of the color reagent (an aqueous 48.0 mmol/L resorcinol solution) and 2.5 μL of H₂O₂ (step 2), and finally 25 μL of the hydrolyzed sample with another 5 μL of water wash (step 3). Absorbance measurements were made every 25 s and the change in absorbance (∆A) at 550 nm was calculated between the initial absorbance and the absorbance after a total incubation time of 15 min. The assay was performed at 37 °C.

Calibration. For calibration purposes, urine samples from healthy individuals were supplemented with the respective aqueous calibrators to a final concentration of respectively 2.0, 5.0, 10.0, 20.0, 50.0, and 100.0 mg/L p-aminophenol. The samples were taken through the whole analytical procedure and the resulting calibration curve...
(ΔA vs concentration) was calculated by using weighted least-squares regression analysis, with a weighing factor of 1/x^2. An intercept significantly different from zero is obtained, as normal human urine always contains a certain amount of p-aminophenol and is due to nonspecific color production (vide infra) of the reagents in the alkaline medium. For every analytical run, a sample for which urine was substituted by doubly distilled water was analyzed in duplicate. The resulting ΔA allows a separate assessment of the contribution of the nonspecific reagent coloration in the overall y-intercept obtained by regression analysis. This value is then used in the calibration and concentration calculations by subtracting it from the individual ΔA values.

VALIDATION PROCEDURES

Precision. The precision of the assay was evaluated by using the NCCLS EP5 procedure with two replicates per specimen per run and two runs per day for 20 days. Estimates of within-run and total standard deviation were determined at low (2.5 mg/L), medium (50.0 mg/L), and high (90.0 mg/L) concentrations. A “blank” urine pool was used to prepare supplemented samples on a daily basis, starting from a freshly prepared stock solution, because unconjugated p-aminophenol is very unstable in solution.

Accuracy. Accuracy was evaluated by analyzing urine samples, supplemented with known concentrations of p-aminophenol (2.5, 50.0, and 90.0 mg/L), on 15 different days. Again, a “blank” urine pool was used to prepare the samples on a daily basis, starting from a freshly prepared stock solution. Recovery of added analyte was calculated.

Sensitivity. The detection limit of the assay, defined as the concentration at a signal-to-noise ratio of 3, was determined by analyzing, in fivefold, a “blank urine” and the same urine supplemented at a concentration of 2.5 mg/L. The difference between the means of both measurement sets (signal) was divided by the difference between the highest and lowest ΔA reading for the “blank” sample (noise). Both samples were serially diluted and reanalyzed until a signal-to-noise ratio of 3 was obtained. The limit of quantification was defined as the lowest point in the calibration curve that can still be measured with acceptable reproducibility (CV <15%).

Specificity. To investigate the specificity of the method, a urine pool (containing 13 mg/L p-aminophenol) was supplemented with several drugs at a concentration of 100 mg/L and related compounds at 50 mg/L. These samples were then batch analyzed, in duplicate, with every five samples an aliquot of the “blank” urine pool. The interference of each compound is calculated as the apparent percent change in the p-aminophenol concentration of the urine pool, relative to the amount added.

STABILITY

A stability study for p-aminophenol was conducted over a 40-day period. A urine pool was aliquoted into 1-mL portions, which were kept either refrigerated (7 °C) or frozen (−20 °C). On a regular basis, a sample of each set was analyzed and the p-aminophenol concentration determined. As calibrator solutions rapidly show discoloration on standing, this degradation process, most probably oxidation, was also investigated. Aqueous calibrator dilutions were prepared, as usual, in concentrations of 25 mg/L and 500 mg/L, aliquoted in 250-μL portions, and kept either refrigerated or frozen. On several days within the 40-day period, a sample of each set was used to supplement a “blank” urine, which was then analyzed to determine the added concentration. In all cases the samples were stored in the dark.

Results and Discussion

COLORIMETRIC ASSAY OF P-AMINOPHENOL

Enzymatic hydrolysis. p-Aminophenol is extensively conjugated before excretion in the urine, and the majority are glucuronide and sulfate conjugates, although glutathione conjugation is also known to occur [21]. We chose to liberate p-aminophenol enzymatically by using a β-glucuronidase/arylsulfatase mixture instead of the more commonly used acid hydrolysis. The latter induces much more potential interferences and would chemically degrade possible urinary paracetamol or phenacetin into p-aminophenol. We used a procedure described for the deconjugation of phenol [22] but reduced the amount of enzyme, as experimental evidence showed the hydrolysis to be essentially complete. The time required for complete hydrolysis was also optimized. Identical urine samples were hydrolyzed in triplicate for 3, 22, 26, and 44 h and immediately analyzed (in duplicate). Analysis of variance of the various measured concentrations showed that the results for the last three hydrolysis time intervals were statistically identical but significantly different from the first (P = 0.01). Overnight hydrolysis of batches of specimens followed by colorimetric analysis the following day was a convenient and reliable approach.

Indophenol reaction. The p-aminophenol color formation reaction is based on the oxidative coupling with an aromatic compound containing an electron-donating group, in an electrophilic aromatic substitution. The resulting quinonimine dyes were already produced in the early decades of industrial dyestuff chemistry [23]. In the serum paracetamol method [19, 20], various color reagents were investigated and the authors finally used 8-hydroxyquinoline. We found this to be unsuitable for our p-aminophenol assay because adaptation to the urine matrix and to the much lower concentrations we aimed to detect inevitably resulted in precipitate formation in the analyzer cuvette, inhibiting the photometric measurement. We also found the reagents to be rather unstable in the alkaline buffer used. Instead, we chose resorcinol as
color reagent combined with a high-pH buffer (CAPS, pH 12) and MnCl₂ as an oxidant to increase the speed of the oxidative coupling reaction. We used three separate reagents, instead of one, combined in the CAPS buffer. Resorcinol is readily oxidized in alkaline medium and MnCl₂ is transformed into MnO, which precipitates. These solutions, in water, are stable for at least 1 month and can be prepared in higher concentrations. This allows the use of smaller volumes in the reaction, thus promoting the color intensity and consequently also the sensitivity. We optimized the overall reaction for concentration of the reagents, the order in which they are mixed, the measurement time, and the final pH of the reaction mixture, which is directly related to the intensity of the color. The criteria for optimization were always the speed of the reaction, thus the time needed for completion, and the linearity in the calibration, especially in the lower concentration range.

The concentration of resorcinol as well as MnCl₂ is the lowest concentration for which the ΔA for a given p-aminophenol concentration no longer increases with increasing reagent concentration. The order in which the reagents are mixed influenced the linearity of the calibration curve. We found that adding the sample in the last step markedly improved the linearity in the lower concentration range. All validation experiments have been done for a measurement time of 10 as well as 15 min. A measurement time of 15 min proved slightly better, and as the Cobas analyzer can start a new pipetting sequence while a previous sample is being measured, an extra 5 min of reaction only results in a marginal increase of analysis time, even for a whole batch of samples. As can be seen from Fig. 1, the final procedure resulted in a fast reaction that is virtually complete within 10 to 15 min. From this figure it is also clear that a certain amount of color formation (increased absorbance reading) starts after addition of the resorcinol (second small square) to the alkaline medium and before sample is added (third small square), because the Cobas analyzer combines every addition step with an absorption measurement. This is the nonspecific color formation, as already mentioned, that is independent of the sample and that is accounted for in the calibration.

Upscaling of the reaction allowed a spectrum to be taken of the indophenol reaction product. Fig. 2 shows this spectrum, and it is clear that a broad maximum is achieved at 576 nm. The Cobas Mira analyzer has filters for a limited number of wavelengths, of which 550 and 600 could be used. We explored both and found that at 550 nm, better linearity was consistently obtained in the calibration process.

ASSAY VALIDATION

Precision. Samples supplemented with p-aminophenol at three different concentrations were analyzed and the supplemented concentration calculated with a complete set of calibrators for every measurement batch. In Table 1 the obtained within-run and total reproducibilities are presented for the three concentrations. CVs are very good for the medium and high concentrations. For the low concentration the reproducibility was somewhat worse, although still very much acceptable. In this respect, a distinct influence of the linear regression calibration was observed because a small change in the response for one of the higher points resulted in a comparatively large effect on the accuracy of the samples with low concentrations. The protocol equally permits separate estimation of day-to-day and between-run reproducibility, and these were all close to zero. We deviated from the NCCLS protocol in one way: Because of the instability of p-aminophenol in aqueous solutions, the supplemented samples were freshly prepared on a daily basis instead of batch prepared once before the whole experiment. Consequently, one aspect of the reproducibility data is due to natural variations in the weighing and diluting process of preparing the samples.

Accuracy. The accuracy of the assay, as assessed by the analytical recovery of calibrators added to urine, was
(mean ± SD, n = 15) 102.8 ± 7.3% for a concentration of 2.5 mg/L, 103.9 ± 3.6% for a concentration of 50.0 mg/L, and 96.8 ± 3.0% for a concentration of 90.0 mg/L. Both the accuracy and precision data illustrate the suitability of the proposed assay for the quantitative analysis of p-aminophenol in urine under normal physiological conditions (below ± 10 mg/L) as well as after exposure.

Calibration curves and linearity. We preferred to construct calibration curves in the same matrix, urine, as the unknown samples to be analyzed. In this way, potential interferences of the matrix, either promoting or inhibiting the color formation, were to a large extent accounted for. Moreover, the use of non-matrix calibrators represents no real advantage in terms of benchwork or analysis time as no extraction step is present. To maximally improve the accuracy and precision in the lower concentration interval, the calibration curves were constructed by using weighted linear regression analysis. A weighing factor of $1/x^2$ emphasizes the importance of the lower calibration concentrations and drastically improves their respective residuals. All of the calibration plots generated gave a good linear response, and a correlation coefficient of $>0.999$ was observed between the $\Delta A$ and the various calibration concentrations. We found a slope of (mean ± SD, n = 5) 0.00529 ± 0.00034 (mean SE of slope 0.00073), an intercept of 0.06167 ± 0.00826 (mean SE of intercept 0.03406), and a mean SE of estimate of 0.06202.

The already-mentioned nonspecific coloration, which is used in the concentration calculations, is an unfortunate result from the discoloration of resorcínol when added to the alkaline medium. Upon using a urine matrix for calibration, the $y$-intercept one gets in regression analysis is made up of both “endogenous” $p$-aminophenol and this nonspecific coloration. When an endogenous compound in supplemented calibrators results in a substantial $y$-intercept, unknowns are corrected accordingly in the calculations. Use of the overall intercept in this case would, however, incorrectly overestimate the unknowns, as it consists of a part, the nonspecific coloration, present in calibrators as well as unknowns for which no correction is needed. It is therefore necessary to assess both contributions independently. The nonspecific coloration can, logically, not be assessed with the urine matrix, as this always contains a certain amount of $p$-aminophenol. It is therefore measured in a water sample together with each calibration, then the resulting $\Delta A$ is subtracted in the calculations from all measured $\Delta A$ values.

In a separate series of experiments, we investigated and confirmed the validity of this procedure. Through the found nonspecific coloration in water samples, the $p$-aminophenol concentration of several different batches of blank urine (from volunteers ages 26–75) could be measured by using the standard calibration approach. Subsequent use of these different urines ($n = 9$) to prepare and analyze eight individual calibration curves then allowed the calculation of the nonspecific coloration for each of these urine batches from the resulting overall $y$-intercept minus the respective previously assessed “endogenous” $p$-aminophenol concentration. All nonspecific coloration measurements (0.035888 ± 0.001940, mean $\Delta A$ ± SD), either in water or in urines of different sources, were equal, disregarding natural variances.

Sensitivity. We calculated the detection limit of the assay to be 0.9 mg/L $p$-aminophenol. By using the aforementioned definition, a concentration of 2.0 mg/L was considered the limit of quantification. Taking into consideration the concentrations of $p$-aminophenol found in individuals without occupational exposure (below ± 10 mg/L) or with possible nonoccupational exposure, which is managed by establishing a biological threshold limit in the order of 30 mg/L [1–3], our objective of a monitoring test to detect groups of workers at risk is more than met. When considering analytical sensitivity, defined as the ability of an analytical procedure to produce a change in signal for a defined change in quantity, one can see from the slope of the calibration curve that the assay does not perform equally well. We found it impossible to enhance the obtained signal for a defined change in quantity through various optimization experiments.

Specificity. The method proved to be highly specific for $p$-aminophenol. We analyzed several commonly used prescription drugs and drugs of abuse as well as some related compounds likely to be potential interferents on the basis of their chemical structure. Table 2 presents the results we found for these compounds, the interference calculated as described before. No significant interference was present, especially not when the within-run variation data are considered. To our knowledge and after an extended computerized literature search, no reference-quality method or generally accepted thoroughly documented alternative providing quantitative data (in urine) was available for a comparison-of-methods study. Earlier screening methods especially lack an in-depth quantitative approach, which precludes sample data comparison.

**APPLICATION OF THE ASSAY**

In an early stage of our research, a stability study was initiated because it was obvious that $p$-aminophenol in solution (especially in prothic solvents such as water and methanol) is very unstable and readily oxidizes. We used

---

**Table 1. Precision evaluation.**

<table>
<thead>
<tr>
<th>$p$-Aminophenol concn. $^a$, mg/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>90.0</td>
<td>0.8</td>
</tr>
<tr>
<td>50.0</td>
<td>2.1</td>
</tr>
<tr>
<td>2.5</td>
<td>7.7</td>
</tr>
</tbody>
</table>

$^a$ In urine.
NCCLS EP-5 protocol was used.
the quantitative aspect of our assay to observe the concentration change in time of samples (urinary or aqueous) kept under various conditions. In Fig. 3A, a graphical representation of the stability of urinary \(p\)-aminophenol is shown. As we analyzed a normal human urine pool, the “endogenous” \(p\)-aminophenol is mainly in the conjugated form. Under these conditions, measured \(p\)-aminophenol concentrations are stable over at least a 40-day period, whether kept frozen or refrigerated. The stability of an aqueous solution of \(p\)-aminophenol is illustrated in Figs. 3B and C. Obviously, the unconjugated compound is much less stable and degrades quickly when kept nonfrozen. The degradation of free \(p\)-aminophenol is most probably due to oxidation to a quinonimine, at least in a first stage, quickly resulting in a visible, brown discoloration. Conjugation, either glucurono- or sulfo-, prevents this initial step, thus protecting the compound. A marked difference was observed when solutions were prepared in solvents such as water, methanol, ethanol, or acetone vs acetonitrile. Very early in our experimental work we found that solutions made in the latter solvent turned brown at a much slower rate. We therefore used this solvent in the preparation of the stock calibrator solution.

### Table 2. Interference evaluation as apparent percent concentration change, relative to amount of interferent added.

<table>
<thead>
<tr>
<th>Related compounds</th>
<th>Apparent percent change</th>
<th>Apparent percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>(m)-Aminobenzoic acid</td>
<td>0.56</td>
<td>(p)-Cresol</td>
</tr>
<tr>
<td>(p)-Aminobenzoic acid</td>
<td>0.15</td>
<td>(p)-Nitrophenol</td>
</tr>
<tr>
<td>(m)-Aminophenol</td>
<td>1.07</td>
<td>Phenol</td>
</tr>
<tr>
<td>(o)-Aminophenol</td>
<td>72.1</td>
<td>Phthalic acid</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>0.11</td>
<td>Salicylic acid</td>
</tr>
<tr>
<td>(o)-Cresol</td>
<td>0.62</td>
<td></td>
</tr>
</tbody>
</table>

**Prescription drugs and drugs of abuse**

- 4-Aminoantipyrine: 0.25
- Acetylsalicylic acid: 0.08
- Amitryptiline: 0.03
- Amphetamine: 0.49
- Methadone: 0.06
- Atenolol: 0.00
- Metoprolol: 0.13
- Bromazepam: 0.10
- Metoclopramide: 0.13
- Bromhexine: 0.46
- Mexiletine: 0.02
- Caffeine: 0.13
- Morphine: 0.38
- Camazepam: 0.05
- Orphenadrine: 0.32
- Carbamazepine: 0.25
- Paracetamol: 0.28
- Chlor Diazepoxide: 0.32
- Ketubamate: 0.29
- Chlorpheniramine: 0.03
- Phencetin: 0.17
- Chlorpromazine: 0.99
- Phenobarbital: 0.11
- Codeine: 0.24
- Phentermine: 0.26
- Desipramine: 0.02
- Phenybutazone: 0.24
- Dextropropoxyphene: 0.15
- Phenytoin: 0.25
- Diazepam: 0.10
- Pholcodine: 0.05
- Dihydrocodeine: 0.07
- Pipamperone: 2.44
- Dihydroergotamine: 0.10
- Piracetam: 0.02
- Diphenhydramine: 0.16
- Piroxicam: 0.09
- Disopyramide: 0.16
- Prednisolone: 0.14
- Disulfiram: 0.41
- Promazine: 0.11
- Dometidone: 0.27
- Promethazine: 2.29
- Doxepine: 0.13
- Propyphenazone: 0.19
- Doxycycline: 0.03
- Quinine: 0.13
- Ephedrine: 0.35
- Sodium diclofenac: 0.19
- Fentanyl: 0.28
- Sulpiride: 0.35
- Flupenthixol: 0.28
- Tetracycline: 0.33
- Furosemide: 0.22
- Theobromine: 1.59
- Glutethimide: 0.04
- Theophylline: 0.11
- Guaiifenesin: 0.15
- Thiouracil: 2.61
- Hexobarbital: 0.19
- Tiidine: 5.0
- Hydrocodeone: 0.06
- Trazodone: 0.45
- Ibuprofen: 0.35
- Tripelennamine: 0.62
- Imipramine: 0.05
- Zopiclone: 0.68
- Indomethacin: −0.02

**Miscellaneous**

- 5-OH tryptamine: 2.6
- Metyldopa: 5.00
- 3-Methyl indole: −0.01
- Nicotine: 0.10
- Ascorbic acid: −0.32
- Tyramine: 2.00
- Indole: −0.05

MDEA, 3,4-methylenedioxy-N-ethylamphetamine.
The final assay procedure is being used in our laboratory in an ongoing occupational health screen. It is essential that samples be taken at the end of the (potential) exposure or within 2 h after exposure because 89% of \( p \)-aminophenol is eliminated within 24 h postexposure [3]. A total of 140 samples have so far been analyzed by using the final analysis scheme, and in Fig. 4, a frequency distribution of the measured concentrations is presented. Although sources differ, an alarming exposure to aniline is suspected when the \( p \)-aminophenol concentration in urine exceeds 50 mg/L, and a reasonable biological threshold limit value is proposed in the order of 30 mg/L [1]. None of the samples examined so far exceeded this limit.

To evaluate the possible influence of normal-dose paracetamol intake on \( p \)-aminophenol excretion, six volunteers (4 men and 2 women, ages 27–75) were asked to take a 500-mg paracetamol dose immediately after producing a blank urine sample. All (at least 4 to up to 8) following urine voidings were collected and analyzed. The results are shown in Fig. 5. The peak \( p \)-aminophenol excretion occurs around 2 to 4 h after intake, and it remains <25 mg/L. Also, the \( p \)-aminophenol concentration quickly normalizes ~6 h after intake. Consequently, one can reasonably conclude that, considering the 50 mg/L alarming exposure limit, paracetamol intake does not interfere with the value of the test for occupational health screening purposes.

The proposed colorimetric assay provides a fast and simple way for the quantitative determination of urinary \( p \)-aminophenol. It differs from the existing \( p \)-aminophenol measurement approaches in its automation, its fully validated quantitative aspect, its operational simplicity, and it being specifically adapted to urine analysis. It can advantageously be used in routine clinical laboratories in toxicology and occupational medicine.

We gratefully acknowledge M. De Bock for her invaluable technical assistance and G. Vergote for his helpful suggestions.

References


