WHAT IS THE RELATIONSHIP BETWEEN FOLIC ACID FROM SUPPLEMENTS AND/OR FORTIFIED FOODS CONSUMED BEFORE AND DURING PREGNANCY AND LACTATION (5 OUTCOMES): SYSTEMATIC REVIEW PROTOCOL

This document describes the protocol for a systematic review to answer the following question: What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and lactation and specific health outcomes? This systematic review is being conducted by the 2020 Dietary Guidelines Advisory Committee, Pregnancy and Lactation Subcommittee and staff from USDA’s Nutrition Evidence Systematic Review (NESR).

NESR methodology for answering a systematic review question involves:
- searching for and selecting articles,
- extracting data and assessing the risk of bias of results from each included article,
- synthesizing the evidence,
- developing a conclusion statement,
- grading the evidence underlying the conclusion statement, and
- recommending future research.

More information about NESR’s systematic review methodology is available on the NESR website: https://nesr.usda.gov/2020-dietary-guidelines-advisory-committee-systematic-reviews.

This document describes the protocol, or plan, for how the systematic review will be conducted. The protocol provides:
- The analytic framework (p. 2) illustrates the overall scope of the question, including the population, the interventions and/or exposures, comparators, and outcomes of interest.
- The literature search and screening plan (p. 6) details the electronic databases and inclusion and exclusion criteria (p. 7) that will be used to search for, screen, and select articles to be included in the systematic review.
- The literature search and screening results (p. 12) includes a list of included articles, and a list of excluded articles with the rationale for exclusion.

This protocol is up-to-date as of: 07/02/2019.

This version of the protocol contains updates to the following sections:
- The analytic framework “key definitions” were edited to define fortification, instead of fortified foods, to ensure consistency across the project, and the “key confounder” anthropometry was revised for clarity, but neither update results in a substantive change in the intent of conduct of the review.
Analytic Framework

The analytic framework (Figure 1) illustrates the overall scope of the systematic review, including the population, the interventions and/or exposures, comparators, and outcomes of interest. It also includes definitions of key terms and identifies key confounders considered in the systematic review. The inclusion and exclusion criteria that follow provide additional information about how parts of the analytic framework will be defined and operationalized for the review.

Figure 1a: Analytic framework: Folic acid and micronutrient status

Systematic Review Question: What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and lactation and micronutrient status?

Key Definitions:

Dietary Supplement - a product (other than tobacco) that is intended to supplement the diet; contains one or more dietary ingredients (including vitamins, minerals, herbs or other botanicals; amino acids, and other substances) or their constituents; is intended to be taken by mouth as a pill, capsule, tablet, or liquid; and is labeled on the front panel as being a dietary supplement. (ODS, Dietary Supplement Health and Education Act, 1994)

Fortification - as defined by the FDA, the deliberate addition of one or more essential nutrients to a food, whether or not it is normally contained in the food. Fortification may be used to prevent or correct a demonstrated deficiency in the population or specific population groups; restore naturally occurring nutrients lost during processing, storage, or handling; or to add a nutrient to a food at the level found in a comparable traditional food. When cereal grains are labeled as enriched, it is mandatory that they be fortified with folic acid.

“Before pregnancy” - includes up to 6 months before pregnancy.

Pre-pregnancy BMI - based on health records up to 1 year before and up to and including 1st trimester.

Gestational weight gain - weight a woman gains during pregnancy. (CDC)

Key Confounders: Age, Race/ethnicity, Socioeconomic status, Smoking, Parity, Anthropometry (pre-pregnancy BMI and gestational weight gain (during pregnancy) or Obesity status (before pregnancy and lactation)).
**Systematic review question:** What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and **risk of gestational diabetes**?

<table>
<thead>
<tr>
<th>Intervention/exposure</th>
<th>vs</th>
<th>Comparator</th>
<th>Intermediate outcomes</th>
<th>Endpoint outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to, including intake of, Folic acid from:</td>
<td>Different level of exposure to, including intake of, Folic acid from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Dietary supplements (including multiple-nutrient supplements)</td>
<td>• Dietary supplements (including multiple-nutrient supplements)</td>
<td>• Fasting glucose</td>
<td>Gestational diabetes</td>
<td></td>
</tr>
<tr>
<td>• Fortified foods</td>
<td>• Fortified foods</td>
<td>• Hemoglobin A1C</td>
<td>Population: Women during pregnancy</td>
<td></td>
</tr>
<tr>
<td>• Dietary supplements + fortified foods</td>
<td>• Dietary supplements + fortified foods</td>
<td>• Glucose tolerance/insulin resistance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Population:** Women before and during pregnancy; healthy and/or at risk for chronic disease

**Key Confounders:** Age, Race/ethnicity, Socioeconomic status, Smoking, Parity, Anthropometry (pre-pregnancy BMI and gestational weight gain during pregnancy or Obesity status (before pregnancy)), Family history of diabetes or pre-diabetes

**Key definitions**

- **Dietary Supplement:** a product (other than tobacco) that is intended to supplement the diet; contains one or more dietary ingredients (including vitamins, minerals, herbs or other botanicals, amino acids, and other substances) or their constituents; is intended to be taken by mouth as a pill, capsule, tablet, or liquid; and is labeled on the front panel as being a dietary supplement. (ODS, Dietary Supplement Health and Education Act, 1994)

- **Fortification:** As defined by the FDA, the deliberate addition of one or more essential nutrients to a food, whether or not it is normally contained in the food. Fortification may be used to prevent or correct a demonstrated deficiency in the population or specific population groups; restore naturally occurring nutrients lost during processing, storage, or handling; or to add a nutrient to a food at the level found in a comparable traditional food. When cereal grains are labeled as enriched, it is mandatory that they be fortified with folic acid.

- **Gestational diabetes:** diabetes occurring during pregnancy in women not previously diagnosed with diabetes (Raghavan et al., AJCN, 2019, P/24 Project)

- **“Before pregnancy”** - includes up to 6 months before pregnancy.

- **Pre-pregnancy BMI** - based on health records up to 1 year before and up to and including 1st trimester

- **Gestational weight gain** - weight a woman gains during pregnancy. (CDC)

**Legend**

- The relationship of interest in the systematic review
- Factors that may impact the relationship of interest in the systematic review

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**Figure 2b: Analytic framework: Folic acid and risk of gestational diabetes**
Question: What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and lactation and specific health outcomes?

Figure 3c: Analytic framework: Folic acid and risk of hypertensive disorders during pregnancy

**Systematic review question:** What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and risk of hypertensive disorders during pregnancy?

**Intervention/exposure** vs Comparator

- Exposure to, including intake of, Folic acid from:
  - Dietary supplements (including multiple-nutrient supplements)
  - Fortified foods
  - Dietary supplements + fortified foods

- Different level of exposure to, including intake of, Folic acid from:
  - Dietary supplements (including multiple-nutrient supplements)
  - Fortified foods
  - Dietary supplements + fortified foods

**Population:** Women before and during pregnancy, healthy and/or at risk for chronic disease.

**Intermediate outcomes**

- Blood pressure (systolic and diastolic)
- Protein in the urine (proteinuria)

**Health outcomes**

- Hypertensive disorders during pregnancy, including:
  - Eclampsia
  - Preeclampsia
  - Gestational hypertension

**Population:** Women during pregnancy

**Key definitions**

- **Dietary Supplement** - a product (other than tobacco) that is intended to supplement the diet, contains one or more dietary ingredients (including vitamins, minerals, herbs or other botanicals, amino acids, and other substances) or their constituents, is intended to be taken by mouth as a pill, capsule, tablet, or liquid, and is labeled on the front panel as being a dietary supplement. (ODS; Dietary Supplement Health and Education Act, 1994)

- **Fortification** - As defined by the FDA, the deliberate addition of one or more essential nutrients to a food, whether or not it is normally contained in the food. Fortification may be used to prevent or correct a demonstrated deficiency in the population or specific population groups, restore naturally occurring nutrients lost during processing, storage, or handling, or to add a nutrient to a food at the level found in a comparable traditional food. When cereal grains are labeled as enriched, it is mandatory that they be fortified with folate.

- **“Before pregnancy”** - includes up to 6 months before pregnancy.

- **Gestational weight gain** - weight a woman gains during pregnancy. (CDC)

**Legend**

- The relationship of interest in the systematic review
- Factors that may impact the relationship of interest in the systematic review
Figure 4d: Analytic framework: Folic acid and human milk composition

**Systematic review question:** What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and lactation and human milk composition?

<table>
<thead>
<tr>
<th>Intervention/exposure vs Comparators</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to, including intake of Folic acid from:</td>
<td>Human milk composition:</td>
</tr>
<tr>
<td>• Dietary supplements (including multiple-nutrient supplements)</td>
<td>• Folate</td>
</tr>
<tr>
<td>• Fortified foods</td>
<td>Population: Women during lactation; healthy and/or at risk for chronic disease</td>
</tr>
<tr>
<td>• Dietary supplements + fortified foods</td>
<td></td>
</tr>
</tbody>
</table>

**Population:** Women during pregnancy and/or lactation, healthy and/or at risk for chronic disease

**Key Confounders:** Age, Race/ethnicity, Socioeconomic status, Smoking, Parity, Anthropometry (pre-pregnancy BMI and gestational weight gain during pregnancy) or Obesity status (before pregnancy and lactation)

**Key definitions**

**Dietary Supplement** - a product (other than tobacco) that, is intended to supplement the diet, contains one or more dietary ingredients (including vitamins, minerals, herbs or other botanicals, amino acids, and other substances) or their constituents; is intended to be taken by mouth as a pill, capsule, tablet, or liquid; and is labeled on the front panel as being a dietary supplement. (ODS, Dietary Supplement Health and Education Act, 1994)

**Fortification** - As defined by the FDA, the deliberate addition of one or more essential nutrients to a food, whether or not it is normally contained in the food. Fortification may be used to prevent or correct a demonstrated deficiency in the population or specific population groups, restore naturally occurring nutrients lost during processing, storage, or handling; or to add a nutrient to a food at the level found in a comparable traditional food.

When cereal grains are labeled as enriched, it is mandatory that they be fortified with folic acid.

**Legend**

- The relationship of interest in the systematic review
- Factors that may impact the relationship of interest in the systematic review

**Question:** What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and lactation and specific health outcomes?
Figure 5e: Analytic framework: Folic acid and developmental milestones, including neurocognitive development

**Systematic review question:** What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and lactation and developmental milestones, including neurocognitive development?

<table>
<thead>
<tr>
<th>Intervention/exposure</th>
<th>vs</th>
<th>Comparator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to, including intake of: Folic acid from:</td>
<td>Different level of exposure to, including intake of: Folic acid from:</td>
<td></td>
</tr>
<tr>
<td>• Dietary supplements (including multiple-nutrient supplements)</td>
<td>• Dietary supplements (including multiple-nutrient supplements)</td>
<td></td>
</tr>
<tr>
<td>• Fortified foods</td>
<td>• Fortified foods</td>
<td></td>
</tr>
<tr>
<td>• Dietary supplements + fortified foods</td>
<td>• Dietary supplements + fortified foods</td>
<td></td>
</tr>
</tbody>
</table>

**Population:** Women before and during pregnancy and/or during lactation; healthy and/or at risk for chronic disease

**Key Confounders:** Age, Race/ethnicity, Socioeconomic status, Smoking, Parity, Anthropometry (pre-pregnancy BMI and gestational weight gain (during pregnancy) or Obesity status (before pregnancy and lactation)), Child sex, Gestational age, Breastfeeding practices

**Outcomes:**
- Developmental domains, examined via milestone achievement and/or scales/indices, including:
  - Cognitive
  - Language/communication
  - Motor/physical
  - Social-emotional
  - Academic performance
  - Attention deficit disorder (ADD) or attention-deficit/hyperactivity disorder (ADHD)
  - Anxiety
  - Depression
  - Autism spectrum disorder (ASD)

**Population:** Infants and toddlers (birth to 24 months), children and adolescents (ages 2-18 years)

**Key definitions:**

**Dietary Supplement** - a product (other than tobacco) that is intended to supplement the diet; contains one or more dietary ingredients (including vitamins, minerals, herbs or other botanicals; amino acids; and other substances) or their constituents; is intended to be taken by mouth as a pill, capsule, tablet, or liquid; and is labeled on the front panel as being a dietary supplement. (ODS: Dietary Supplement Health and Education Act, 1994)

**Fortification** - as defined by the FDA, the deliberate addition of one or more essential nutrients to a food, whether or not it is normally contained in the food. Fortification may be used to prevent or correct a demonstrated deficiency in the population or specific population groups, restore naturally occurring nutrients lost during processing, storage, or handling; or to add a nutrient to a food at the level found in a comparable traditional food. When cereals are labeled as enriched, it is mandatory that they be fortified with folic acid.

“Before pregnancy” - includes up to 6 months before pregnancy

**Pre-pregnancy BMI** - based on health records up to 1 year before and up to and including 1st trimester

**Gestational weight gain** - weight a woman gains during pregnancy. (CDC)

**Legend**

- The relationship of interest in the systematic review
- Factors that may impact the relationship of interest in the systematic review

LITERATURE SEARCH AND SCREENING PLAN

**Electronic databases**

Listed below are the databases that will be searched to identify all potentially relevant articles that have been published to address the systematic review question. Additional details regarding the search strategy will be published upon completion of the review, and are available upon request prior to publication.

- Pubmed
- Cochrane
- Embase
- Cumulative Index to Nursing and Allied Health Literature (CINAHL)
### Inclusion and exclusion criteria

This table provides the inclusion and exclusion criteria for the systematic review. The inclusion and exclusion criteria are a set of characteristics used to determine which articles identified in the literature search will be included or excluded in the systematic review.

**Table 1. Inclusion and exclusion criteria**

<table>
<thead>
<tr>
<th>Category</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study design</strong></td>
<td>• Randomized controlled trials</td>
<td>• Uncontrolled trials</td>
</tr>
<tr>
<td></td>
<td>• Non-randomized controlled trials including quasi-experimental and controlled before-and-after studies</td>
<td>• Case-control studies</td>
</tr>
<tr>
<td></td>
<td>• Prospective cohort studies</td>
<td>• Cross-sectional studies (for outcomes: micronutrient status, gestational diabetes, hypertensive disorders, developmental milestones, including neurocognitive health)</td>
</tr>
<tr>
<td></td>
<td>• Retrospective cohort studies</td>
<td>• Narrative reviews</td>
</tr>
<tr>
<td></td>
<td>• Nested case-control studies</td>
<td>• Systematic reviews</td>
</tr>
<tr>
<td></td>
<td>• Uncontrolled before-and-after studies</td>
<td>• Meta-analyses</td>
</tr>
<tr>
<td></td>
<td>• Cross-sectional studies (for outcomes: human milk composition)</td>
<td></td>
</tr>
</tbody>
</table>

**Intervention/exposure**

- Exposure to, including intake of, folic acid from:
  - Dietary supplements (including multiple-nutrient supplements)
  - Fortified foods
  - Dietary supplements + fortified foods

- Exposure to multiple-micronutrient supplements in which nutrients other than the nutrient of interest vary

**Comparator**

- Different levels of exposure to, including intake of, folic acid from:
  - Dietary supplements (including multiple-nutrient supplements)
  - Fortified foods
  - Dietary supplements + fortified foods

- No comparator
- Exposure to multiple-micronutrient supplements in which nutrients other than the nutrient of interest vary

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**Question:** What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and lactation and specific health outcomes?
<table>
<thead>
<tr>
<th>Category</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome: Micronutrient status</td>
<td>• Folate • Vitamin B12 • Hemoglobin • Mean Corpuscular Volume (MCV) • Red Blood Cell Distribution Width (RDW)</td>
<td>• N/A</td>
</tr>
<tr>
<td>Outcome: Gestational diabetes</td>
<td>• Gestational diabetes • Fasting glucose • Hemoglobin A1C • Glucose tolerance/insulin resistance • Oral Glucose Tolerance Test</td>
<td>• N/A</td>
</tr>
<tr>
<td>Outcome: Hypertensive disorders</td>
<td>• Eclampsia • Preeclampsia • Gestational hypertension • Blood pressure (systolic and diastolic) • Protein in the urine (proteinuria)</td>
<td>• N/A</td>
</tr>
<tr>
<td>Outcome: Human milk composition</td>
<td>• Folate</td>
<td>• N/A</td>
</tr>
<tr>
<td>Outcome: Developmental milestones, including neurocognitive health</td>
<td>• Developmental domains, examined via milestone achievement and/or scales/indices, including: o Cognitive, o Language/communication, o Movement/physical, o Social-emotional o Academic performance • Attention deficit disorder (ADD) or attention-deficit/hyperactivity disorder (ADHD) • Anxiety • Depression • Autism spectrum disorder (ASD)</td>
<td>• N/A</td>
</tr>
<tr>
<td>Date of publication (for all outcomes)</td>
<td>• January 1980 – June/July 2019</td>
<td>• Articles published prior to January 1980</td>
</tr>
<tr>
<td>Category</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Publication status</td>
<td>• Articles that have been peer-reviewed</td>
<td>• Articles that have not been peer-reviewed and are not published in peer-reviewed journals, including unpublished data, manuscripts, reports, abstracts, and conference proceedings</td>
</tr>
<tr>
<td>Language of publication</td>
<td>• Articles published in English</td>
<td>• Articles published in languages other than English</td>
</tr>
<tr>
<td>Country</td>
<td>• Studies conducted in countries ranked as high or very high human development</td>
<td>• Studies conducted in countries ranked as medium or lower human development</td>
</tr>
<tr>
<td>Study participants</td>
<td>• Human participants</td>
<td>• Non-human participants (e.g., animal or in-vitro models)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Studies that exclusively enroll women who became pregnant using Assisted Reproductive Technologies</td>
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<tr>
<td></td>
<td></td>
<td>• Studies that exclusively enroll women with multiple gestation pregnancies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Men</td>
</tr>
<tr>
<td>Life stage of study participants</td>
<td>• Women up to 6 months before pregnancy</td>
<td>• N/A</td>
</tr>
<tr>
<td></td>
<td>• Women during pregnancy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Women during lactation</td>
<td></td>
</tr>
</tbody>
</table>

1 The Human Development classification was based on the Human Development Index (HDI) ranking from the year the study intervention occurred or data were collected (UN Development Program. HDI 1990-2017 HDRO calculations based on data from UNDESA (2017a), UNESCO Institute for Statistics (2018), United Nations Statistics Division (2018b), World Bank (2018b), Barro and Lee (2016) and IMF (2018). Available from: http://hdr.undp.org/en/data). If the study did not report the year in which the intervention occurred or data were collected, the HDI classification for the year of publication was applied. HDI values are available from 1980, and then from 1990 to present. If a study was conducted prior to 1990, the HDI classification from 1990 was applied. When a country was not included in the HDI ranking, the current country classification from the World Bank was used instead (The World Bank. World Bank country and lending groups. Available from: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-country-and-lending-groups).
<table>
<thead>
<tr>
<th>Category</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life stage of study participants - outcomes: micronutrient status</td>
<td>• Women during pregnancy</td>
<td>• N/A</td>
</tr>
<tr>
<td>Life stage of study participants - outcomes: gestational diabetes</td>
<td>• Women during pregnancy</td>
<td>• N/A</td>
</tr>
<tr>
<td>Life stage of study participants - outcomes: hypertensive disorders</td>
<td>• Women during pregnancy</td>
<td>• N/A</td>
</tr>
<tr>
<td>Life stage of study participants - outcomes: milk composition</td>
<td>• Women during lactation</td>
<td>• N/A</td>
</tr>
<tr>
<td>Life stage of study participants - outcomes: dev milestones including</td>
<td>• Infants and toddlers (birth – 24 months)</td>
<td>• Adults (19 – 64 years)</td>
</tr>
<tr>
<td></td>
<td>• Children and adolescents (2 – 18 years)</td>
<td>• Older adults (65 years and older)</td>
</tr>
</tbody>
</table>

Question: What is the relationship between folic acid from supplements and/or fortified foods consumed before and during pregnancy and lactation and specific health outcomes?
<table>
<thead>
<tr>
<th>Category</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
</table>
| Health status of study participants | • Studies that enroll participants who are healthy and/or at risk for chronic disease, including those with obesity  
• Studies that enroll some participants diagnosed with a disease or with the health outcome of interest:  
  o Gestational diabetes  
  o Hypertensive disorders of pregnancy  
  o Neurocognitive disorders (ADD, ADHD, anxiety, depression, or ASD)  
  o Studies that enroll some participants who are deficient in folate  
• Studies that enroll some mothers with infants who are born preterm (<37 weeks and 0/7 days gestational age)  
• Studies that enroll mothers diagnosed with the outcome of interest that is to be examined in the infant/child (developmental milestones, including neurocognitive development) | • Studies that exclusively enroll participants diagnosed with a disease, or hospitalized with an illness or injury. (For this criterion, studies that exclusively enroll participants with obesity will not be excluded.)  
• Studies that exclusively enroll participants with the outcome of interest (gestational diabetes, hypertensive disorders of pregnancy) (i.e., studies that aim to treat participants who have already been diagnosed with the outcome of interest)  
• Studies that exclusively enroll infants born preterm (gestational age <37 weeks and 0/7 days), infants with low birth weight (<2500g), and/or infants born small for gestational age |
LITERATURE SEARCH AND SCREENING RESULTS

This protocol will be updated with the literature search and screening results after the search and screening plan has been finalized and implemented.

The flow chart (Figure 2) below illustrates the literature search and screening results for articles examining the systematic review question. The results of the electronic database searches, after removal of duplicates, were screened independently by two NESR analysts using a step-wise process by reviewing titles, abstracts, and full-texts to determine which articles met the inclusion criteria. A manual search was done to find articles that were not identified when searching the electronic databases; all manually identified articles are also screened to determine whether they meet criteria for inclusion.

Figure 6: Flow chart of literature search and screening results (To be added)

Included Articles (To be added)

1. Ref

Excluded Articles (To be added)

The table below lists the articles excluded after full-text screening. At least one reason for exclusion is provided for each article, which may not reflect all possible reasons. Information about articles excluded after title and abstract screening is available upon request.

Table 2. Excluded articles

<table>
<thead>
<tr>
<th>Citation</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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</tbody>
</table>