Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements

Patrick Kelly, Joseph McPartlin, Michael Goggins, Donald G Weir, and John M Scott

ABSTRACT  Periconceptual consumption of folic acid has been shown to decrease the incidence of neural tube defects. The strategy of universal fortification of staple foodstuffs with folic acid presents the possibility of life-long exposure to unmetabolized folic acid. Chief among the risks of exposure to folic acid in the circulation is that of masking the diagnosis of cobalamin deficiency in pernicious anemia and the progression of neurologic disease. Other effects are unknown. For instance, the effect of in vivo chronic exposure of adult and fetal cells to the synthetic form of the vitamin has never been investigated at the population level. This study examined the acute appearance of unmetabolized folic acid in serum in response to the consumption of some fortified foodstuffs by young and elderly volunteers. Subjects on a 5-d regimen of fortified ready-to-eat-cereal and bread in addition to their normal diet had a threshold intake of 266 μg folic acid per meal at which unaltered folic acid appeared in the serum. Subjects given folic acid in either isotonic saline, milk, or white bread also had a threshold > 200 μg. From patterns of food consumption in the United States, the implementation of flour fortification at 1.4 mg/kg is unlikely to lead to folic acid appearance in serum, assuming that consumption is spread throughout the day. Increasing this level of fortification, however, as has been advocated by some agencies, may result in the repeated appearance of folic acid in serum over many years, particularly in consumers in nontargeted populations of large amounts of fortified foods. The "safe level of intake" of 1 mg folate/d set by the US Food and Drug Administration may cause a serum folic acid effect. Furthermore, a repeated serum folic acid response is likely to be found in many women complying with the advice to take 400 μg folic acid/d to prevent the occurrence of neural tube defects.  


KEY WORDS  Food fortification, supplements, folic acid, serum, safety, metabolism threshold, humans, neural tube defects

INTRODUCTION  

In response to the findings of several studies establishing the relation between folate intake and the incidence of neural tube defects, there is broad international agreement on the advisability of increasing the amount of folate in the diet of women of childbearing age (1). The strategies for supplying women with folate in the periconceptual period include supplementa-

See corresponding editorial on page 1889.
unmetabolized folic acid in the circulation poses a problem, particularly in the effective diagnosis of cobalamin deficiency of pernicious anemia, although the potential for harm may exist (4), for example, in chronic exposure of fetal as well as adult cells to a synthetic form of the vitamin not normally encountered. Finally, the remarkable success of chemotherapy based on antifolates, whose mode of action is to limit folate availability to tumor cells, must raise concern about efforts to increase exposure to folate, particularly to unmetabolized folic acid.

The current US Food and Drug Administration (FDA) proposal for fortification of grain products (1.4 mg/kg flour) is estimated to deliver an additional 100 μg folic acid/d to the average US diet, although consumers of high amounts of grain products are likely to be exposed to higher amounts (5). Simultaneously, a safe upper limit of 1000 μg/d was set by the FDA, which admitted that the effects of long-term exposure of body tissues to elevated blood concentrations of folic acid have not been evaluated (6). Moreover, the Centers for Disease Control and Prevention Folic Acid Working Group (7) proposed fortification at 3.5 mg (and even 7.0 mg/kg flour to ensure that many more women would consume the recommended 400 μg/d.

In light of these issues, this study was undertaken to determine the threshold dose of folic acid fortification in a range of foodstuffs eaten under normal dietary conditions above which unmetabolized folic acid was found in serum postprandially.

**SUBJECTS AND METHODS**

**Subjects**

Subjects were recruited from two population groups based on age. One group consisted of healthy volunteers aged 18–42 y (x ± SD: age 26.04 ± 5.67 y; n = 5 males, 18 females) recruited from university students and laboratory staff. Another group consisted of healthy elderly volunteers aged 63–82 y (70.47 ± 5.36 y; n = 5 males, 25 females) recruited from the long-stay geriatric patient population at St James’ Hospital, Dublin. These recruits were assessed by routine physical examination, medical history, and relevant laboratory analyses before admittance to the study, which was approved by the Hospital Ethics Committee. Subjects gave informed consent before being admitted into the study.

**Experimental protocols**

**Experiment 1: folic acid absorption from fortified RTEC and bread under normal dietary conditions in subjects aged 18–42 y**

A range-finding study was undertaken to determine the threshold dose of oral folic acid intake under normal dietary conditions above which unmetabolized folic acid appeared in serum. Fourteen healthy volunteers aged 18–42 y were recruited to eat both fortified RTEC and bread as part of their daily diet for 5 consecutive days. Before the study began a blood sample was taken to determine red cell folate and serum folic acid concentrations. The subjects were then grouped into seven pairs, each pair given folic acid as fortified RTEC and bread at total daily intakes of 90, 400, 800, 900, 1000, 1100, and 1200 μg. The lowest intake represents the amount of folic acid per serving supplied commercially in cornflakes (Kellogg’s, Manchester, United Kingdom), as described on the box. The total daily amount of folic acid was divided between one portion of cornflakes (25 g) and two portions of white bread (30 g each). The bread (commercially available white milk bread) was fortified by applying microliter amounts of a freshly prepared folic acid solution (10 g/L) to 10 evenly spaced regions of individual bread slices with a micropipette in amounts ranging from 266 to 800 μg per two slices (2 × 30 g). The bread was then foil-wrapped, stored frozen, then allowed to thaw at room temperature on the eve of consumption. Additional incremental amounts of folic acid (totaling 133–400 μg; **Table 1**) were added to cornflakes in the same manner as described for the bread. The cornflake portions were stored at room temperature in sealed portions in the dark before use. Each subject was provided with their daily ration of fortified food the evening before consumption with instructions that it be eaten at 0800 (cornflakes), 1300 (bread), and 1800

---

**TABLE 1** Folic acid and total folate in serum of subjects consuming folic acid under normal dietary conditions

<table>
<thead>
<tr>
<th>Subject</th>
<th>Red cell folate</th>
<th>Oral folic acid intake</th>
<th>Total folate</th>
<th>Fasting</th>
<th>Postprandial†</th>
<th>Folic acid, postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg/L</td>
<td>mg</td>
<td>μg/d</td>
<td>μg/L</td>
<td></td>
<td>μg/L</td>
</tr>
<tr>
<td>1</td>
<td>195</td>
<td>90</td>
<td>000</td>
<td>90</td>
<td>10.75</td>
<td>11.50</td>
</tr>
<tr>
<td>2</td>
<td>156</td>
<td>90</td>
<td>000</td>
<td>90</td>
<td>8.75</td>
<td>15.00</td>
</tr>
<tr>
<td>3</td>
<td>162</td>
<td>133</td>
<td>266</td>
<td>400</td>
<td>4.75</td>
<td>9.00</td>
</tr>
<tr>
<td>4</td>
<td>158</td>
<td>133</td>
<td>266</td>
<td>400</td>
<td>7.25</td>
<td>16.00</td>
</tr>
<tr>
<td>5</td>
<td>164</td>
<td>266</td>
<td>533</td>
<td>800</td>
<td>7.50</td>
<td>19.75</td>
</tr>
<tr>
<td>6</td>
<td>167</td>
<td>266</td>
<td>533</td>
<td>800</td>
<td>15.50</td>
<td>19.50</td>
</tr>
<tr>
<td>7</td>
<td>164</td>
<td>300</td>
<td>600</td>
<td>900</td>
<td>9.00</td>
<td>18.50</td>
</tr>
<tr>
<td>8</td>
<td>153</td>
<td>300</td>
<td>600</td>
<td>900</td>
<td>6.00</td>
<td>19.25</td>
</tr>
<tr>
<td>9</td>
<td>306</td>
<td>333</td>
<td>666</td>
<td>1000</td>
<td>19.00</td>
<td>28.50</td>
</tr>
<tr>
<td>10</td>
<td>162</td>
<td>333</td>
<td>666</td>
<td>1000</td>
<td>8.00</td>
<td>27.50</td>
</tr>
<tr>
<td>11</td>
<td>327</td>
<td>366</td>
<td>733</td>
<td>1100</td>
<td>12.50</td>
<td>22.50</td>
</tr>
<tr>
<td>12</td>
<td>195</td>
<td>366</td>
<td>733</td>
<td>1100</td>
<td>—</td>
<td>17.50</td>
</tr>
<tr>
<td>13</td>
<td>195</td>
<td>400</td>
<td>800</td>
<td>1200</td>
<td>4.75</td>
<td>24.00</td>
</tr>
<tr>
<td>14</td>
<td>160</td>
<td>400</td>
<td>800</td>
<td>1200</td>
<td>14.00</td>
<td>19.25</td>
</tr>
</tbody>
</table>

† Significantly different from fasting total folate, P < 0.0001 (paired t test).
(bread) daily. The fortified food was incorporated into the subjects' normal dietary pattern for 4 consecutive days. Subjects were asked to avoid other folic acid–fortified food during this period (a comprehensive list of fortified foods available on the Irish market, including RTECs and certain brands of milk and bread, was issued to each subject). On the final morning of the study a blood sample was taken 2.25 h after the consumption of the last portion of fortified cornflakes.

Experiment 2: folic acid absorption from isotonic saline in subjects aged 22–40 y

Preliminary work from this laboratory showed that the time of maximum appearance of folic acid in serum of healthy volunteers after an oral dose in isotonic saline (Dioralyte; Rhone Poulenc Rorer, Dublin) was 80 min (max). On the basis of this time-course study, six volunteers (aged 22–40 y) were recruited to each take a single dose of folic acid in Dioralyte (50 mL) at three doses: 400, 300, and 200 µg, separated by a 2-wk interval. Volunteers were asked to eschew folate-fortified foods for the duration of the study. Fasting blood samples were taken for total serum folate and serum folic acid assay before and at max after each folic acid bolus. Subsequently, five additional volunteers (aged 21–35 y) were recruited to repeat the regimen at the 200-µg dose.

Experiment 3: folic acid absorption from bread in an elderly population

This aspect of the study tried to determine the amount of folic acid fortification in bread at which unmetabolized vitamin appears in the circulation in a healthy geriatric population. Commercially available white milk bread was fortified with folic acid in amounts ranging from 150 to 600 µg per two slices (2 × 30 g) as described above. Thirty long-stay geriatric patients (63–82 y) were recruited for this experiment. Blood samples were taken beforehand for red cell folate and serum folate and serum folic acid concentrations. After pretreatment with a 400-µg folic acid tablet daily for 18 d, each subject was given a constant dose of folic acid in the specially fortified bread slices for 3 consecutive days at 0830 and 1800 daily along with a folic acid–free meal consisting of tea and additional toasted bread (30 g) and butter. The remainder of their daily normal diet for the duration of the experiment was folic acid–free. On the final morning of the study fasting serum and red cell samples were taken for folate analysis and each subject was given their total daily dose of folic acid on bread (2 × 30 g) in the usual manner. A second blood sample was taken 2.25 h later for serum folic acid analysis.

Experiment 4: folic acid absorption from milk in elderly subjects

Sixteen elderly patients (63–80 y) were recruited to determine serum folic acid concentrations after long-term intake of folic acid–fortified food. As part of their normal diet these subjects were routinely given fortified milk (380 µg folic acid/L) and breakfast cereal. These subjects were therefore estimated to have a daily folic acid intake within the range 172–190 µg in addition to their natural folate intake. After an overnight fast a blood sample was taken to test red cell folate concentration. Each recruit was given commercially available low-fat milk (200 mL) fortified with folic acid (200 µg), which was consumed simultaneously with a folic acid–free meal consisting of tea and toasted white bread (60 g), butter, and preserves. A second blood sample was collected 2.25 h later. Aliquots of serum taken before and after the study were assayed for both folic acid and total folate.

Sample analysis

Blood samples were collected from volunteers before and after the folic acid treatment procedures. The blood was allowed to clot and the separated serum was stored at −20 °C. Sera were assayed for total folate by using the Lactobacillus casei assay described previously (8). Serum folic acid concentrations were assayed by a procedure that involved deproteinization of the serum sample with perchlorate before HPLC fractionation of folic acid and L. casei assay of chromatographic fractions (3). Red cell folate samples were prepared by 1:10 dilution of whole blood with ascorbic acid (1% wt:vol) and assayed by L. casei.

Statistical analysis

Descriptive statistics, t tests (paired and unpaired), two-way analysis of variance (ANOVA), and post hoc Scheffé analyses were performed by using the DATA DESK statistics program for Macintosh, version 4.1 (Data Descriptions Inc, Ithaca, NY).

RESULTS

Experiment 1

As can be seen in Table 1, all participants were folate replete at the start of the experiment as determined by their red cell folate concentrations (normal range: >150 µg/L). There was a significant increase in total serum folate for all subjects postprandially. Unmetabolized folic acid was measured in the postprandial serum sample of both subjects given 800 µg folate/d (but whose final meal before sampling contained 266 µg) and in all other subjects consuming higher doses.

Experiment 2

Unaltered serum folic acid was found in four of the six subjects taking 400 µg, three of six taking 300 µg, and in none of those taking 200 µg (Table 2). Of five additional subjects recruited to take 200 µg, none showed unmetabolized folic acid in their sera. Two-way ANOVA using both subject and group as independent variables showed a difference between groups in their unmetabolized folic acid response that was almost significant (P = 0.059). For the total serum folate response, two-way ANOVA showed a nonsignificant difference between groups. Post hoc analysis (Scheffé) showed that the effect was attributable to the difference between the groups consuming 200 and 400 µg/d (P = 0.06).

Experiment 3

The results of this experiment are shown in Table 3. Serum from subjects given ≤200 µg/d contained undetectable amounts of folic acid. Subjects with folic acid in their sera included three of those five consuming 300 µg/d, four of the six consuming 400 µg/d, and all subjects consuming either 500 or 600 µg/d. Two-way ANOVA using both subject and group as independent variables showed a significant difference between groups in their unmetabolized folic acid response (P = 0.0015). Post hoc analysis
showed that differences in treatments were significant only between the 600- and 200-μg/d regimens \((P = 0.011)\). Two-way ANOVA of the total folate response showed no significant difference among treatment groups.

**Experiment 4**

A significant rise in total serum folate was noted postprandially (Table 4). However, folic acid was not found in any subject’s serum.

**DISCUSSION**

The introduction of fortification of food with folic acid has been welcomed as an effective measure to diminish the incidence of neural tube defects. Other benefits thought to accrue from increased folate intake include protection against arterial disease (9). The proposed amount of fortification set by the FDA, calculated to deliver on average an additional 100 μg folic acid/d to the US diet, represents the most considered approach to date in terms of efficacy and safety (6). Safety concerns about fortification programs must relate primarily to the presence on some occasions of metabolically unaltered folic acid in the circulation with oral consumption of this synthetic form of the vitamin.

Butterworth and Tamura (10) and Dickinson (11), among others, have reviewed safety and toxicity considerations and concluded that folic acid is intrinsically safe under most circumstances even in supraphysiologic quantities. Clinical treatments, for instance, that involve the use of antiepileptics or antifolates in noncancer applications appear to take place safely with no significant reduction in drug efficacy when folic acid is administered simultaneously (12-14). However, caution is required when considering the implications of the efficacy of antifolate therapy in other clinical settings. The remarkable historical success of antifolate drugs as cancer chemotherapeutic agents must raise concerns about efforts to increase the exposure of the population at large to more folate. Even less information is available on the possible interference of folic acid with antifolate-based cancer chemotherapy. One of the main modes of acquired resistance of tumors to methotrexate treatment is the amplification of expression of the enzyme dihydrofolate reductase (15), one of the substrates for which is folic acid. Neither is epidemiologic evidence forthcoming on the risks of potential chronic exposure of fetal tissue to a synthetic substance, i.e., folic acid, as a consequence of fortification.

The most publicized safety risk concerns the masking of the neurologic sequelae of cobalamin deficiency in patients with pernicious anemia (11). On physiologic and biochemical grounds, it may be shown that the risk in pernicious anemia arises not from naturally occurring folate but from folic acid...
TABLE 4
Folic acid and total folates in serum of subjects before and after ingestion of 200 μg folic acid in milk

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total folate</th>
<th>Folic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg/L</td>
<td>μg/L</td>
</tr>
<tr>
<td>Red cell folate</td>
<td>Fasting</td>
<td>Postprandial</td>
</tr>
<tr>
<td>1</td>
<td>315</td>
<td>10.40</td>
</tr>
<tr>
<td>2</td>
<td>382</td>
<td>4.60</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>2.85</td>
</tr>
<tr>
<td>4</td>
<td>152</td>
<td>3.25</td>
</tr>
<tr>
<td>5</td>
<td>545</td>
<td>15.20</td>
</tr>
<tr>
<td>6</td>
<td>345</td>
<td>8.20</td>
</tr>
<tr>
<td>7</td>
<td>468</td>
<td>9.60</td>
</tr>
<tr>
<td>8</td>
<td>186</td>
<td>2.70</td>
</tr>
<tr>
<td>9</td>
<td>120</td>
<td>2.70</td>
</tr>
<tr>
<td>10</td>
<td>265</td>
<td>10.80</td>
</tr>
<tr>
<td>11</td>
<td>394</td>
<td>10.80</td>
</tr>
<tr>
<td>12</td>
<td>537</td>
<td>10.80</td>
</tr>
<tr>
<td>13</td>
<td>385</td>
<td>7.20</td>
</tr>
<tr>
<td>14</td>
<td>348</td>
<td>5.60</td>
</tr>
<tr>
<td>15</td>
<td>235</td>
<td>1.20</td>
</tr>
<tr>
<td>16</td>
<td>572</td>
<td>7.80</td>
</tr>
</tbody>
</table>

Significantly different from fasting total folate, P = 0.0014 (paired t test).

presentation to tissues. Intestinal folate transport takes place in the jejunum mainly by a saturable, pH-dependent process (16) exhibiting transport Michaelis-Menten kinetic values adequate for dealing with normal amounts of endogenous dietary folate. Before entry into the portal blood system, folic acid undergoes reduction and methylation to 5-methyltetrahydrofolate. This circulating form of the vitamin is metabolically inactive until it is demethylated to tetrahydrofolate (THF) intracellularly—forming methionine from homocysteine in the process. THF then becomes available as a cofactor for the biosynthesis of DNA precursors. The demethylation of 5-methyltetrahydrofolate is catalyzed by the enzyme methionine synthase, which requires cobalamin as cofactor. On the basis of the known substrate specificity of polyglutamate synthetase, it is improbable that in cobalamin deficiency 5-methyltetrahydrofolate would be retained by the cell before removal of the methyl group by methionine synthetase because this form of folate is a weak candidate for polyglutamylation (17). Thus, in cobalamin deficiency, marrow regeneration will fail to occur on treatment with 5-methyltetrahydrofolate.

Above certain amounts of supplemental folic acid, however, a nonsaturable transport mechanism involving passive diffusion of the unaltered vitamin complements the normal absorption mechanism, as shown indirectly by Lukeck et al (2) and Kelly et al (3).

In contrast with the cellular uptake of 5-methyltetrahydrofolate, folic acid is incorporated into metabolically active tissue folates on entry into the cell through reduction by the enzyme dihydrofolate reductase to THF. This form of folate is readily polyglutamylated and retained by the cell for DNA precursor biosynthesis. A hematologic response is thus precipitated not only in folate but also in cobalamin deficiency because the enzyme methionine synthase is not required for the folate cofactors involved in purine and pyrimidine biosynthesis.

Several in vitro studies highlighted the contrasting effects of folic acid and 5-methyltetrahydrofolate in vitamin B-12 deficiency. Tisman and Herbert (18) showed that in bone marrow cells from normal individuals, the uptake of 5-methyltetrahydrofolate was about twice that of folic acid. In bone marrow from pernicious anemia patients on the other hand, the uptake of 5-methyltetrahydrofolate was not significantly greater than that of folic acid, although the uptake of both species was diminished. Metz et al (19), Ganeshaguru and Hoffbrand (20), and Zittoun et al (21) showed that folic acid partially corrected the deoxyuridine suppression abnormality in bone marrow from patients with cobalamin deficiency, whereas 5-methyltetrahydrofolate was ineffective.

One likely explanation for the refractoriness of folic acid as opposed to 5-methyltetrahydrofolate to the treatment of the neurologic consequences of cobalamin deficiency is that with direct folic acid assimilation by the cell an additional function of folate is largely foregone, namely, its capacity to synthesize methionine and subsequently S-adenosylmethionine for methylation purposes (22). The ability of folate to act as a donor of methyl groups in this case is impaired because of diminished activity of the cobalamin-requiring enzyme methionine synthase. Clinical data abound on the deleterious neurologic consequences of folic acid treatment of patients with pernicious anemia in the period before the cobalamin-folate interrelation was elucidated toward the end of the 1950s (11). There is no suggestion that folic acid itself is other than intrinsically safe in this regard, it merely has the capacity to mask the underlying progression of neurologic disease.

In the present study we looked at the acute response in serum to the consumption of folic acid from several foodstuffs. Short-term increases in plasma folate have been used previously as a measure of folate bioavailability (23). Although valuable comparative data on the effect, for instance, of a particular test vehicle or foodstuff may be gathered from time-course response curves (24, 25), one drawback associated with their construction is that folic acid doses > 1 mg are necessary to obtain a measurable acute serum response. Previous studies using 3 mg folic acid indicated the max of both folic acid and 5-methyltetrahydrofolate to be 1.3 h when folic acid was administered orally in saline solution compared with 2.25 h in the case of folic acid in bread (3). In the present study we wished to detect the presence or absence of unmethylated folic acid in serum at concentrations comparable with that of normal circulating 5-methyltetrahydrofolate in response to doses ≤ 1 mg. Therefore, the response was determined by a single postprandial blood sample drawn at the appropriate max for the test meal.

Two caveats must apply to the current study of the acute serum response to oral folic acid. First, to avoid the problem of thermal destruction of folic acid during food production, folic acid in solution was applied and air-dried onto the food after processing. Indeterminate decomposition of folic acid due to processing would have obfuscated the result, particularly when more than one foodstuff was involved. Second, the levels of fortification we used in our study were greater per slice of bread than that which the proposed FDA fortification allows for. This was necessary because acute-response measurements required the determination of serum folic acid concentrations at a time as near as possible to the max. Folic acid was thus delivered in comparatively small food bulk to facilitate ease of consumption over a 10-min period, particularly for the elderly subjects.

Compared with the FDA proposal for fortification at 1.4 mg folic acid/kg (calculated to deliver 30 μg folic acid per slice), the
amounts used here per gram bread, for instance, were substantially higher. Faced with this constraint of overfortifying, it nevertheless required a bolus in isotonic saline, bread, and milk > 200 μg to elicit an acute folic acid response in serum. Similar threshold doses for isotonic saline and bread indicate greater bioavailability of folate in bread than had been reported previously (26). No significant dose-response effects on serum folic acid concentrations were found in the sample of subjects throughout these experiments except at the highest bread fortification (Table 3).

What are the implications, however, of the frequency of occurrence of unmetabolized folic acid in serum in the relatively small numbers examined here for the recent debate surrounding the US fortification program? In the United States, nontargeted, high bread-consuming groups such as 11–18-y-old males at the 95th percentile of consumption currently consume ~800 μg total folate/d (5). The 1.4-mg/kg fortification program may be calculated to increase that amount to ~960 μg/d if the Centers for Disease Control and Prevention recommendation of 3.5 mg/kg flour (7) were to be implemented this would increase consumption to ~1200 μg/d. Similarly increased intakes would apply to high-consuming males and females in the 4–10-y age group and in males > 51 y. These estimations of increased intake of folic acid make it unlikely, however, that the proposed fortification for the US population will lead to the repeated acute appearance of unmetabolized folic acid in serum because the fortified food is likely to be consumed at more than one sitting per day. However, adherence to the FDA’s “safe level of intake” of 1000 μg folate/d by normal consumers through a combination of food folate and supplements may produce unmetabolized folic acid in serum because the major proportion of this amount is likely to be supplemental folic acid. Similarly, on the basis of the current results, compliance with the recommendations for women of childbearing age to take 400 μg/d to prevent neural tube defect pregnancies would most likely result in repeated folic acid appearance in the maternal and fetal circulation for many consumers.

The results of the present study present a dilemma in which, on the one hand, it appears that folic acid supplementation (by tablet) would appear to be the only effective route to achieve protection against neural tube defects (27), and on the other, that such supplementation could result in unmetabolized folic acid appearing in the circulation. A fortification program, such as that being undertaken currently by the US government, can overcome this dilemma by delivering folic acid to the vast majority of the population in a projected daily dose regimen that does not exceed the threshold described here.


REFERENCES

5. Crane NT, Wilson DB, Cook A, Lewis CJ, Yetley EA, Rader JI.