Interlaboratory Evaluation of Salicylate Interference in Colorimetric Acetaminophen Methods and Its Clinical Significance

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Serum specimens with concentrations simulating an overdose of salicylate and acetaminophen were submitted to laboratories participating in an external quality-control program, to evaluate both the magnitude of salicylate interference in colorimetric acetaminophen methods and the clinical significance of the interference. The apparent acetaminophen concentration determined by nitration methods was increased by about 0.70 mg/L per milligram of salicylate per deciliter. Of those laboratories using nitration procedures, 25% do not routinely correct for salicylate and 66% use the (incorrect) correction factor provided by a kit manufacturer. Laboratory data, as they would have been reported to physicians, were used to estimate the acetaminophen half-life and were also applied to a nomogram used to assess the probability of hepatotoxicity. Interference by salicylate in the simulated overdose of 10 g (total dose) of each drug falsely indicated impending hepatic necrosis unless the appropriate correction factor was used. Laboratories using nitration procedures should screen samples submitted for acetaminophen assay for the presence of salicylate and, if present, either use a method specific for acetaminophen or utilize a correction factor determined in-house.

Acetaminophen, although introduced as an effective antipyretic in 1893, was not widely used until after 1949, when Brodie and Axelrod (1) demonstrated that acetaminophen is the active metabolite of phenacetin. In 1966, reports of severe hepatotoxicity associated with acetaminophen overdose were published (2, 3) and in 1971 Prescott et al. (4) presented criteria based upon serum concentrations and estimates of half-life (t_{1/2}) to be used when assessing the prognosis in overdose cases. In 1973, the biochemical mechanism of toxicity was elucidated (5), leading to effective treatment with sulfhydryl compounds such as N-acetylcysteine and methionine (6). These compounds decrease the cytochrome-P450-mediated arylation of hepatic tissue macromolecules, thus preventing necrosis (7).

Because treatment is effective only if initiated within 16 h of the time of drug ingestion (8), rapid and accurate methods are required for measuring acetaminophen. Some procedures, however, suffer from interference by salicylate, and the magnitude of the error introduced by the presence of salicylate is a matter of some controversy (9-11). Co-ingestion of aspirin and acetaminophen is not unlikely. Indeed, many analgesic/antipyretic medications contain both drugs (12). In an investigation of acetaminophen methods, 25% of the specimens collected from patients abusing acetaminophen also contained salicylate (13).

Specimens designed (by pharmacokinetic models) to simulate an overdose of both aspirin and acetaminophen were used in an interlaboratory survey designed to evaluate the magnitude of salicylate interference in commonly used methods for acetaminophen determination and the clinical significance of the interference.

Materials and Methods

Specimen preparation: The pharmacokinetic models of salicylate (14) and acetaminophen (15) disposition in humans in this study are characterized by parallel first-order and Michaelis–Menten kinetics. The metabolism of each drug is not influenced by the presence of the other (16), and the models predict accurately the time course of drug in the body (14, 17).

The predicted time course of plasma salicylate and acetaminophen concentrations in a 70-kg man after a 10-g overdose of each drug (see Figure 1 below) was assessed through numerical integration of the respective model differential equations. For numerical integration I used an International Mathematical-Statistical Libraries (IMSL, Houston, TX 77036-5085) subroutine involving fifth- and sixth-order Runge–Kutta formulas.

Acetaminophen disposition was determined by using Slatery and Levy's pharmacokinetic constants (17), an apparent volume of distribution (V_d) of 950 mL/kg, and an absorption rate constant of 2.2 min^{-1}. Salicylate disposition was determined by using kinetic constants of Levy et al. (14, subject A), an absorption rate constant of 0.2 min^{-1}, and an apparent V_d of 186 mL/kg. Acetylsalicylic acid (aspirin) is rapidly hydrolyzed (t_{1/2} = 15 min) to salicylic acid (18), and my simulation assumed that the parent drug was released into the circulation solely as salicylic acid.

I used the predicted salicylate and acetaminophen concentrations 4, 8, 12, 16, and 24 h after drug ingestion to prepare serum samples simulating the overdose. Samples were prepared by adding appropriate weights of acetaminophen and salicylate to pooled bovine serum, assuring dissolution before aliquoting. Samples were shipped frozen to those laboratories participating in the New York State Therapeutic Substance Monitoring/Quantitative Toxicology proficiency testing program that use a colorimetric method for acetaminophen quantitation. Currently, 75 of the 276 laboratories participating in the program perform in-house acetaminophen analysis and 33 utilize colorimetric procedures. The samples were labeled 4, 8, 12, 16, and 24 HR, and the laboratory directors were informed that the samples simulated an overdose of both salicylate and acetaminophen. Laboratories were requested to report the salicylate and acetaminophen concentrations, correcting the latter for salicylate interference if necessary.

These laboratories were also challenged, in the course of routine proficiency testing, with serum samples containing acetaminophen but no salicylate. Data from these surveys were used to evaluate the analytical performance of methods included in this study, as summarized in Table 1.

Methods: The commercially available colorimetric acetaminophen methods utilized by participants in this study were: Acetaminophen Rapid Stat® Diagnostic Kit (Lancer Division of Sherwood Medical, St. Louis, MO 63103), Acetaminophen Test Set (Stanbio Laboratory, Inc., San Antonio, TX 78202), Serum Acetaminophen Assay (Quantimetrix Corp., Hawthorne, CA 90250), and the acetaminophen assay of Sigma Chemical Co., St. Louis, MO 63178. The Lancer and Sigma methods are modifications of the Glynn–Kendal

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Table 1. Linear Regression Analysis of Interlaboratory Acetaminophen Results Obtained from Analysis of Samples Containing No Salicylate

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>Mean slope (and SD)</th>
<th>y-intercept (and SD)</th>
<th>s_y</th>
<th>Corr. coeff. (r)</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMT</td>
<td>25</td>
<td>0.97 (0.01)</td>
<td>-1.8 (1.7)</td>
<td>1.9</td>
<td>1.000</td>
<td>9.7</td>
</tr>
<tr>
<td>Stanbio</td>
<td>12</td>
<td>1.01 (0.02)</td>
<td>-0.4 (3.8)</td>
<td>4.2</td>
<td>0.999</td>
<td>13.7</td>
</tr>
<tr>
<td>Sigma</td>
<td>4</td>
<td>1.02 (0.04)</td>
<td>10.9 (6.2)</td>
<td>7.0</td>
<td>0.998</td>
<td>8.1</td>
</tr>
<tr>
<td>Lio, chromat.</td>
<td>8</td>
<td>1.02 (0.02)</td>
<td>-5.8 (2.9)</td>
<td>3.3</td>
<td>0.999</td>
<td>14.4</td>
</tr>
<tr>
<td>Lancer</td>
<td>12</td>
<td>1.06 (0.04)</td>
<td>2.3 (5.9)</td>
<td>6.7</td>
<td>0.998</td>
<td>14.7</td>
</tr>
</tbody>
</table>

Acetaminophen data were obtained from the New York State Toxicology proficiency testing program for the testing year 1983-1984. Nine specimens were assayed and the range of acetaminophen concentrations was 50-245 mg/L. Regression analysis was performed using the method means (y) and the mean of reference laboratory data (x). N is the number of laboratories using the method; CV is the mean coefficient of variation for results submitted in the three surveys conducted in the testing year. The Quantimetrix method was not included in the analysis, because too few laboratories were using the protocol when the specimens were submitted.

(19) procedure in which serum proteins are acid-precipitated and the supernatant fluid is reacted with sodium nitrite. The absorbance of the resulting nitrophenol at alkaline pH is determined at 405 nm and is proportional to the specimen's acetaminophen concentration. The Quantimetrix assay is based upon the reaction of acetaminophen with Folin-Ciocalteau reagent, yielding an indophenol with a color intensity proportional to the acetaminophen concentration. The Stanbio procedure is essentially that of Liu and Oka (20) wherein acetaminophen, extracted from serum with ethyl acetate, reduces ferric-5,6,8-tripryridyl-s-triazine complex (ferric-TPTZ) to its blue ferrous form. Absorbance at 595 nm is proportional to the acetaminophen concentration.

Two laboratories using the exxrrror Acetaminophen Assay (Syva Co., Palo Alto, CA 94303-0847) were also included in the study. In this technique, the drug in the patient's specimen and drug covalently bound to glucose-6-phosphate dehydrogenase (EC 1.1.1.49) compete for acetaminophen-specific antibody-binding sites. The competitive binding results in enzyme activity proportional to the specimen's acetaminophen concentration; the resulting NADH is monitored at 340 nm.

I used a "high-pressure" liquid chromatographic method for acetaminophen developed in our laboratory to verify the integrity of the samples. Sample, buffered with sodium acetate (1 mol/L, pH 5.8), was extracted with chloroform/isopropanol solvent (9/1 by vol) containing the internal standard β-hydroxyethyltheophylline, and the extract was evaporated. The residue was reconstituted with a mobile phase consisting of sodium phosphate buffer (40 mmol/L, pH 4.5), methanol, and acetonitrile (82/14/4 by vol). The extract was chromatographed on a reversed-phase column (UltraspHERE-ODS, 15 cm x 4.6 mm; Beckman Instruments, Inc., Irvine, CA 92713) and the effluent was monitored at 254 nm. For peak-height data reduction I used a Model 4100 Computing Integrator (Spectra-Physics Laboratories, Piscataway, NJ 08854).

The various methods for salicylate quantitation, and the percentages of laboratories using them, were: DuPont acs, 30%; Lancer Salicylate Rapid Stat®, 23%; Quantimetrix Salicylate Assay, 7%; Trinder (21), 23%; Keller (22), 10%; and Natelson (23), 7%.

Clinical evaluation of acetaminophen results: Results for acetaminophen as they would have been reported to physicians were applied to a nomogram used in clinical practice to assess the probability of hepatotoxicity (24). The nomogram is a semilogarithmic plot of serum acetaminophen concentration vs hours after drug ingestion; concentrations above a line joining coordinates of 200 mg/L at 4 h and 50 mg/L at 12 h are prognostic of hepatotoxicity and indicate the need for immediate antidotal treatment.

The acetaminophen elimination kinetics predicted by the model used in this study are not exponential; however, the simulated data could easily be taken for such. To conform with current clinical practice, estimates of f1/2 were made on the assumption of first-order elimination kinetics for concentrations measured 8, 12, and 16 h after ingestion. The elimination rate constant (k) was determined from the slope of the least-squares line of the data for ln acetaminophen concentration vs time and t1/2 was calculated from the expression t1/2 = 0.693/k. Half-lives exceeding 4 h are indicative of probable risk for hepatotoxicity (4).

Results

In the computer simulation of an acetaminophen overdose, the concentration in serum was greatest (126 mg/L) at 2 h and 9.5 mg/L was eliminated from the body with a t1/2 of 3.9 h (Figure 1). The acetaminophen concentrations in serum are all below the treatment decision line, indicating no need for antidotal therapy—the 3.9-h t1/2 supports the prognosis of no hepatotoxicity. According to Reed et al. (10), when a

![Probable Hepatic Toxicity Associated with Acetaminophen Toxicity](https://via.placeholder.com/150)

**Fig. 1.** Time courses of plasma salicylate and acetaminophen concentrations in a 70-kg man after ingestion of 10 g of each drug, as predicted by pharmacokinetic models of acetaminophen (15) and salicylate (14) disposition.

Apparent acetaminophen concentrations were determined by incrementing the acetaminophen concentration by a factor of 0.75 mg/L, per mg salicylate/dL. The time courses are plotted on a nomogram used to assess the risk of hepatotoxicity associated with acetaminophen overdoses (24). Concentrations above the straight line are prognostic of probable toxicity. The time course of acetaminophen and salicylate concentrations used in the interlaboratory evaluation of acetaminophen methods is denoted by solid symbols. □, salicylate, mg/dL; ○ acetaminophen, mg/L; △, apparent acetaminophen, mg/L.

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commercially available nitration procedure is used, the acetaminophen concentration is increased 0.75 mg/L per milligram of salicylate per deciliter. I used this factor to calculate the time course of apparent acetaminophen concentration (Figure 1). It is evident that specimens collected more than 7 h after ingestion would yield analytical results prognostic of hepatotoxicity. The difference between the true and apparent acetaminophen concentrations increases as the ratio of serum salicylate to acetaminophen concentrations increases. The net effect is a flattening of the acetaminophen elimination curve, yielding an apparent half-life of 8.9 h, which is prognostic of severe hepatic coma.

Specimens with salicylate and acetaminophen concentrations equal to those in the simulation at 4, 8, 12, 16, and 24 h were submitted to laboratories to determine if their procedures adequately compensate for the salicylate interference. Salicylate concentrations reported by the laboratories agreed well with gravimetric target concentrations, and no problems were evident with any of the methods. The interlaboratory CV was <7% (Table 2).

Liquid-chromatographic and EMIT assays could accurately account for the acetaminophen in each sample (Table 2). The Stanbio procedure, which exhibited no bias with survey specimens void of salicylate (Table 1), gave consistently high values for specimens containing salicylate, equivalent to 0.1 mg of acetaminophen per liter per milligram of salicylate per deciliter, a value that agrees well with Liu and Oka's (20) estimate of 0.07 mg/L. Data plotted on the nomogram were prognostic of low risk. However, as the salicylate/acetaminophen ratio increased, the discrepancy between the Stanbio data and the actual time course increased (Figure 2), artificially prolonging the half-life to 5.0 h. The method's sensitivity is reportedly 25 mg/L, yet each laboratory reported quantitative results for the 16- and 24-h specimens. This did not have an adverse effect on the half-life estimate, however (Table 2). Salicylate interference in the indophenol procedure, as stated by the kit manufacturer (25), corresponds to about 0.5 mg of acetaminophen per liter per milligram of salicylate per deciliter. Laboratories accurately compensated for the interference for the 4-, 8-, and 12-h specimens, but the 16-h (and 24-h) results were significantly high, resulting in a calculated half-life of 7.3 h. The calculated half-life is decreased to 5.6 h if acetaminophen concentrations lower than the method sensitivity (25 mg/L) are excluded from the determination (Table 2).

The time courses of apparent acetaminophen concentration determined by the Lancer and Sigma nitration procedures were nearly identical (Table 2), very similar to the simulated apparent acetaminophen time course calculated with an interference factor of 0.75 mg/L for each milligram of salicylate per deciliter (Figure 1). Application of the Lancer data to the nomogram resulted in a false prognosis of hepatotoxicity with specimens collected 8 h after ingestion unless compensation for analytical error was made (Figure 2). The clinical impression was not changed by using the 0.15 mg/L factor recommended by the kit manufacturer (25). Analysis of acetaminophen samples void of salicylate revealed a proportional error of 6% and a systematic bias of 2.3 mg/L (Table 1), which agrees well with the values of 5.4% and 1.0 mg/L obtained from a correlation between the Lancer procedure and liquid chromatography (25). Part of the bias noted with specimens in this study therefore is attributable to inherent analytical error. The increment in apparent acetaminophen concentration as a result of interference by salicylate was determined to be 0.7 mg/L per milligram of salicylate per deciliter. The time course of acetaminophen disposition, when corrected for salicylate interference by using this factor, agreed well with the actual time course (Figure 2).

The systematic bias associated with the Sigma protocol for specimens void of salicylate was 10.5 mg of acetaminophen per liter (Table 1). With this inherent analytical error taken into account, the correction factor for salicylate interference was determined to be 0.5 mg of acetaminophen per liter for each milligram of salicylate per deciliter, which agrees with the manufacturer's quoted factor (27). The interlaboratory imprecision of the Sigma method, however, was excessive: two of the four laboratories reported accurate and clinically acceptable results, but results from the other were approximately 40% high.

Four of the 15 laboratories using nitration procedures indicated that they do not correct for salicylate interference, six laboratories using the Lancer kit employ the 0.15 factor.

Table 2. Summary of Interlaboratory Salicylate and Acetaminophen Results and Acetaminophen Half-Life Estimates

<table>
<thead>
<tr>
<th>Drug, anal. method</th>
<th>No. labs</th>
<th>4 (mean and SD)</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>24</th>
<th>Half-life, h</th>
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<tbody>
<tr>
<td>Salicylate, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorimetric</td>
<td>30</td>
<td>40.1 (2.6)</td>
<td>53.4 (2.7)</td>
<td>55.4 (2.5)</td>
<td>53.4 (2.5)</td>
<td>44.7 (2.3)</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen, mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gavemetric</td>
<td>103.0</td>
<td>63.0</td>
<td>34.0</td>
<td>15.0</td>
<td>0.0</td>
<td>3.9*</td>
<td></td>
</tr>
<tr>
<td>Lg. chromatog.</td>
<td>99.5</td>
<td>81.0</td>
<td>34.3</td>
<td>17.2</td>
<td>0.0</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>EMIT</td>
<td>37.2</td>
<td>62.5</td>
<td>35.6 (0.9)</td>
<td>17.0 (0.6)</td>
<td>0.0 (0.0)</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Stanbio</td>
<td>104.0</td>
<td>71.6</td>
<td>42.1 (7.6)</td>
<td>23.5 (9.7)</td>
<td>5.2 (7.1)</td>
<td>5.0 (5.2)</td>
<td></td>
</tr>
<tr>
<td>Lancer (apparent)</td>
<td>131.7</td>
<td>98.7 (16.3)</td>
<td>73.8 (15.6)</td>
<td>57.4 (16.4)</td>
<td>35.6 (15.6)</td>
<td>10.2</td>
<td></td>
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<tr>
<td>Correction factor</td>
<td></td>
<td></td>
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<tr>
<td>0.15</td>
<td>6</td>
<td>131.6 (16.4)</td>
<td>88.6 (19.4)</td>
<td>67.4 (14.3)</td>
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<td>30.8 (19.3)</td>
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<tr>
<td>0.75</td>
<td>1</td>
<td>116.0</td>
<td>58.0</td>
<td>&lt;40.0</td>
<td>&lt;40.0</td>
<td>0.0</td>
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<tr>
<td>0.90</td>
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<td>97.0</td>
<td>45.0</td>
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<tr>
<td>0.95</td>
<td>1</td>
<td>65.5</td>
<td>37.1</td>
<td>3.5</td>
<td>0.0</td>
<td>0.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Sigma (apparent)</td>
<td>4</td>
<td>135.3 (27.9)</td>
<td>98.3 (21.5)</td>
<td>73.2 (18.7)</td>
<td>55.3 (14.3)</td>
<td>31.2 (4.1)</td>
<td>9.5</td>
</tr>
<tr>
<td>(corrected)</td>
<td>2</td>
<td>123.5 (41.7)</td>
<td>80.5 (31.8)</td>
<td>53.5 (28.1)</td>
<td>35.0 (19.8)</td>
<td>12.2 (4.0)</td>
<td>6.7 (6.8)</td>
</tr>
<tr>
<td>Quantimetrix (apparent)</td>
<td>2</td>
<td>108.0 (2.8)</td>
<td>84.5 (3.5)</td>
<td>61.5 (0.7)</td>
<td>32.5 (2.1)</td>
<td>38.0 (2.8)</td>
<td>7.0</td>
</tr>
<tr>
<td>(corrected)</td>
<td>2</td>
<td>87.0 (4.2)</td>
<td>58.0 (2.8)</td>
<td>35.5 (2.1)</td>
<td>27.0 (4.2)</td>
<td>15.0 (1.4)</td>
<td>7.3 (6.3)</td>
</tr>
</tbody>
</table>

*a Half-life was determined by using the mean acetaminophen concentration at 8, 12, and 16 h (see Materials and Methods); in healthy adults, the half-life is 2–4 h.

*b Half-life was determined by using the mean acetaminophen concentration at 8 and 12 h; the concentration reported for the 16-h specimen is below the method sensitivity specified by the manufacturer (see Results).

c Correction factors for salicylate interference expressed as mg acetaminophen/mL per mg salicylate/dL. Two laboratories do not correct for salicylate interference.

d Correction factors were specified by the manufacturer.

The 0.15 factor is specified by the manufacturer; the remaining factors are those determined by laboratory evaluation.

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and three performed in-house evaluations to determine the extent of interference (Table 2). The laboratories using factors of 0.8 and 0.95 appear to have grossly overestimated the interference; however, the apparent acetaminophen concentrations reported by the laboratories were significantly lower than the group means, accounting in part for the low corrected acetaminophen concentrations. The 12-, 16-, and 24-h results are significantly lower than the Rapid Stat method sensitivity of 40 mg/L (20 mg/L if an increased sample volume is used) (25), yet laboratories appear to be reporting them to physicians. The one laboratory using the 0.75 factor compensated accurately for salicylate interference and reported appropriately the acetaminophen concentrations lower than the method sensitivity as "less than."

Discussion

Acetaminophen, an effective and safe antipyretic-analgesic agent when taken in therapeutic doses, may cause anorexia, nausea, vomiting, diaphoresis, hepatic centrilobular necrosis, and acute renal tubular necrosis when an overdose is ingested (28, 29). Through the mid 1960s and 1970s, the pattern of serious consequences associated with acetaminophen (paracetamol) overdose was evolving in the United Kingdom (30). Meanwhile, the United States was slow to recognize the potential toxicity of acetaminophen. As of 1975, only one article describing the clinical course of an acetaminophen overdose case had been published in the U.S. (31). Since 1976, however, over 9000 cases of overdose have been registered with the nationwide multiclinic open study conducted by the Rocky Mountain Poison Center (32).

Acetaminophen is eliminated from the body principally by biotransformation to sulfate and glucuronide conjugates; these metabolites are innocuous and are excreted in urine along with small amounts of unchanged acetaminophen (33, 34). Approximately 4% of the acetaminophen is oxidized to an electrophilic arylated metabolite thought to be N-acetylbenzoquinoneimine (35). This metabolite is normally inactivated by conjugation with reduced glutathione and excreted into the urine as cysteine and mercapturic acid conjugates. When hepatic glutathione stores are diminished to 20–30% of normal, as may occur with acetaminophen doses of approximately 15 g in the 70-kg man, the arylated metabolite begins to bind covalently to cellular proteins, causing necrosis (36). In cases of severe acetaminophen overdose, the detoxification process is compromised within hours of drug ingestion (5), although hepatotoxicity does not become evident clinically for up to three days (4).

Clinical management consists of early recognition of patients at risk for hepatotoxicity and prompt treatment with agents such as oral N-acetylcysteine. The need for rapid, reliable determination of acetaminophen in emergencies resulted in a proliferation of analytical techniques, some of which are now considered inappropriate (37). Among the limitations of selected colorimetric methods is poor sensitivity. Acetaminophen is cleared rapidly from the body. Sixteen hours after ingestion, when antidotal therapy is still likely to be beneficial, concentrations of 25 mg/L or higher are associated with possible hepatic toxicity; estimates of half-life may require quantification of concentrations less than 25 mg/L. As evidenced by this interlaboratory survey, many laboratories are unfamiliar with the sensitivity limitation of their acetaminophen method. Results obtained from the analysis of specimens with acetaminophen concentrations that are below the method sensitivity are likely to be biased positively and, in some cases, may produce clinically significant increases in the calculated half-life.

Interference by salicylate also compromises the reliability of some methods for acetaminophen. As demonstrated in the computer simulation of a concurrent acetaminophen and salicylate overdose, if the plasma salicylate/acetaminophen ratio is high, the salicylate interference is likely to be significant, resulting in increased estimates of half-life and potentially false impressions of high risk for hepatotoxicity. Salicylate is metabolized by parallel first-order and capacity-limited pathways; and in overdose cases the t1/2 increases significantly, resulting in prolonged high salicylate concentrations (14). Acetaminophen, on the other hand, is cleared relatively rapidly from the body, even when overdoses are ingested. The metabolic patterns of these drugs therefore contribute to the possibility of a high plasma salicylate/acetaminophen ratio and reinforce the importance of correcting for any interference.

Several investigators have noted the need for a correction factor to compensate for salicylate interference in nitrinitation procedures (8–11); others have proposed an approach to eliminate the salicylate interference (38–44). Nevertheless, in the present study, acetaminophen results reported by 11 of the 15 laboratories using nitrination procedures would likely have resulted in an unnecessary three-day course of antidotal therapy. Laboratories reporting the inaccurate results indicated that either (a) they did not believe the interference to be clinically significant or (b) they used a correction factor provided by the kit manufacturer.

The relatively high interlaboratory imprecision of the nitrination methods was also apparent and was probably a result of variability among laboratories in performing the
nitrification reaction. The magnitude of salicylate interference is sensitive both to the length of time that nitrification is allowed to proceed and to the strength of the nitrite reagent (41, 42, 44). Stringent control of assay variables is required to minimize salicylate effects on the assay.

Salicylate also interfered with the indophenol procedure, but laboratories compensated for the interference accurately as long as the acetaminophen concentration exceeded the assay sensitivity limit of 25 mg/L. Acetaminophen concentrations below this limit, however, may be overestimated.

The ferric-TPTZ procedure is least affected by salicylate, and laboratories using this method do not routinely correct for salicylate interference. However, when the plasma salicylate/acetaminophen ratio is very high, this method may also produce inaccurate results, which appear clinically significant and which should be corrected for salicylate interference.

The American Academy of Pediatrics Committee on Drugs has recently acknowledged the potential problems associated with salicylate interference and has urged that physicians be notified of any methodology limitations before the results are interpreted (45).

Ideally, acetaminophen methods should be specific and sensitive. If a laboratory must utilize nonspecific methods because of instrumentation or personnel limitations, every sample should be screened for the presence of interferents. If an interferent is found, the laboratory should utilize a method specific for acetaminophen or should correct for the analytical error on the basis of in-house evaluations of the magnitude of interference.

References

25. Quantimetrix Serum Acetaminophen Assay kit instructions.
27. Sigma Technical Bulletin No. 430 (11–82).