The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the Pharmacy Compounding Advisory Committee (advisory committee). We are bringing certain compounding issues to this advisory committee to obtain the committee’s advice. The background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division, Office, or Agency.
Table of Contents

I. Introduction .......................................................................................... 3
   A. Bulk Drug Substances That Can Be Used by Compounders under Section 503A .. 3
   B. Drug Products and Categories of Drug Products that Present Demonstrable
      Difficulties for Compounding .................................................................. 4

II. Substances Nominated for Inclusion on the 503A Bulks List .................. 5

III. Drug Products and Categories of Drug Products That Present Demonstrable
     Difficulties for Compounding .................................................................. 6
     A. Oral Solid Modified Release Drug Products That Employ Coated Systems .... 7

IV. Draft Points to Consider ....................................................................... 7
I. Introduction

Section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act) describes the conditions that must be satisfied for human drug products compounded by a licensed pharmacist in a State-licensed pharmacy or Federal facility, or by a licensed physician, to be exempt from the following three sections of the FD&C Act: section 505 (concerning the approval of drugs under new drug applications (NDAs) or abbreviated new drug applications (ANDAs)); section 502(f)(1) (concerning the labeling of drugs with adequate directions for use); and section 501(a)(2)(B) (concerning current good manufacturing practice (CGMP) requirements).

The Drug Quality and Security Act added a new section 503B to the FD&C Act, under which a compounding pharmacy may elect to register as an outsourcing facility. Outsourcing facilities, as defined in section 503B of the FD&C Act, are facilities that meet certain conditions described in section 503B, including registration with FDA as an outsourcing facility. If these conditions are satisfied, a drug product compounded for human use by or under the direct supervision of a licensed pharmacist in an outsourcing facility is exempt from three sections of the FD&C Act: (1) Section 502(f)(1) (concerning the labeling of drugs with adequate directions for use); (2) section 505 (concerning the approval of human drug products under NDAs or ANDAs); and (3) section 582 (concerning the requirements of the Drug Supply Chain Security Act). Outsourcing facilities remain subject to CGMP requirements. Outsourcing facilities can compound drugs with or without receiving patient specific prescriptions or orders.

A. Bulk Drug Substances That Can Be Used by Compounders under Section 503A

One of the conditions that must be met for a compounded drug product to qualify for the exemptions in section 503A of the FD&C Act is that a licensed pharmacist or licensed physician compounds the drug product using bulk drug substances that meet one of the following criteria:

1. Comply with the standards of an applicable United States Pharmacopeia (USP) or National Formulary (NF) monograph, if a monograph exists, and the USP chapter on pharmacy compounding;
2. If such a monograph does not exist, are drug substances that are components of drugs approved by the Secretary; or
(3) If such a monograph does not exist and the drug substances are not components of drugs approved by the Secretary, appear on a list developed by the Secretary through regulations issued by the Secretary under subsection (c) of section 503A. 

(See section 503A(b)(1)(A)(i) of the FD&C Act).

FDA is considering those substances nominated for inclusion on the list of bulk drug substances that can be used to compound drug products under section 503A of the FD&C Act (503A Bulks List). As discussed at the February 2015 PCAC meeting, and as proposed in the Notice of Proposed Rulemaking published in the Federal Register of December 16, 2016 (81 FR 91071), FDA has proposed the following criteria to evaluate the nominated substances:

(1) The physical and chemical characterization of the substance;
(2) Any safety issues raised by the use of the substance in compounded drug products;
(3) The available evidence of effectiveness or lack of effectiveness of a drug product compounded with the substance, if any such evidence exists; and
(4) Historical use of the substance in compounded drug products, including information about the medical condition(s) the substance has been used to treat and any references in peer-reviewed medical literature.

In evaluating the candidates for the 503A Bulks List under these criteria, the Agency has proposed to use a balancing test. Specifically, the Agency has proposed to consider each criterion in the context of the others and to balance them, on a substance-by-substance basis, to decide whether a particular substance is appropriate for inclusion on the list.

B. Drug Products and Categories of Drug Products that Present Demonstrable Difficulties for Compounding

Both sections 503A and 503B of the FD&C Act require compounded drug products to satisfy several conditions to qualify for the statutory exemptions from the FD&C Act listed in each section.

One of the conditions for the exemptions under section 503A is that the compounded drug product is not a drug product that “presents demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety or effectiveness of that drug product.” See section 503A(b)(3).
Similarly, one of the conditions for the exemptions under section 503B is that the compounded drug product “is not identified (directly or as part of a category of drugs) on a list published by the Secretary . . . of drugs or categories of drugs that present demonstrable difficulties for compounding that are reasonably likely to lead to an adverse effect on the safety or effectiveness of the drug or category of drugs, taking into account the risks and benefits to patients,” or “is compounded in accordance with all applicable conditions identified…as conditions that are necessary to prevent the drug or category of drugs from presenting [such] demonstrable difficulties.” See section 503B(a)(6).

FDA is considering those substances nominated for inclusion on the list of drug products and categories of drug products that present demonstrable difficulties for compounding (Difficult to Compound List). As discussed at the November 2016 PCAC meeting, FDA proposes to apply six criteria to evaluate whether drug products or categories of drug products are difficult to compound under sections 503A and/or 503B of the FD&C Act:

(1) The complexity of the formulation;
(2) The complexity of the drug delivery mechanism;
(3) The complexity of the dosage form;
(4) The complexity of the bioavailability issues;
(5) The complexity of the compounding process; and
(6) The physicochemical or analytical testing complexity.

II. Substances Nominated for Inclusion on the 503A Bulks List (in order of discussion at the meeting)

A. Nicotinamide Adenine Dinucleotide (Tab 1)

1. Nomination (Tab 1a)
   (a) Fagron

2. FDA Review (Tab 1b)

B. Nicotinamide Adenine Dinucleotide Disodium Reduced (Tab 2)

1. Nominations (Tab 2a)
   (a) Fagron
   (b) International Academy of Compounding Pharmacists
2. FDA Review (Tab 2b)

C. Nettle (Tab 3)

1. Nominations (Tab 3a)
   (a) National Community Pharmacists Association
   (b) International Academy of Compounding Pharmacists

2. FDA Review (Tab 3b)

B. Ubiquinol (Tab 4)

1. Nomination (Tab 4a)
   (a) Fagron

2. FDA Review (Tab 4b)

C. Vanadyl Sulfate (Tab 5)

1. Nominations (Tab 5a)
   (a) McGuff Compounding Pharmacy Services, Inc.
   (b) Integrative Medical Consortium
   (c) Alliance for Natural Health USA
   (d) American Association of Naturopathic Physicians

2. FDA Review (Tab 5b)

D. Artemisinin (Tab 6)

1. Nominations (Tab 6a)
   (a) McGuff Compounding Pharmacy Services, Inc.
   (b) Alliance for Natural Health USA
   (c) American Association of Naturopathic Physicians
   (d) Integrative Medical Consortium

2. FDA Review (Tab 6b)

III. Drug Products and Categories of Drug Products That Present Demonstrable Difficulties for Compounding
A. Oral Solid Modified Release Drug Products That Employ Coated Systems (Tab 7)

1. Nominations (Tab 7a)
   (a) Public Citizen’s Health Research Group
   (b) AbbVie
   (c) GlaxoSmithKline
   (d) Biogen Idec

2. FDA Review (Tab 7b)

IV. Draft Points to Consider

A. May 8, 2017, a.m. session

Draft Points for the PCAC to Consider Regarding Whether to Include Certain Bulk Drug Substances on the 503A Bulks List

1. FDA is proposing that nicotinamide adenine dinucleotide NOT be included on the 503A Bulks List. Should nicotinamide adenine dinucleotide be placed on the list?

2. FDA is proposing that nicotinamide adenine dinucleotide disodium reduced NOT be included on the 503A Bulks List. Should nicotinamide adenine dinucleotide disodium reduced be placed on the list?

B. May 8, 2017, p.m. session

Draft Points for the PCAC to Consider Regarding Whether to Include Certain Bulk Drug Substances on the 503A Bulks List

1. FDA is proposing that nettle NOT be included on the 503A Bulks List. Should nettle be placed on the list?

2. FDA is proposing that ubiquinol NOT be included on the 503A Bulks List. Should ubiquinol be placed on the list?

3. FDA is proposing that vanadyl sulfate NOT be included on the 503A Bulks List. Should vanadyl sulfate be placed on the list?
C. May 9, 2017, a.m. session

Draft Points for the PCAC to Consider Regarding Whether to Include Certain Bulk Drug Substances on the 503A Bulks List

1. FDA is proposing that artemisinin NOT be included on the 503A Bulks List. Should artemisinin be placed on the list?

Draft Points for the PCAC to Consider Regarding Whether to Include Certain Drug Products or Categories of Drug Products on the Difficult to Compound List

2. FDA is proposing that oral solid modified release drug products that employ coated systems be INCLUDED on the Difficult to Compound List under sections 503A and 503B of the FD&C Act. Should oral solid modified release drug products that employ coated systems be placed on the list?
Tab 1

Nicotinamide Adenine Dinucleotide
Tab 1a

Nicotinamide Adenine Dinucleotide Nominations
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the name of the nominated ingredient?</td>
<td>Nicotinamide Adenine Dinucleotide(NAD)</td>
</tr>
<tr>
<td>Is the ingredient listed in any of the three sections of the Orange Book?</td>
<td>The nominated substance was searched for in all three sections of the Orange Book located at <a href="http://www.accessdata.fda.gov/">http://www.accessdata.fda.gov/</a> scripts/cder/ob/docs/queryai.cfm. The nominated substance does not appear in any section searches of the Orange Book.</td>
</tr>
<tr>
<td>Were any monographs for the ingredient found in the USP or NF monographs?</td>
<td>The nominated substance was searched for at <a href="http://www.uspnf.com">http://www.uspnf.com</a>. The nominated substance is not the subject of a USP or NF monograph.</td>
</tr>
<tr>
<td>What is the chemical name of the substance?</td>
<td>1-(3-Carbamoylpyridinio)-β-D-ribofuranoside 5-(adenosine-5’-pyrophosphate)</td>
</tr>
<tr>
<td>What is the common name of the substance?</td>
<td>Nadide; Coenzyme I; Coenzima I; Coenzym I; Co-I; Difosfopiridina nucleotido; Diphosphopyridine Nucleotide; DPN; NAD; Nadida; Nadidum; Nicotinamida adenina dinucleot</td>
</tr>
<tr>
<td><strong>Does the substance have a UNII Code?</strong></td>
<td>0U46U6E8UK</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>What is the chemical grade of the substance?</strong></td>
<td>no grade</td>
</tr>
</tbody>
</table>
| **What is the strength, quality, stability, and purity of the ingredient?** | Purity: ≥ 95.0%  
NAD+ Content: (report)  
Water Content: < 8.0%  
ε at 260 nm, pH 7.5: (18.0 +/- 0.5) x 10^3  
Ratio at pH 7.5:  
- A250/A260: 0.83 +/- 0.03  
- A280/A260: 0.21 +/- 0.02  
ε when reduced with alcohol dehydrogenase at 340 nm, pH 10: (6.3 +/- 0.2) x 10^3  
Ratio when reduced with alcohol A340/A260 dehydrogenase at pH 10: 0.43 +/- 0.01 |
<p>| <strong>How is the ingredient supplied?</strong> | Powder |
| <strong>Is the substance recognized in foreign pharmacopeias or registered in other countries?</strong> | DPN (Alclin, S.Afr.) , Enada (Health &amp; Diet Food Co., UK) , Enervon-C (United American, Philipp.) , Gameval (Alter, Spain) , Gynaegnost (Schwarzhaupt, Ger.) , Nad (Medical, Spain) , Nicodrasi (Bruco, Ital.) , Piruvasi (Isnardi, Ital.) , Visal (Alter, Spain) |
| <strong>Has information been submitted about the substance to the USP for consideration of monograph development?</strong> | No USP Monograph submission found. |
| <strong>What dosage form(s) will be compounded using the bulk drug substance?</strong> | Capsules |
| <strong>What strength(s) will be compounded from the nominated substance?</strong> | 5mg |
| <strong>What are the anticipated route(s) of administration of the compounded drug product(s)?</strong> | Oral |</p>
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has the bulk drug substance been used previously to compound drug product(s)?</td>
<td>Capsules</td>
</tr>
<tr>
<td>What is the proposed use for the drug product(s) to be compounded with the nominated substance?</td>
<td>NAD is an essential nutrient and supplementation has been shown useful in reducing fatigue in Multiple Sclerosis</td>
</tr>
<tr>
<td>Is there any other relevant information?</td>
<td>All relevant information was expressed in the above questions</td>
</tr>
</tbody>
</table>
General Background on Bulk Drug Substance

**Ingredient Name**  
beta-Nicotinamide Adenine Dinucleotide Disodium Salt, Trihydrate, Reduced

**Chemical/Common Name**  
beta-NADH Disodium Salt; beta-NADH; Reduced NAD; Reduced DPN

**Identifying Codes**  
606-68-8

**Chemical Grade**  
Provided by FDA Registered Supplier/COA

**Description of Strength, Quality, Stability, and Purity**  
Provided by FDA Registered Supplier/COA

**How Supplied**  
Varies based upon compounding requirement

**Recognition in Formularies**  
Not Listed in USP/NF for this specific salt/form

Information on Compounded Bulk Drug Preparation

**Dosage Form**  
Varies based upon compounding requirement/prescription

**Strength**  
Varies based upon compounding requirement/prescription

**Route of Administration**  
Varies based upon compounding requirement/prescription

**Bibliography**  
(where available)

Past and Proposed Use

The very nature of a compounded preparation for an individual patient prescription as provided for within FDCA 503A means that the purpose for which it is prescribed is determined by the health professional authorized to issue that prescription. FDA’s request for this information is an insurmountable hurdle that has not been requested by the PCAC.
Tab 1b

FDA Review of Nicotinamide
Adenine Dinucleotide
DATE: March 31, 2017

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TO: Pharmacy Compounding Advisory Committee

SUBJECT: Review of Nicotinamide Adenine Dinucleotide for Inclusion on the 503A Bulk Drug Substances List
I. INTRODUCTION

Nicotinamide adenine dinucleotide (NAD) has been nominated for inclusion on the list of bulk drug substances for use in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for use in “reducing fatigue in multiple sclerosis” (MS). The nominated route of administration and dosage form is oral capsules.

We have reviewed publicly available data on the physicochemical characteristics, safety, effectiveness, and historical use in compounding of this substance. For the reasons discussed below, we believe the evaluation criteria weigh against placing NAD on the list of bulk drug substances that can be used to compound drug products in accordance with section 503A of the FD&C Act (503A Bulks List).

II. EVALUATION CRITERIA

A. Is the substance well-characterized, physically and chemically, such that it is appropriate for use in compounding?

![NAD molecule](image)

NAD is a prevalent coenzyme in animal and human systems that consists of two nucleotide moieties. NAD is the oxidized form of the coenzyme. The nomination identified 5 mg oral capsules as the anticipated compounded strength and dosage form.


1. Stability of the active pharmaceutical ingredient (API) and likely dosage forms

NAD is a reactive chemical and degrades rapidly because the pyrophosphate linkage is susceptible to hydrolysis upon heating (e.g., to 40 °C), an increase of pH, and exposure to light (Anderson et al. 1962; Dawson et al. 1986; Lowry et al. 1961; Wang et al. 2014). NAD solid is very hygroscopic and should be stored desiccated. According to the Sigma-Aldrich product...
information,\textsuperscript{1} neutral or slightly acidic NAD aqueous solutions are stable for 6 months at -70 °C and 2 weeks at 0 °C (pH 6.0 – 7.5). NAD is very labile in alkaline solutions (pH 8.0) as well. Therefore, NAD is unlikely to be stable under normal storage conditions when compounded as capsules.

2. **Probable routes of API synthesis**

NAD is typically manufactured via yeast fermentation (Budavari et al. ed. 1989). Other routes of chemical synthesis have been reported, but none of them is practical for large-scale production.

3. **Likely impurities**

Likely impurities may include:

1. Bioburden, such as residual yeast
2. Residual solvents and reagents used in the purification
3. Byproducts from the degradation of NAD, such as nicotinamide mononucleotide (NMN) and adenosine monophosphate (AMP) (Wang et al. 2014)

4. **Toxicity of those likely impurities**

Impurities are unlikely to be significantly toxic. Further characterization of the toxicity of these substances is not warranted.

5. **Physicochemical characteristics pertinent to product performance, such as particle size and polymorphism**

NAD is a white or almost white powder that is freely soluble in water. According to the Sigma-Aldrich product information, crystalline NAD is less hygroscopic and electrostatic than amorphous NAD. No further information on the influence of particle size and polymorphism on bioavailability was found in the literature.

6. **Any other information about the substance that may be relevant, such as whether the API is poorly characterized or difficult to characterize**

NAD is characterized with nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, and mass spectrometry.

\textsuperscript{1} Available at http://www.sigmaaldrich.com/catalog/product/sigma/n5655?lang=en&region=US. Sigma-Aldrich manufactures chemicals for use in scientific research, biotechnology, and pharmaceutical development. According to its website, a Sigma-Aldrich specification is “a list of test methods, references to analytical procedures, and appropriate acceptance criteria that are numerical limits, ranges, or other criteria for the test described. It establishes the set of criteria to which a material should conform to be considered acceptable for its intended use.” See http://www.sigmaaldrich.com/united-kingdom-technicalservices/specifications.html. Throughout this review, we rely on information in the Sigma-Aldrich product information sheet where necessary to supplement information from other sources.
Conclusions: NAD is a dinucleotide coenzyme found endogenously in animals and humans. The bulk substance substantially degrades when exposed to light, moisture, alkaline pH, or standard room temperatures; therefore, it will not be stable under ordinary storage conditions. The nominated compound is easily characterized with various analytical techniques, and the preparation of this compound has been well developed.

B. Are there concerns about the safety of the substance for use in compounding?

1. Nonclinical assessment

The following databases were consulted regarding NAD in the preparation of this portion of this review: Embase, PubMed, TOXNET, and Web of Science. Given the limited available toxicology information about NAD, and in an attempt to better define potential safety concerns associated with NAD, the toxicological profiles of related compounds were considered. Toxicology aspects of NAD precursors, including nicotinamide riboside, nicotinamide, and nicotinic acid, are summarized in this review, although an exhaustive review of all available toxicology data for these related compounds was not conducted.

a. Pharmacology of the drug substance

NAD is an endogenous substance that is formed either by de novo synthesis or by recycling/salvage pathways (Sauve, 2008; Penberthy and Tsunoda, 2009; Bender, 2015). When produced via de novo synthesis, NAD is formed by conversion of tryptophan to NAD; when produced through the recycling/salvage pathway, NAD is synthesized from precursors such as nicotinic acid, nicotinamide riboside, and nicotinamide. Decomposition of NAD forms nicotinamide that can be converted to nicotinamide mononucleotide, which in turn can be recycled to form NAD.

NAD plays a central role in energy metabolism and oxidative phosphorylation and is a key component of many metabolic pathways for carbohydrates, lipids, and amino acids (Penberthy and Tsunoda, 2009). NAD is a coenzyme in adenosine triphosphate (ATP) production (glycolysis, tricarboxylic acid (TCA) cycle, and electron transport chain) through the NAD+/NADH2 redox state (Penberthy and Tsunoda, 2009). NAD is reduced to NADH in glycolysis, fermentation (via lactate dehydrogenase) and TCA cycle (Yang and Sauve, 2016). NADH is oxidized back to NAD in oxidative phosphorylation and fermentation (via lactate dehydrogenase).

In addition to its coenzyme role, NAD acts as a substrate for enzymes that add or remove chemical groups from proteins in post-translational modifications leading to changes in protein functions. In this interaction, NAD is the source of adenosine diphosphate (ADP)-ribose for the

---

2 NADH is a coenzyme composed of ribosylnicotinamide 5'-diphosphate coupled to adenosine 5'-phosphate by pyrophosphate linkage. It is found widely in nature and is involved in numerous enzymatic reactions in which it serves as an electron carrier by being alternately oxidized (NAD+) and reduced (NADH). Nicotine adenine dinucleotide disodium reduced (NADH) is a distinct substance and will be considered separately for inclusion on the 503A bulk drug substances list.
ADP-ribosylation of proteins and poly-ADP-ribosylation of nucleoproteins (Penberthy and Tsunoda, 2009). Enzymes that use NAD as a substrate include poly-ADP-ribose polymerase-I (PARP-1), which is essential for DNA repair; cADPR-ribose synthases (CD38 and CD157), which produce mediators of calcium signaling involved in lymphocyte chemotaxis or microglia activation; and the sirtuins lysine deacetylases, which improve mitochondrial adaptation (Khan et al. 2007; Penberthy and Tsunoda, 2009).

Nonclinical studies have been conducted to assess NAD’s involvement in a wide range of biological processes and the potential benefit of exogenous NAD administration (Beaudoin, 1976; Ying et al. 2007; Sheng et al. 2012; Wang et al. 2014; Wang et al. 2016). For example, administration of NAD (50 mg/animal) showed protection against 2-amino-1,3,4-thiadiazole-induced teratogenicity in rats (Beaudoin, 1976). NAD administration via intraperitoneal (IP) injection decreased doxorubicin-induced liver damage in mice (Wang et al. 2014). Sheng et al. (2012) reported that NAD administration attenuated X-ray-induced DNA damage in rats. In a rat model of transient focal ischemia, intranasal NAD dosing 2 hours after ischemia decreased infarct formation at 24 and 72 hours (Ying et al. 2007).

We identified one study of NAD in an experimental animal model of MS, autoimmune encephalomyelitis (EAE) induced with myelin oligodendrocyteglycoprotein (MOG). In this study, mice (n=8/group) received a daily dose of NAD at 250 mg/kg via intraperitoneal injection or a control formulation for 25 days. The mean onset time of clinical signs was delayed from 14 to 22 days post-immunization with MOG, and clinical severity was reported to have been reduced in the NAD-treated group compared to control during the treatment period. The histological analyses of the lumbar spinal cords collected on day 25 revealed that NAD supplementation was associated with reduced inflammation, demyelination, and axonal damage in the spinal cords of the NAD-treated mice compared to control mice. Higher expression levels of p-AMPK and a sirtuin (SIRT1), and a suppressed pro-inflammatory T-cell response, were observed in NAD treated mice compared to controls (Wang et al. 2016).

b. Pharmacokinetics

A limited number of nonclinical studies were found in the literature that described the pharmacokinetics of NAD. Because both NADH and NAD coexist in the body and are converted from one to the other in many metabolic reactions, data on the pharmacokinetics of both enzymes are provided in this section.

Gross and Henderson (1983) assessed intestinal contents (fluid and tissue) following oral dosing of 14C labeled-NAD using a vascularity perfused rat intestine model and a whole in vivo rat model (unspecified dose and number of animals). The decline of labeled-NAD in the intestinal fluid and the increase in hydrolysis products in intestinal tissue after dosing was interpreted to mean that NAD was absorbed and converted to nicotinamide riboside and nicotinamide. NAD administration resulted in a significant increase in urinary excretion of nicotinamide and its metabolites. A single dose of NAD (5 μmol/mouse) was given to mice (n=5 per group) via intraperitoneal injection or orally, and urine samples were continuously collected for 4 days. Following a single intraperitoneal dose of NAD, urinary excretion of nicotinamide was increased by approximately 550 nmol/day. A single oral dose of NAD increased urinary excretion of nicotinamide by 900 nmol/day.
The bioavailability of NAD was measured in the central nervous system of rats using NADH fluorescence signal, representing the concentration of NADH in the cortex (Rex et al. 2002). When rats were administered IV doses of NAD (10 and 50 mg/kg), a significant increase in fluorescence intensity of NADH was observed in the cortex. However, intraperitoneal doses of NAD (10 and 50 mg/kg) were not associated with increases in the NADH fluorescence signal.

In a 21-day repeat dose toxicity study, nicotinamide was administered to rats (500 mg/kg), and an increase in NAD levels was seen in the liver of nicotinamide-treated animals compared to the saline-treated group (Jaus et al. 1977). NAD levels in urine did not change over time (i.e., similar results were seen following the single dose and the 21-day dose), suggesting that NAD does not accumulate after repeated exposure.

Endogenous NAD and NADH coexist in the body and are converted from one to the other in many metabolic reactions. However, little is known about whether the metabolic fates of NAD and NADH are similar or whether administration of one compound results in alterations in the systemic levels of the other.

No human pharmacokinetic information or data were found for NAD.

c. Acute toxicity

The acute toxicity of NAD in mice was investigated in a study of the antihypoxic activity of eight compounds, including NAD, nicotinamide adenine dinucleotide phosphate (NADP), guanosine, inosine, adenosine, ATP, ADP, and adenosine monophosphate (AMP) (Eliseev and Marikhina, 1986). The compounds were dissolved in physiological solution and administered intraperitoneally at doses of 10, 30, 100, 300, and 1000 mg/kg 30 minutes before placing mice in a hermetically sealed chamber to induce hypoxia. The resistance of mice to hypoxia was increased significantly in the presence of the coenzymes NAD and NADP. NAD administration extended by several minutes the lifespan of mice that experienced CO2-induced hypoxia compared to the vehicle treated mice. In the same study, a group of mice (n=6/dose group) received NAD via intraperitoneal injection at 1000, 2000, 3000, 4000 or 5000 mg/kg. The LD50 of NAD was calculated to be 4333 mg/kg.

No other acute toxicity studies were found for NAD.

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3 *Acute toxicity* refers to adverse effects observed following administration of a single dose of a substance, or multiple doses given within a short period (approximately 24 hours). Endpoints captured in acute toxicity studies usually include mortality and gross clinical observations. Acute toxicity data are usually superseded by data obtained from longer term toxicity studies.

4 The number of animals was estimated since the authors did not specify the exact number of the sample size for each treatment group. The reviewers assumed that the reported sample size (n=240) refer to the total number of animals included in all treatment groups and back calculated the number of animals/treatment arm based on the number of groups used in this experiment.
The oral LD$_{50}$ of nicotinic acid, a precursor for NAD, was reported to be approximately 4300 mg/kg in mice and approximately 5100 mg/kg in rats (Unna, 1939).

The effect of nicotinamide, a precursor and a byproduct of NAD, seems to be more potent. The oral LD$_{50}$ of nicotinamide was reported to be approximately 2100 mg/kg and approximately 2700 mg/kg in mice and rats, respectively (Unna, 1939).

d. Repeat dose toxicity

No repeat dose toxicity data were found for NAD.

The toxicity profile of nicotinamide riboside, a precursor and byproduct of NAD during intestinal absorption, was reported in 14-day and 90-day rat oral toxicity studies (Conze et al. 2016). Under the conditions of this study, no mortality was noted up to 5000 mg/kg. At lower doses, target organs of toxicity included the liver, kidney, ovaries, and testes. The lowest observed adverse effect level for nicotinamide riboside was 1000 mg/kg/day, and the no observed adverse effect level was 300 mg/kg/day.

Dosing with nicotinamide via intraperitoneal injection (500 mg/kg) for 21 days caused an increase in NAD levels in the liver and affected other metabolic processes among treated rats (Jaus et al. 1977). The presence of high levels of NAD in the liver was associated with irreversible liver damage, which persisted up to 5 weeks after the end of the dosing period. The histological findings seemed to be more exaggerated among treated female rats. Histopathology of the treated rat livers showed cell enlargement and glycogen deposition with an increase in total hepatic lipids.

e. Genotoxicity

No genotoxicity data were found for NAD. The safety of Niagen, a synthetic form of nicotinamide riboside, was studied in a bacterial reverse mutagenesis assay (Ames), an in vitro chromosome aberration assay, and an in vivo micronucleus assay. Under the conditions of the study, nicotinamide riboside was not genotoxic (Conze et al. 2016). Nicotinamide was negative in the Ames test when tested up to a maximum concentration of 50 mg/plate. When tested in the chromosomal aberration assay using Chinese Hamster fibroblast cell lines, nicotinamide tested negative when used up to a concentration of 2mg/ml (Ishidate et al. 1984), but was positive when tested in a Chinese Hamster lung cell line at a concentration of 3mg/ml where it induced large structural chromosomal aberrations (Ishidate et al. 1988). Nicotinic acid was found negative in the Ames assay when tested up to a maximum concentration of 10 mg/plate and negative in the

---

5 Repeated-dose toxicity studies consist of in vivo animal studies that seek to evaluate the toxicity of the test substance by observing the changes that emerge in clinical observations, clinical chemistry, gross pathology, and histology endpoints when the test substance is repetitively administered daily for a predetermined period of time.

6 The genotoxicity assessment battery usually consists of a gene mutagenicity assay (for single dose trials) and a variety of clastogenicity/genotoxicity assays. To support multiple dose administration in humans, additional genotoxicity testing assessment is usually conducted to detect chromosomal damage in mammalian systems.
chromosomal aberration tests when tested up to a maximum concentration of 2mg/ml in Chinese Hamster fibroblast cell lines (Ishidate et al. 1984).

f. Developmental and reproductive toxicity

No developmental or reproductive toxicity data were found for NAD.

Nicotinamide was not found to be teratogenic in a chick embryo model when tested at 2 to 19 mg/egg (Knip et al. 2000).

g. Carcinogenicity

No carcinogenicity data were found for NAD.

Nicotinamide tested negative for carcinogenic activity when administered to rats via intraperitoneal injection (up to 350 mg/kg) or to mice in drinking water (1%) for a life-long exposure (Knip et al. 2000).

h. Toxicokinetics

No toxicokinetic data (AUC, Tmax, Cmax, t_{1/2}) were found for NAD.

**Conclusion:** NAD is an endogenously synthesized substance whose role in metabolic processes has been studied in various proof-of-concept animal disease models. The limited data that were found in the literature show that NAD can be absorbed in the small intestine in rodents and is hydrolyzed to various related compounds. The toxicity profile of NAD associated with the nominated route of exposure (i.e., oral) cannot be confirmed due to the lack of oral toxicity studies conducted with NAD. When NAD is administered via the intravenous route, it is metabolized to NADH, which can reach the brain; the significance of this finding is not understood in terms of human health. Potential toxicities associated with the administration of NAD, particularly on a chronic basis, are uncharacterized due to lack of animal data (e.g., repeat

---

7 Developmental and reproductive toxicity studies are usually designed to assess the potential adverse effects in humans of both sexes and include females from various age groups that will be exposed to the proposed substance. **Developmental toxicity or teratogenicity** refers to adverse effects (can include embryo-fetal mortality, structural abnormalities, functional impairment, or alterations to growth) and can occur in pups either as a result of the exposure of their parents to the substance, before the pups’ birth, or by direct exposure of the pups to the substance after birth.

8 Studies that assess cancer risk in animals are used as predictive tools to evaluate the potential for drugs to result in tumors when used by humans on a chronic basis. **Carcinogenicity studies** are conducted if the clinical use is expected to be continuous for a minimum of 6 months of life, or if intermittent clinical use is expected to total 6 months or more of life.

9 **Toxicokinetics (TK)** is the field of study where the relationship between the systemic exposure of a substance in experimental animals and the level of toxicity in exposed animals is evaluated. In the context of drug development, TK calculation is used to establish the relationship between systemic exposures in toxicology experiments in animals and the corresponding exposures in humans.
dose toxicity, carcinogenicity, and developmental/reproductive toxicity) found in the literature for NAD.

The toxicological profiles of several NAD precursors, including nicotinamide riboside, nicotinamide, and nicotinic acid, were included in the review because of their involvement in the pharmacological action of NAD. The limited data reported in the literature for the precursors seem to point to a hepatotoxic signal for nicotinamide when used at high doses in the general toxicity study. This finding did not correlate with liver tumor findings in the carcinogenicity study. The correlation of nicotinamide and NAD toxicity is unknown. Overall, the nonclinical safety data for NAD are inadequate to evaluate NAD, particularly for treatment of a chronic condition.

2. Clinical assessment - human safety

The following databases were consulted in preparation of the clinical portion of this review: PubMed, Embase, Cochrane Library, and ClinicalTrials.gov. There are no trials listed on the website ClinicalTrials.gov in which NAD is reported as a treatment.

a. Reported adverse reactions (FAERS, CAERS)

The Office of Surveillance and Epidemiology conducted a search of the FDA Adverse Events Reporting System (FAERS) database for reports of adverse events for NAD through October 25, 2016, and retrieved one case report, from a foreign source, in which NAD was listed as a single ingredient product. A 23-year-old male was reported to have been hospitalized for an acute episode of mania while receiving unspecified amounts of multiple (approximately 45) herbal medicines and dietary supplements, including NAD (Long, 2016). The subject was treated with benzodiazepines and later recovered. The adverse event could not be attributed to NAD.

The Center for Food Safety and Nutrition (CFSAN) collects reports of adverse events involving food, cosmetics, and dietary supplements in the CFSAN Adverse Event Reporting System (CAERS). A search of CAERS was conducted for adverse events associated with NAD on November 3, 2016. No adverse events were identified where NAD was listed as a single ingredient. Adverse events in the multi-ingredient reports could not be attributed to NAD.

b. Clinical trials assessing safety

We identified six controlled clinical trials that evaluated the efficacy of NAD supplementation in alcohol withdrawal symptoms (Hekimian et al. 1966; Rappaport, 1969; Smith et al. 1971), schizophrenia (Kline et al. 1967; Meltzer et al. 1969) and psoriasis (Wozniacka et al. 2007). Three alcohol withdrawal trials included a total of 27 patients. One reported that doses of 100 mg of NAD administered intramuscularly (IM) led to “no pronounced side effects” (Hekimian et al. 1966). No safety or tolerance data were reported for the other two alcohol withdrawal trials, which dosed NAD at 3 mg orally daily for 3 weeks (Rappaport, 1969; Smith et al. 1971). No safety or tolerance data were reported for the two trials of NAD in the treatment of a total of 20 schizophrenic patients, in which NAD was dosed at 2 g daily for 14 to 21 days, or 1 g for 10
days followed by 2 g for 10 days (total of 20 days treatment), respectively (Kline et al. 1967; Meltzer et al. 1969).

In one trial, it was observed that 250 mg capsules of NAD were too big to be swallowed. and these were reformulated to 125 mg (Kline et al. 1967). Topical ointments of 0.3 or 1% NAD were reported to have been well tolerated, with no observed adverse or undesirable effects, in 26 patients treated twice daily for 4 weeks (Wozniacka et al. 2007).

c. The availability of alternative approved therapies that may be as safe or safer

There are no drugs approved by FDA specifically for treating MS-related fatigue. There are multiple FDA-approved MS disease modifying drugs that have been shown to delay disease progression measured in part using tools such as the Expanded Disability Status Scale (EDSS), and may improve other MS symptoms, including primary fatigue. The National Multiple Sclerosis Society publishes a list of FDA-approved disease modifying drugs for the treatment of MS. It can be found at [http://www.nationalmssociety.org/Treating-MS/Medications](http://www.nationalmssociety.org/Treating-MS/Medications). It includes the following:

- Injectable medications
  - Avonex (interferon beta-1a)
  - Betaseron (interferon beta-1b)
  - Copaxone, Glatopa (glatiramer acetate)
  - Extavia (interferon beta-1b)
  - Plegridy (peginterferon beta-1a)
  - Rebif (interferon beta-1a)
  - Zinbryta (daclizumab)
- Oral medications
  - Aubagio (teriflunomide)
  - Gilenya (fingolimod)
  - Tecfidera (dimethyl fumarate)
- Infused medications
  - Lemtrada (alemtuzumab)
  - Novantrone (mitoxantrone)
  - Tysabri (natalizumab)

Amantadine and modafinil are FDA-approved drugs that are identified in at least one consensus-based clinical practice guideline for off-label use to treat MS-related fatigue. Other FDA approved drugs are indicated to treat various secondary factors, which may contribute to MS-related fatigue (e.g., depression).

**Conclusions:** NAD is an endogenous substance; however, there are minimal clinical data on which to identify a safe dose of exogenously administered NAD. Likewise, there are insufficient

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10 FDA is not endorsing the products, services, or information contained on this website and makes no claims, promises, or guarantees about the completeness, accuracy, currency, content or quality of information contained on this website.
data to determine whether NAD may interact with MS disease-modifying therapies or other drugs. Therefore, we have insufficient information on which to evaluate the safety of NAD for use in compounded drug products. There are FDA-approved products indicated for the treatment of MS that have been demonstrated to be safe.

C. Are there concerns about whether a substance is effective for a particular use?

The following databases were consulted in the preparation of this section: PubMed, Embase, Cochrane Library, and ClinicalTrials.gov. There are no trials listed on the website ClinicalTrials.gov in which NAD is reported as a treatment.

The exact mechanism by which NAD might exert an effect in the treatment of MS-related fatigue is not known. A recent review article describes the pathophysiology for primary and secondary fatigue in MS (Rottoli et al. 2016). Primary fatigue is considered to be related to the underlying central nervous system (CNS) abnormalities of MS. Proposed mechanisms of fatigue related to CNS disturbances may involve the immune system or sequela from CNS damage (Braley and Chervin, 2010; Induruwa et al. 2012; Rottoli et al. 2016). Secondary factors that may contribute to MS-related fatigue include pain, depression, sleep disorders, coping ability, and degree of psychosocial support (Braley and Chervin, 2010; Induruwa et al. 2012; Rottoli et al. 2016). The causes of fatigue must be thoroughly evaluated in each patient to determine the appropriate treatment, which may include pharmacologic or non-pharmacological interventions.

1. Reports of trials, clinical evidence, and anecdotal reports of effectiveness, or lack of effectiveness, of the bulk drug substance

Our review did not identify any published studies evaluating the effects of exogenous NAD administration in the treatment of MS-related fatigue or any other aspect of the disease. In addition, we did not identify any published studies that evaluated the effects of nicotinamide, nicotinic acid, or nicotinamide riboside administration to treat MS-related fatigue or any other aspect of the disease.

One published study suggests that NAD levels are decreased in MS patients and may be associated with disease progression. A study compared serum NAD and NADH levels in healthy controls (n=99) to three groups of clinically stable MS patients: (1) relapsing remitting MS (RRMS) (n=209), (2) secondary progressive MS (SPMS) (n=136), and (3) primary progressive MS (PPMS) (n=51). MS patients had serum NAD levels at least 50% lower than controls (17.9 mcg/mL), corrected for age and gender. Within the three MS sub-groups, NAD levels were higher in RRMS (9.973 mcg/mL) compared to SPMS (7.872 mcg/mL) and PPMS (6.372 mcg/mL). MS patients also had a two-fold increase in NADH levels and at least three-fold reduction in the NAD/NADH ratio as compared to controls (Braidy et al. 2013).

2. Whether the product compounded with this bulk drug substance is intended to be used in a serious or life-threatening disease
The National Multiple Sclerosis Society provides information about the disease and its incidence, symptoms, and pathogenesis at [http://www.nationalmssociety.org/](http://www.nationalmssociety.org/). MS is a serious immune-mediated disease of the central nervous system that results in myelin destruction and axonal degeneration in the brain and spinal cord. The worldwide prevalence of MS is estimated to be 2.3 million patients. Fatigue is intrinsic to MS and is the most frequently reported symptom in MS, which is reported to affect up to 80% of patients. Additionally, 50 to 60% of MS patients describe fatigue as one of their most troubling symptoms, regardless of their disease course or level of disability. MS-related fatigue is defined by a subjective sense of exhaustion, lack of energy, or tiredness.

3. **Whether there are any alternative approved therapies that may be as effective or more effective.**

See Section II. B. 2. c. above

**Conclusions:** There are no published studies that support the use of NAD for the treatment of fatigue in patients with multiple sclerosis. Therefore, we have insufficient information on which to evaluate the effectiveness for NAD for its proposed use. MS is a serious condition for which FDA-approved products are available.

**D. Has the substance been used historically in compounding?**

The following databases were consulted in preparation of this section: PubMed, Natural Medicines, European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, USP/NF, and Google.

1. **Length of time the substance has been used in pharmacy compounding**

The coenzyme NAD (first called cozymase) was first discovered by Arthur Harden and William Young in 1906 (Harden and Young, 1906). There is insufficient information available to determine how long NAD has been used in pharmacy compounding.

2. **The medical condition(s) it has been used to treat**

Results from a Google search using the terms *nicotinamide adenine dinucleotide compounding pharmacy* and *NAD compounding pharmacy* indicate that NAD is/has been compounded in both topical and injectable formulations. Based on internet searches, NAD is being prepared as a topical ointment for use in rosacea. In addition, clinics across the U.S. advertise intravenous NAD for addiction recovery, among other claims.

3. **How widespread its use has been**

Insufficient data are available from which to draw conclusions about the extent of use of NAD in compounded drug products.

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11 See fn 10.
4. Recognition of the substance in other countries or foreign pharmacopeias


Conclusions: Information is insufficient to determine the historical use of NAD in pharmacy compounding. Based on internet searches, NAD appears to be available as a compounded product in both topical and intravenous forms.

III. RECOMMENDATION

We have balanced the criteria described in section II above to evaluate NAD for the 503A Bulks List. After considering the information currently available, a balancing of the criteria weighs against NAD being placed on that list based on the following:

1. NAD is well characterized physically and chemically. However, it is susceptible to substantial degradation when exposed to light, moisture, alkaline pH, or standard room temperatures. Unless multiple compensatory measures to improve its stability are implemented, NAD will likely be unstable when compounded in a capsule, the proposed dosage form.

2. Nonclinical data found in the literature are inadequate to characterize the potential toxicity profile for NAD, particularly for use in a chronic disease such as MS. Similarly, we did not find sufficient clinical data about NAD to evaluate whether it is safe for use in compounded drug products.

3. We found no published information regarding the clinical evaluation of the use of NAD supplementation in the treatment of MS-related fatigue or MS. Therefore, we have insufficient information on the efficacy of NAD to support the nominated use.

4. Information is insufficient to determine the length or extent of historical use of NAD in compounded drug products. Based on internet searches, NAD appears to be available as a compounded product in both topical and intravenous forms.

Based on the information the Agency has considered, as described above, a balancing of the four evaluation criteria weighs against NAD being added to the 503A Bulks List.
REFERENCES


Tab 2

Nicotinamide Adenine Dinucleotide
Disodium Reduced
<table>
<thead>
<tr>
<th>Tab 2a</th>
</tr>
</thead>
</table>

Nicotinamide Adenine Dinucleotide Disodium Reduced Nominations
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the name of the nominated ingredient?</td>
<td>Nicotinamide Adenine Dinucleotide Na2 Reduced (NADH)</td>
</tr>
<tr>
<td>Is the ingredient an active ingredient that meets the definition of “bulk drug substance” in § 207.3(a)(4)?</td>
<td>Yes, Nicotinamide Adenine Dinucleotide Na2 reduced (NADH) is an active ingredient as defined in 207.3(a)(4) because when added to a pharmacologic dosage form it produces a pharmacological effect. References for Nicotinamide Adenine Dinucleotide Na2 reduced (NADH) pharmacological actions are provided Marwan Maalouf, Patrick G. Sullivan, Laurie Davis, Do Young Kim, Jong M. Rho. Ketones inhibit mitochondrial production of reactive oxygen species production following Glutamate exotoxicity by increasing NADH oxidation. Neuroscience. Author manuscript; available in PMC 2007 May 5. Published in final edited form as: Neuroscience. 2007 March 2; 145(1): 256–264. Published online 2007 January 18. doi: 10.1016/j.neuroscience.2006.11.065 <a href="http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1865572/">http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1865572/</a></td>
</tr>
<tr>
<td>Is the ingredient listed in any of the three sections of the Orange Book?</td>
<td>The nominated substance was searched for in all three sections of the Orange Book located at <a href="http://www.accessdata.fda.gov/scripts/cder/ob/docs/queryai.cfm">http://www.accessdata.fda.gov/scripts/cder/ob/docs/queryai.cfm</a>. The nominated substance does not appear in any section searches of the Orange Book.</td>
</tr>
<tr>
<td>Were any monographs for the ingredient found in the USP or NF monographs?</td>
<td>The nominated substance was searched for at <a href="http://www.uspnf.com">http://www.uspnf.com</a>. The nominated substance is not the subject of a USP or NF monograph.</td>
</tr>
<tr>
<td>What is the chemical name of the substance?</td>
<td>1-(3-Carbamoylpyridinio)-β-D-ribofuranoside 5-(adenosine-5'-pyrophosphate) disodium reduced</td>
</tr>
<tr>
<td>What is the common name of the substance?</td>
<td>β-NADH; reduced DPN; Reduced nadide</td>
</tr>
<tr>
<td>Question</td>
<td>Answer</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Does the substance have a UNII Code?</td>
<td>8295030YNC</td>
</tr>
<tr>
<td>What is the chemical grade of the substance?</td>
<td>no grade</td>
</tr>
</tbody>
</table>
| What is the strength, quality, stability, and purity of the ingredient? | Purity: ≥ 95%  
NADH Contents: (Report)  
Sodium Contents: 6.5% ± 1.5%  
Water Contents: < 8.0%  
Spectral Data (at 10mM Tris Solution): ε at 260 nm: (14.4 ± 0.5) x 103  
14.5 x 103 L/mole/cm  
ε at 340 nm: (6.3 ± 0.2) x 103 6.4 x 103 L/mole/cm  
Ratio at pH 10 A250 / A260: 0.82 ± 0.03 0.80  
A280 / A260: 0.23 ± 0.02 0.24  
A340 / A260: 0.43 ± 0.01 0.44 |
<p>| How is the ingredient supplied?                                         | Powder                                                                |
| Is the substance recognized in foreign pharmacopeias or registered in other countries? | No foreign pharmacopeia monographs or registrations found.             |
| Has information been submitted about the substance to the USP for consideration of monograph development? | No USP Monograph submission found.                                     |
| What dosage form(s) will be compounded using the bulk drug substance?  | Capsules                                                               |
| What strength(s) will be compounded from the nominated substance?       | 5-20mg                                                                |
| What are the anticipated route(s) of administration of the compounded drug product(s)? | Oral                                                                  |</p>
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has the bulk drug substance been used previously to</td>
<td>Capsules</td>
</tr>
<tr>
<td>What is the proposed use for the drug product(s) to be</td>
<td>Has been useful in treating depression, fatigue and jetlag</td>
</tr>
<tr>
<td><strong>What is the reason for use of a compounded drug product rather than an FDA-approved product?</strong></td>
<td>No FDA approved Nicotinamide Adenine Dinucleotide reduced (NADH) preparation. Chronic fatigue is a debilitating condition of unknown origin. It presents with extreme fatigue and may also present with muscle pain, joint pain, tender nodes, unrefreshing sleep and impaired memory or concentration. There are no FDA approved medications for this condition. NADH has shown improvement in oral administration. (M.L. Santaella, I Font, and O.M. Disdier (2004) Comparison of Oral Nicotinamide Adenine Dinucleotide (NADH) Versus Conventional Therapy for Chronic Fatigue Syndrome P.R. Health Sci J. Jun;23(2):89-93 ) Nicotinamide has profound effects on inflammatory skin disorders such as acne, rosacea, and hyperpigmentation. There are FDA approved preparations for inflammatory skin disorders but they consist mainly of antibiotics, retinoids, and steroids. These items can have resistance from bacteria develop, cause skin thinning and stretch marks develop with long term use and cause irritation and burning. NADH has shown promise in these areas with lower side effect profile. (A. Wozniacka, A. Sysa-Jedzejowska, J. Adamus and J. Gebicki. (2002) Topical Application of NADH for the treatment of Rosacea and Contact Dermatitis Clinical and Experimental Dermatology 28 61-63)</td>
</tr>
<tr>
<td><strong>Is there any other relevant information?</strong></td>
<td>All relevant information was expressed in the above questions</td>
</tr>
</tbody>
</table>
Appendix 1: Nicotinamide Adenine Dinucleotide Na2 Reduced (NADH) Nomination from Fagron

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3206923/

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2258638/

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1413662/

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1865572/

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1163582/

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3259196/
### General Background on Bulk Drug Substance

<table>
<thead>
<tr>
<th><strong>Ingredient Name</strong></th>
<th>beta-Nicotinamide Adenine Dinucleotide Disodium Salt, Trihydrate, Reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical/Common Name</strong></td>
<td>beta-NADH Disodium Salt; beta-NADH; Reduced NAD; Reduced DPN</td>
</tr>
<tr>
<td><strong>Identifying Codes</strong></td>
<td>606-68-8</td>
</tr>
<tr>
<td><strong>Chemical Grade</strong></td>
<td>Provided by FDA Registered Supplier/COA</td>
</tr>
<tr>
<td><strong>Description of Strength, Quality, Stability, and Purity</strong></td>
<td>Provided by FDA Registered Supplier/COA</td>
</tr>
<tr>
<td><strong>How Supplied</strong></td>
<td>Varies based upon compounding requirement</td>
</tr>
<tr>
<td><strong>Recognition in Formularies (including foreign recognition)</strong></td>
<td>Not Listed in USP/NF for this specific salt/form</td>
</tr>
</tbody>
</table>

### Information on Compounded Bulk Drug Preparation

<table>
<thead>
<tr>
<th><strong>Dosage Form</strong></th>
<th>Varies based upon compounding requirement/prescription</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strength</strong></td>
<td>Varies based upon compounding requirement/prescription</td>
</tr>
<tr>
<td><strong>Route of Administration</strong></td>
<td>Varies based upon compounding requirement/prescription</td>
</tr>
<tr>
<td><strong>Bibliography (where available)</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Past and Proposed Use

The very nature of a compounded preparation for an individual patient prescription as provided for within FDCA 503A means that the purpose for which it is prescribed is determined by the health professional authorized to issue that prescription. FDA’s request for this information is an insurmountable hurdle that has not been requested by the PCAC.
Tab 2b

FDA Review of Nicotinamide Adenine Dinucleotide Disodium Reduced
DATE: March 31, 2017

FROM: Ben Zhang PhD
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Charles Ganley, MD
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Frances Gail Bormel, RPh, JD
Director, Division of Prescription Drugs, Office of Unapproved Drugs and Labeling Compliance

TO: Pharmacy Compounding Advisory Committee

SUBJECT: Review of Nicotinamide Adenine Dinucleotide Disodium Reduced for Inclusion on the 503A Bulk Drug Substances List
I. INTRODUCTION

Nicotinamide adenine dinucleotide disodium reduced (NADH) has been nominated for inclusion on the list of bulk drug substances for use in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for use in the treatment of depression, fatigue, including chronic fatigue syndrome (CFS), and jetlag. The nominated route of administration and dosage form is oral capsules. This review focuses on only its use in the treatment of CFS because adequate support was not provided for the other nominated uses.

We have reviewed available data on the physicochemical characteristics, safety, effectiveness, and historical use in compounding of this substance. For the reasons discussed below, we believe the evaluation criteria weigh against placing NADH on the list of bulk drug substances that can be used to compound drug products in accordance with section 503A of the FD&C Act (503A Bulks List).

II. EVALUATION CRITERIA

A. Is the substance well characterized, physically and chemically, such that it is appropriate for use in compounding?

NADH is an important coenzyme that consists of two nucleotide moieties. NADH is involved in numerous enzymatic reactions where it serves as an electron carrier. NADH is the reduced form of the coenzyme. This compound is currently marketed as a dietary supplement as capsules (5 mg, 10 mg, and 20 mg) and tablets (5 mg, 10 mg, and 20 mg).

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1 Recent scientific advances have led to proposals to change the name of the disease of interest from CFS to myalgic encephalomyelitis, myalgic encephalomyelitis/CFS, or systemic exertion intolerance disease. Based on the nomination, the disease is referred to as CFS in this review; however, the review encompasses information derived from published literature related to the disease called by any of the related names.
Databases searched for information on NADH in regard to Section A of this review included PubMed, SciFinder, Analytical Profiles of Drug Substances, the European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, and United States Pharmacopoeia (USP)/NF.

1. Stability of the API and likely dosage forms

NADH is extremely reactive and can oxidize very rapidly when exposed to air. Degradation also happens very quickly upon exposure to light and heat (Wu et al. 1986). At 41°C, the half-life of NADH in pure water is 400 minutes at pH 6 and 5.5 minutes at pH 4. It is easily oxidized to generate hydrogen peroxide and nicotinamide adenine dinucleotide (NAD),2 which can then be further degraded into nicotinamide mononucleotide (NMN) and adenosine monophosphate (AMP) (Benofsky et al. 1982). According to Sigma-Aldrich product information,3 the solid form of NADH should be stored at -20°C, protected from air and light, and desiccated. Aqueous solutions of NADH are even more unstable than the solid form of NADH. Acidic and basic conditions can accelerate the degradation.

While certain formulation techniques have been reported to improve the stability of NADH, supporting data are not available to allow for verification of these claims. For example, it has been reported that certain formulation techniques can improve the stability of NADH at room temperature when kept away from light, and such techniques usually involve addition of stabilizers to the formulation of the compressed solid dosage form of NADH. In one formulation, a mixture consisting of 5% NADH (wt %), 5% (wt %) poly-(l-vinyl-2-pyrolidone) as a stabilizer and 90% (wt %) D-mannitol as a filler was compressed into 100 mg tablets and was then coated with a coating mixture of cellulose acetate phthalate, magnesium stearate, ethyl phthalate, acetone, and water. The coating was reported to provide stability under acidic conditions so that the tablet can pass through gastric acidic pH without breaking down (Birkmayer et al. 1994). The patent on this formulation technique claims that the resulting tablets are stable for at least 24 months when kept away from light; however, no supporting stability data were provided.4 Therefore, the publicly available data do not demonstrate that such techniques can effectively enhance the stability of NADH under ordinary storage conditions. Even if specific techniques can enhance the stability of the formulated tablets (or pills), the

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2 NAD is a distinct substance. It was nominated and is being considered separately for inclusion on the 503A Bulks List.

3 Available at http://www.sigmaaldrich.com/catalog/product/sigma/n8129?lang=en&region=US. Sigma-Aldrich manufactures chemicals for use in scientific research, biotechnology, and pharmaceutical development. According to its website, a Sigma-Aldrich specification is "a list of test methods, references to analytical procedures, and appropriate acceptance criteria that are numerical limits, ranges, or other criteria for the test described. It establishes the set of criteria to which a material should conform to be considered acceptable for its intended use.” See http://www.sigmaaldrich.com/united-kingdom-technicalservices/specifications.html. Throughout this review, we rely on information in the Sigma-Aldrich product information sheet where necessary to supplement information from other sources.

4 The enteric coated NADH tablets obtained from such techniques are marketed as dietary supplements. FDA has not approved any new drug applications for enteric coated NADH tablets.
manufacture of enteric coated tablets needs to be tightly controlled to ensure that the coating tablets contain a consistent amount of functional enteric coating and perform as intended.\footnote{This Committee will also be considering whether certain types of oral solid modified release drug products that employ coated systems should be included on the Difficult to Compound List.}

2. \textit{Probable routes of API synthesis}

NADH is usually prepared by the reduction of NAD, which can be obtained from fermentation of yeasts. This reaction is usually carried out either by enzymatic catalysts or by microbial cells (Šilhánková et al. 1992).

3. \textit{Likely impurities}

Likely impurities may include:

1. Bioburden, such as residual yeast if the reaction is carried out via microbial reduction
2. Residual enzymes and other reaction intermediates
3. NAD, either from the starting material or from the oxidation of NADH, and other degradation products such as ribose and adenosine diphosphate (ADP). (Benofsky et al. 1982).

4. \textit{Toxicity of those likely impurities}

Impurities are unlikely to be significantly toxic. Further characterization of the toxicity of the impurities is not warranted, pending the availability of additional information about the likelihood of their presence and amounts in the bulk drug substance.

5. \textit{Physicochemical characteristics pertinent to product performance, such as particle size and polymorphism}

NADH is a white or almost white powder, soluble in water. No further information on the influence of particle size and polymorphism on bioavailability were found in the literature.

6. \textit{Any other information about the substance that may be relevant, such as whether the API is poorly characterized or difficult to characterize}

NADH is characterized with nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and mass spectrometry (MS).

\textbf{Conclusions:} NADH is the reduced form of the coenzyme NAD and is found endogenously in animals and humans. The bulk substance degrades when exposed to light, moisture, acidity, or standard room temperatures; therefore, it will not be stable under ordinary storage conditions. The preparation of compressed NADH tablets with stabilizers and enteric coating of the tablets has been reported, albeit without supporting data, to greatly enhance the stability when kept...
away from light. However, enteric coating requires specialized equipment and quality control to ensure that the active pharmaceutical ingredient can survive the gastric environment, and the stability enhancement for the use period will need to be further demonstrated. The nominated compound is easily characterized with various analytical techniques, and the preparation of this compound has been well developed.

B. Are there concerns about the safety of the substance for use in compounding?

1. Nonclinical assessment

The following database(s) were consulted regarding NADH in the preparation of this portion of this review: Embase, PubMed, TOXNET, and Web of Science.

a. General pharmacology of the drug substance

NADH (also referred to as β-NADH; reduced DPN; reduced nadide) is a reduced form of NAD. NAD is an endogenous substance involved in a wide range of biological reactions such as energy metabolism, adenosine triphosphate (ATP) production, and post-translational protein modifications (Sauve, 2008). NAD is reduced to NADH in glycolysis, fermentation (via lactate dehydrogenase), and the tricarboxylic acid cycle (Yang and Sauve, 2016). In contrast, NADH is oxidized back to NAD in oxidative phosphorylation and fermentation (via lactate dehydrogenase). The ratio of NAD/NADH plays an important role in regulating the activity of various enzymes, including those involved in glycolysis, tricarboxylic acid cycle, and fatty acid oxidation (Yang and Sauve, 2016). The intracellular redox status, especially in the mitochondria and nucleus, appears to also be regulated by the NAD/NADH ratio.

Due to the involvement of NADH in a wide range of biological processes and the role of NAD/NADH ratio in regulating mitochondrial functions, several animal studies have explored the potential therapeutic benefit of NADH administration. For example, NADH microinjected into the cortex or caudate nucleus induced jumping behavior in rats (Shen and Lin, 1985). Wistar rats given NADH (≥5mg/kg) via intraperitoneal injection showed a reduction in immobility and an increase in swimming behavior in the forced swim test (Rex et al. 2004a). In another study, 20-month old, learning impaired Wistar rats injected with NADH intraperitoneally for 10 days (10-100 mg/kg) showed an improvement in the water maze performance (Rex et al. 2004b). In addition to displaying improvement in the cognitive and motor functions mentioned above, hypertensive rats fed oral doses of NADH (5mg) for 10 weeks had lower systolic blood pressure, total cholesterol, LDL-cholesterol, and renal lipid peroxidation compared to placebo (Bushehri et al. 1998).

With regard to the nominated use, CFS is characterized by persistent fatigue accompanied by additional diverse symptoms. The etiology of CFS is not known. The mechanism by which NADH would exert its effect in the treatment of CFS is not known. Investigators hypothesize that oxidative stress and mitochondrial dysfunction, which may reduce the rate of adenosine-5-triphosphate (ATP) synthesis, are important in the pathogenesis of CFS (Castro-Marrero et al. 2013). Because NADH is involved in cellular ATP production via mitochondrial oxidative phosphorylation, it has been hypothesized that NADH supplementation could improve cellular ATP production, which, in turn, may improve symptoms of fatigue in CFS.
b. Pharmacokinetics (PK)

A limited number of nonclinical studies were found in the literature that described the pharmacokinetics of NADH. Because both NADH and NAD coexist in the body and are converted from one to the other in many metabolic reactions, data on the pharmacokinetics of both enzymes are provided in this section.

The pharmacokinetic profile of NADH was assessed in mice based on the hypothesis that exogenously administered NADH is metabolized to nicotinamide and excreted in the urine (Kimura et al. 2006). A single intraperitoneal dose of NADH (5 μmol/mouse) was given to mice (n=5 per group) and urine was collected continuously for 4 days. Under the conditions of the study, urinary excretion of nicotinamide was increased from 250 nmol/day to 800 nmol/day. Dosing of NADH via the oral route did not produce an increase in the nicotinamide level in urine. Although there is no direct evidence that nicotinamide in the urine is an appropriate biomarker for NADH metabolism, the Kimura study suggested that NADH may be systemically absorbed via the intraperitoneal route, but not oral route of administration. The study also suggested that poor oral absorption may be due to the instability of NADH at low pH. Following incubation of NADH in an acidic solution, which mimicked gastric juice milieu, NADH was rapidly converted to uncharacterized compounds, suggesting that NADH is unstable in an acid environment and, therefore, not absorbed in the gastrointestinal tract. In the same study, both intraperitoneal and oral doses of NAD were associated with increased urinary excretion of nicotinamide, which is used in the synthesis of NADH.

To further characterize the absorption of NADH in rodents, the absorption of NADH in the small intestine using the everted sac technique was investigated (Rex and Fink, 2008). In this experiment, the small intestine was dissected out, everted, ligated on both ends and immersed in a tyrode solution.6 The concentration of NADH in the fluid was measured by HPLC with fluorescence detection and laser-induced fluorescence spectroscopy. The concentration of NADH in the serosal fluid of the gut sac increased (0.56 ± 0.05, 2.79 ± 0.26, and 4.72 ± 0.80 mg/L) with increasing concentrations of NADH in the tyrode solution (10, 50, and 100 mg/L). The absorption rate of NADH was 5% and was independent of the external concentration of the tyrode solution tested (10, 50, or 100 mg/L). The NADH fluorescence intensity analysis revealed that the NADH absorption occurred within the first few minutes of incubation and reached a plateau after 20 to 30 minutes. Under the conditions of this in vitro experiment, intact NADH was absorbed in the small intestine in rats, suggesting that, if protected from the acidic gastric environment, NADH can be absorbed in the small intestine.

The bioavailability of NADH was measured in the central nervous system (CNS) of rats administered NADH via the oral, intraperitoneal, and intravenous routes of administration using a fluorescence based spectroscopy assay (Rex et al. 2002). When rats were given an oral dose of NADH (51 mg/kg), a slight increase in NADH fluorescence intensity was observed. Intraperitoneal doses of 10 mg/kg showed no increase in fluorescence intensity, but 50 mg/kg doses resulted in an increase of approximately 10% of the maximal change in fluorescence.

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6 Tyrode solution is a buffer isotonic with interstitial fluid.
intensity. Intravenous doses of 10 mg/kg increased fluorescence to 17% of maximal change and 50 mg/kg doses increased fluorescence to 20% of maximal change. In the same study, intravenous but not intraperitoneal doses of NAD, caused an increase in the NADH fluorescence in the CNS. Endogenous NADH and NAD coexist in the body and are converted from one to the other in many metabolic reactions. However, little is known about whether the metabolic fates of NADH and NAD are similar or whether administration of one compound results in alterations in the systemic levels of the other.

No human pharmacokinetic information or data were found for NADH.

c. Acute toxicity

The acute toxicity of NADH was investigated in a 14-day dog model where NADH was administered using either oral ENADA tablets (20, 100, and 150 mg/kg) or via intravenous infusion. The intravenous dosing regimen consisted of 2 phases: a maximum tolerated dose (MTD) phase followed by a fixed dose phase. In the MTD phase, animals were treated with increasing doses of NADH starting with 100 mg/kg/day for the first 4 days, followed by 200 mg/kg/day for 3 days, 500 mg/kg/day for 4 days, and 1000 mg/kg/day on the final fifth day to establish the MTD. At the end of the MTD phase, control animals that had received saline solution were used to evaluate the potential toxicity of the established MTD. These animals received 500 mg NADH/kg/day (MTD) for 14 days in the fixed dose phase (Birkmayer et al., 2004).

Treatment with NADH via the oral route for 14 days in the dog model did not show any overt signs of toxicological effects. Clinical signs were limited to a transient change in stool formation, which was observed on day 3-7 in males treated with ≥100 mg/kg. Necropsy findings included increases in the adjusted organ to body weights of the adrenal glands, heart, kidney, liver, brain, and thyroid, which were more pronounced in males. Histopathological findings were limited to urinary cystitis, which occurred in some treated females. None of the findings in this study were considered to be of biological significance by the investigator due to the lack of a dose response in the incidence and severity of the toxicity (Birkmayer et al., 2004).

Treatment with NADH via the intravenous route in the dog model resulted in a number of adverse clinical signs, including subdued behavior, pale gums, cold (and less frequently warm) ears, blood-shot eyes, dry nose, and increased respiratory rate during dose administration. After dosing, all treated dogs had tremors and cold foot pads. All treated dogs vocalized and occasionally appeared agitated during dosing. Other signs of toxicity seen included salivation,

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7 *Acute toxicity* refers to adverse effects observed following administration of a single dose of a substance, or multiple doses given within a short period (approximately 24 hours). Endpoints captured in acute toxicity studies usually include mortality and gross clinical observations. Acute toxicity data are usually superseded by data obtained from longer term toxicity studies.

8 ENADA: Enteric-coated tablet contains P-NADH (nicotinamide adenine dinucleotide - reduced form) 5 mg. Other ingredients are: i) (1) cl-mannitol 54.30 mg; ii) sodium bicarbonate 6.0 mg; iii) microcrystalline cellulose 1.36 mg. iv) magnesium stearate 1 mg; and v) sodium ascorbate 0.34 mg. The total tablet weight was 68.00 mg. Coating material was methacrylic acid copolymer.
lip licking, red eyes, awkward gait, increased heart rate, panting, and the appearance of the third eyelid during dosing. A dose-related decrease in body weight and food consumption was seen among dogs treated with \( \geq 500 \text{ mg/kg/day} \) via the intravenous route of exposure. Under the conditions of this study, NADH showed some adverse effects on the cardiovascular system where a decrease in mean arterial (systolic) blood pressure was seen 60 minutes after dosing. Blood pressure returned to the initial values 120 minutes after dosing and remained at this level until 360 minutes. The drop in the mean arterial blood pressure is consistent with the compensatory initial increase in heart rate after 10 minutes of NADH infusion on day 1 of the fixed dose phase, followed by a decrease in heart rate at 60 minutes. Heart rates remained higher at all-time points evaluated when compared with the pre-dose rate (Birkmayer et al. 2004).

Treatment related histopathological signs were limited to the findings of mixed, mainly mononuclear, inflammatory perivascular infiltrate cuffing of blood vessels in the medulla oblongata of the brain in all dogs together with a focus of inflammatory cells in the thalamus (only seen in one female). The histopathology findings at injection sites (low-grade dermatitis, phlebitis/periphlebitis, and subcutaneous hemorrhage) were consistent with repeated intravenous injections with no evidence of local irritation or other toxicity.

d. Repeat dose toxicity

A 26-week repeat dose toxicity study was conducted in rats (n=20/sex/group) treated with either ENADA tablets containing NADH (5 mg/day) or placebo control (Birkmayer and Nadlinger, 2002). The only potential toxicity observed in this study was in one treated female that showed slight bilateral ocular lens opacity upon examination on week 25 and another treated female that was euthanized on week 24 for humane reasons due to eye lesions. The investigator concluded that this type of eye lesion is a common finding and considered to be unrelated to treatment.

No differences in body weight between the placebo and the ENADA-treated males were observed. In the second half of the treatment period (weeks 13-26), females treated with NADH gained significantly (\( p < 0.05 \)) more body weight than the controls. Food consumption in the treated males was similar to that in controls. From approximately week 15 onwards, treated females consumed up to 10% more food than the controls. No differences were observed between the control and the treated groups in terms of hematology or clinical chemistry parameters. There was no apparent treatment-related effect on urine analysis parameters or on absolute or relative organ weight. No macroscopic evidence of specific target organ toxicity associated with the test drug was observed. Histological assessment of treated females included ovarian and renal findings. Treated females showed acyclic ovaries arrested in the follicular phase when compared to control females (number of animals affected was not provided). In the kidney, the incidence of glomerulonephropathy was higher in treated females compared to their control counterparts. The investigators concluded that the incidence of both acyclic ovaries and glomerulonephropathy is not treatment related since both tend to increase with age in rats of this strain and also because the renal findings were limited to treated females (but not males) (Birkmayer et al. 2002).

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9 Repeated-dose toxicity studies consist of in vivo animal studies that seek to evaluate the toxicity of the test substance by observing the changes that emerge in clinical observations, clinical chemistry, gross pathology, and histology endpoints when the test substance is repetitively administered daily for a predetermined period of time.
Conclusions regarding the toxicity profile of NADH after chronic exposure cannot be drawn from this study because the study used a single treatment dose and no apparent treatment related toxicity was achieved.

e. Genotoxicity

No genotoxicity data were found for NADH.

f. Developmental and reproductive toxicity

No developmental or reproductive toxicity data were found for NADH.

g. Carcinogenicity

No carcinogenicity data were found for NADH.

h. Toxicokinetics

No toxicokinetic data (AUC, Tmax, Cmax, t1/2) were found for NADH.

Conclusions: NADH is an endogenously synthesized substance that functions as an electron carrier in numerous enzymatic reactions. The limited data found in the literature suggest that NADH is absorbed and can reach the brain via the intraperitoneal and intravenous routes of exposure in rodents. Rodents in an in vivo model absorbed little to no NADH given orally. In vitro evidence suggested that NADH could be absorbed in the small intestine of rodents if protected from exposure to gastric acid, which can inactivate it. The literature contained either inadequate nonclinical data (insufficient animal number, only a single dose tested, toxicology parameters not captured, etc.) or no data (genotoxicity, carcinogenicity, and reproductive toxicity) to characterize the potential toxicities associated with the administration of NADH, particularly for chronic use.

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10 The genotoxicity assessment battery usually consists of a gene mutagenicity assay (for single dose trials) and a variety of clastogenicity/genotoxicity assays. To support multiple dose administration in humans, additional genotoxicity testing assessment is usually conducted to detect chromosomal damage in mammalian systems.

11 Developmental and reproductive toxicity studies are usually designed to assess the potential adverse effects in humans of both sexes and include females from various age groups that will be exposed to the proposed substance. Developmental toxicity or teratogenicity refers to adverse effects (can include embryo-fetal mortality, structural abnormalities, functional impairment, or alterations to growth) and can occur in pups either as a result of the exposure of their parents to the substance, before the pups’ birth, or by direct exposure of the pups to the substance after birth.

12 Studies that assess cancer risk in animals are used as predictive tools to evaluate the potential for drugs to result in tumors when used by humans on a chronic basis. Carcinogenicity studies are conducted if the clinical use is expected to be continuous for a minimum of 6 months of life, or if intermittent clinical use is expected to total 6 months or more of life.
2. Human safety

The following databases were consulted in the preparation of this section: PubMed, Embase, Cochrane Library, and ClinicalTrials.gov. One trial of NADH was identified in ClinicalTrials.gov and is included in this review.

a. Reported adverse reactions

The Office of Surveillance and Epidemiology conducted a search of the FDA Adverse Event Reporting System (FAERS) database for reports of adverse events for NADH through October 25, 2016. One U.S. case report was found in which NADH was listed as a single ingredient product. A 62-year-old male consumer reported decreased therapeutic effect of Meridia (sibutramine, appetite suppressant) and inquired about the potential of the 15 other drug, herbal or dietary supplements he was taking, including NADH 5 mg for “mood,” to cause the decreased effect (Long, 2016).

The Center for Food Safety and Nutrition (CFSAN) collects reports of adverse events involving food, cosmetics, and dietary supplements in the CFSAN Adverse Event Reporting System (CAERS). A search of CAERS was conducted for adverse events associated with NADH on November 30, 2016. CFSAN’s search of the CAERS database did not identify any adverse events where NADH was listed as a single ingredient product.

b. Clinical trials assessing safety

No serious adverse events were identified in the clinical efficacy studies of NADH discussed in Section II C. In the randomized, double-blind, placebo-controlled crossover study in 35 patients with CFS (Forsyth et al. 1999) discussed in Section II.C.1, the adverse events reported to be related to NADH were considered non-serious and included single cases of “being overly stimulated, mild loss of appetite, heartburn, increased incidence of gas, and an odd taste and dryness reported on the first day of taking the drug.” In the randomized trial discussed in Section II.C.1 comparing NADH treatment to nutritional and psychological (“other”) therapy in 31 patients with CFS (Santaella et al. 2004), no adverse events were reported with the use of NADH.

We identified 12 clinical studies that evaluated the efficacy of NADH supplementation in Parkinson's disease, Alzheimer's disease, depression, jet lag, physical and mental performance, and rosacea and contact dermatitis. None of the four studies in Parkinson’s patients reported on safety outcomes (Birkmayer and Birkmayer, 1989; Birkmayer et al. 1993; Dizdar et al. 1994; Kuhn et al. 1996), and one study in depression did not report safety outcomes (Birkmayer and Birkmayer, 1991a). In the remaining seven studies, it was reported that no patients experienced adverse events:

- In three Alzheimer’s studies, 55 patients received NADH 10 mg orally daily for 8 to 24 weeks (Birkmayer, 1996; Rainer et al. 2000; Demarin et al. 2004).
- In one depression trial, 15 patients received NADH 10 to 20 mg administered intravenously three times per week, NADH 1 to 6 mg intramuscularly three times per
week, or 5 to 10 mg orally administered daily or every other day for 14 to 84 days (Birkmayer and Birkmayer, 1991b).

- In a study of NADH for the treatment of jet lag, 18 subjects received one 20 mg sublingual dose of NADH (Birkmayer et al. 2002).
- Eight healthy subjects were included in a study that examined NADH effects on physical and mental performance (Mero et al. 2008). The NADH dosage and duration was 30 mg per day for 4 weeks. No adverse events were reported.
- Nineteen patients were included in a trial that examined the use of NADH to treat rosacea and contact dermatitis (Wozniacka et al. 2003). The dosage and duration was two to three grams of 1% NADH applied topically twice daily for 2 weeks.

c. The availability of alternative approved therapies that may be as safe or safer

There are no FDA-approved drugs to treat CFS or, specifically, the symptom of fatigue in CFS.

The NICE 2007 Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) Guideline (Baker and Shaw, 2007) states that there is insufficient evidence for the use of NADH supplements for CFS, and, therefore, they should not be prescribed to treat the symptoms of the condition. However, the Guideline further states that some people with CFS/ME have found supplements, such as NADH, to be helpful as a part of a self-management strategy for their symptoms.

The Centers for Disease Control and Prevention recommends that the treatment plan for each CFS patient should be tailored to address symptoms that are most disruptive or disabling (e.g., pain, depression, sleeplessness), but does not mention the use of NADH.

Conclusions: Although no serious adverse events were observed in the available clinical trials, there was an insufficient amount of clinical safety data available for review. In addition, dosing was variable among the trials and systematic adverse event collection was not reported to have been conducted in many of the trials.

C. Are there concerns about whether a substance is effective for a particular use?

The following databases were consulted in preparation of the clinical portion of this review: PubMed, Embase, Cochrane Library, and ClinicalTrials.gov. One trial of NADH was identified in ClinicalTrials.gov and is included in this review.

1. Reports of trials, clinical evidence, and anecdotal reports of effectiveness, or lack of effectiveness, of the bulk drug substance

The etiology of CFS is not known; among the many possible precipitants, viruses, immune dysfunction, endocrine-metabolic dysfunction, and neuropsychiatric factors have been most thoroughly studied (Gluckman, 2015). In addition, the potential mechanism by which NADH might reduce fatigue associated with CFS is unknown.

We identified four clinical studies that evaluated the efficacy of NADH in CFS. One was available only as an abstract; because the full publication is not available in English we were
unable to evaluate the study for purposes of this review (Alegre et al. 2010). It is noted that safety outcomes were not reported in the abstract. According to ClinicalTrials.gov, study NCT02063126 evaluated the efficacy of NADH in CFS. It is not possible to understand the effects of NADH in this study because the study design is confounded by the concomitant administration of Coenzyme Q10 (CoQ10) (Castro-Marrero et al. 2015). No serious adverse events were reported to have occurred in this study. Given the instability of the bulk substance NADH, the potential bioavailability of the test formulations used in any of these four studies is unclear.

The efficacy of NADH (ENADA) was evaluated in a randomized, double-blind, placebo-controlled crossover study in 35 patients with CFS (Forsyth et al. 1999). Patients meeting the CDC CFS diagnostic criteria (see Appendix) were randomized to receive oral NADH 10 mg per day or placebo for 4 weeks, followed by a 4-week washout during which no drug was given, and 4 weeks of the alternate regimen. The study analysis comprised 26 patients (17 females and 9 males; mean age 39.6 years). The investigators employed a self-generated subjective 50-item symptom questionnaire that scored severity on a scale from 1 (none of the time) to 4 (all of the time). Examples of symptoms assessed included fatigue, memory and concentration difficulties, muscle aches, muscle weakness, joint aches, lymphadenopathy, headaches, sore throat, sleep disturbance, and mood changes. Improvement was defined as a 10 percent improvement (i.e., one point decrease in 10 question responses or 2 point decrease in 5 question responses). It is noted that this analysis is not specific to the symptom of fatigue. At 12 weeks, 8 of 26 patients (31 percent) showed a 10 percent improvement while on NADH; 2 of 26 patients (8 percent) showed a 10 percent improvement while on placebo. No differences were noted in laboratory measurements (e.g., serum immunoglobulin concentrations, lymphocyte subsets, or oxidoreductase activity). No interference was observed between NADH and other concomitant medications taken during the study period, however, other concomitant medications were not reported. As mentioned in Section II.B.2.b, adverse events reported to be related to NADH were considered non-serious and included single cases of “being overly stimulated, mild loss of appetite, heartburn, increased incidence of gas, and an odd taste and dryness reported on the first day of taking the drug.”

The efficacy of NADH was evaluated in a randomized trial comparing NADH treatment to nutritional and psychological (“other”) therapy in 31 patients with CFS (Santaella et al. 2004). Patients meeting the CFS diagnostic criteria from the Centers for Disease Control and Prevention were randomly assigned to receive either oral NADH or nutritional and psychological therapy for 24 months. Study endpoints were measured at 0, 3, 6, 9, and 12 months of therapy. The investigators employed a subjective symptom scoring questionnaire that scored severity of a variety of symptoms, on a scale of 1 (minimum) to 4 (maximum). The symptoms rated in the questionnaire are not named in the publication. Of the 31 enrolled patients, 11 did not maintain a minimum of 12 months of uninterrupted therapy and were excluded from study analysis. The study analysis comprised 20 patients (NADH=12; other=8). Seven of the 12 patients in the NADH group received 5 mg throughout the treatment period, and the remaining subjects were started on 5 mg and were titrated up to 10 mg “if their symptoms did not improve with the starting dose.” Eighteen patients were female and two were male with a mean age of 31 years. Nine of the 20 evaluable patients received concomitant treatment with anti-diabetics, antidepressants, anxiolytics, or antihistamines. The identity of the medications and the
associated treatment group were not provided. NADH treated patients showed a reduction from baseline in the mean symptom score from 3.8 at baseline to 2.2 at 3 months. There were no statistically significant differences between NADH treatment and other therapy at any assessment time point. As mentioned in Section II.B.2.b, no adverse events were reported with the use of NADH.

2. *Whether the product compounded with this bulk drug substance is intended to be used in a serious or life-threatening disease*

Chronic fatigue syndrome is a serious condition.

3. *Whether there are any alternative approved therapies that may be as effective or more effective.*

There are no FDA-approved drugs to treat CFS. See section II.B.2.c.

**Conclusions:** There are insufficient data to establish that NADH is efficacious in treating CFS. The two evaluable trials that were identified in the published literature each involved a small number of patients, evaluated many symptoms such that the effect of NADH specifically on fatigue could not be established, and failed to show statistically significant outcomes for efficacy metrics.

**D. Has the substance been used historically in compounding?**

The following databases were consulted in preparation of this section: PubMed, Natural Medicines, European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, USP/NF, and Google.

1. *Length of time the substance has been used in pharmacy compounding*

There is insufficient information available to determine how long NADH has been used in pharmacy compounding.

2. *The medical condition(s) it has been used to treat*

According to the Natural Medicines Database, NADH is used orally for improving mental clarity, alertness and concentration; improving memory; chronic fatigue syndrome; depression; jet lag; Alzheimer’s disease; Parkinson’s disease; improving athletic endurance; enhancing energy and reducing aging, among other conditions (Natural Database, 2015).

Results from a Google search using the terms *nicotinamide adenine dinucleotide reduced compounding pharmacy* and *NADH compounding pharmacy* indicate that NADH is and has been compounded in oral, topical, and injectable formulations. In an article published in the International Journal of Pharmaceutical Compounding, authors describe the use of NADH in the treatment of Parkinson’s disease and Alzheimer’s disease (Galsnapp and Schaefer, 2000). The
article provides a formula for compounding NADH capsules, but the authors do not provide any information on the stability of the capsules. The results of internet searches indicate NADH is compounded into a topical ointment for use in treating contact dermatitis and rosacea. In addition, at least one clinic advertised using NADH as a component of intravenous nutrition to “improve metabolic output,” among other claims.

3. How widespread its use has been

Insufficient data are available from which to draw conclusions about the extent of use of NADH in compounded drug products.

4. Recognition of the substance in other countries or foreign pharmacopeias


Conclusions: Information is insufficient to determine the historical use of NADH in pharmacy compounding. Based on internet searches, NADH appears to be available as a compounded drug product in the United States in oral, topical, and intravenous forms.

III. RECOMMENDATION

We have balanced the criteria described in section II above to evaluate NADH for the 503A Bulks List. After considering the information currently available, a balancing of the criteria weighs against NADH being placed on that list based on the following:

1. NADH is well characterized physically and chemically. However, it is susceptible to substantial degradation when exposed to light, moisture, acid pH, or standard room temperatures. Unless multiple compensatory measures (such as the addition of stabilizers, compression into tablets, and enteric coating) are implemented and shown to improve its stability, NADH is unlikely to be stable when compounded in a capsule, the proposed dosage form.

2. Although we found no reports of serious adverse events, the clinical safety data available for review was minimal. Nonclinical data reported in the literature suggest that NADH is not stable in an acid medium and is likely to be degraded before absorption after oral dosing. Nonclinical safety data are insufficient to characterize the potential toxicity profile for NADH, particularly for use in a chronic disease such as CFS.

3. The available clinical efficacy data regarding administration of NADH to patients with CFS failed to provide an assessment of fatigue specifically and failed to show statistically

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13 According to the formula, the capsules contain NADH and lactose.
significant improvement on assessment scales of multiple other symptoms. Therefore, the information on the efficacy of NADH for the nominated use is insufficient.

4. There is insufficient information to determine the historical use of NADH in pharmacy compounding.

Based on the information the Agency has considered, a balancing of the four evaluation criteria weighs against NADH being added to the 503A Bulks List.
REFERENCES


APPENDIX: CENTER FOR DISEASE CONTROL AND PREVENTION – CFS DIAGNOSTIC CRITERIA\textsuperscript{14}

The individual has severe chronic fatigue for 6 or more consecutive months that is not due to ongoing exertion or other medical conditions associated with fatigue (these other conditions need to be ruled out by a doctor after diagnostic tests have been conducted). The fatigue significantly interferes with daily activities and work.

AND

The individual concurrently has four or more of the following eight symptoms:

1. Post-exertion malaise lasting more than 24 hours
2. Unrefreshing sleep
3. Significant impairment of short-term memory or concentration
4. Muscle pain
5. Multi-joint pain without swelling or redness
6. Headaches of a new type, pattern, or severity
7. Tender cervical or axillary lymph nodes
8. A sore throat that is frequent or recurring

\textsuperscript{14} Available at \url{https://www.cdc.gov/cfs/general/index.html}. 
Tab 3

Nettle
Tab 3a

Nettle Nominations
<table>
<thead>
<tr>
<th>Ingredient Name</th>
<th>Nettle leaf (Urtica dioica subsp. dioica leaf)</th>
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<tr>
<td>Chemical Name</td>
<td>nettle leaf (Urtica dioica subsp. dioica leaf)</td>
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<tr>
<td>Common Name</td>
<td>Nettle leaf</td>
</tr>
<tr>
<td>UNII Code</td>
<td>232L6DS3Y4</td>
</tr>
<tr>
<td>Description of strength, quality, stability and purity</td>
<td>From PCCA Database MSDS: Product is 100% by weight and stable. Should be protected from strong oxidizing agents.</td>
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<tr>
<td>Ingredient Format(s)</td>
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<tr>
<td>Recognition in Pharmacopeias</td>
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<tr>
<td>Final Compounded Formulation Dosage Form(s)</td>
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</tr>
<tr>
<td>Final Compounded Formulation Strength</td>
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<tr>
<td>Final Compounded Formulation Route(s) of Administration</td>
<td></td>
</tr>
<tr>
<td>Final Compounded Formulation Clinical Rationale and History of Past Use</td>
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</table>
Bulk Drug Substances for Consideration by the FDA's Pharmacy Compounding Advisory Committee

Submitted by the International Academy of Compounding Pharmacists

General Background on Bulk Drug Substance

**Ingredient Name**
nettles

**Chemical/Common Name**
nettles

**Identifying Codes**
0GG6WIU2KW

**Chemical Grade**
Provided by FDA Registered Supplier/COA

**Description of Strength, Quality, Stability, and Purity**
Provided by FDA Registered Supplier/COA

**How Supplied**
Varies based upon compounding requirement

**Recognition in Formularies**
Not Listed in USP/NF/NF Listed

Information on Compounded Bulk Drug Preparation

**Dosage Form**
Varies based upon compounding requirement/prescription

**Strength**
Varies based upon compounding requirement/prescription

**Route of Administration**
Varies based upon compounding requirement/prescription

**Bibliography**
digestive aid


Past and Proposed Use

The very nature of a compounded preparation for an individual patient prescription as provided for within FDCA 503A means that the purpose for which it is prescribed is determined by the health professional authorized to issue that prescription. FDA’s request for this information is an insurmountable hurdle that has not been requested by the PCAC.
Tab 3b

FDA Review of Nettle
DATE: March 31, 2017

FROM: Cassandra Taylor, PhD
Chemist, Botanical Review Team, Office of Pharmaceutical Quality (OPQ)

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SUBJECT: Review of Nettle (Urtica dioica) for Inclusion on the 503A Bulk Drug Substances List
I. INTRODUCTION

Nettle (Urtica dioica L.)\(^1\) has been nominated for inclusion on the list of bulk drug substances for use in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for glycemic control.\(^2\) No route of administration or formulation was proposed in the nominations. This review addresses clinical use via oral administration only and summarizes the safety and efficacy considerations for this substance based fully on publicly available information.

We have reviewed available data on the physicochemical characteristics, safety, effectiveness, and historical use in compounding of this substance. For the reasons discussed below, we believe the evaluation criteria weigh against placing nettle on the list of bulk drug substances that can be used to compound drug products in accordance with section 503A of the FD&C Act (503A Bulks List).

II. EVALUATION CRITERIA

A. Is the substance well characterized, physically and chemically, such that it is appropriate for use in compounding?

The databases searched for information on nettle leaf in regard to Section A of this review included U.S. Pharmacopeia (USP)/NF, World Health Organization Monographs, Natural Medicines, American Herbal Products Association (AHPA), European Medicines Agency (EMA), PubMed, and SciFinder Scholar.

The nominated substance, nettle leaf (the leaf of Urtica dioica L.),\(^3\) does not have a well-characterized physicochemical profile, and we are unaware of any adequate quality control mechanism supporting its use as a compounded drug. Summarized below are descriptions of the botanical raw material, known classes of compounds, and human experience relevant to quality control and human use of nettle leaf (also called stinging nettle).

Nettle leaf is a botanical raw material and is not well characterized because the major components, especially the major active component(s), are yet to be identified. There are insufficient data to suggest that the herb has a defined mechanism of action for treating certain diseases (Tyler, 1994).

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\(^1\) The names nettle and Urtica dioica (UD) may be used interchangeably in this review. If a given study specified the plant part and/or preparation, it is reported as stated in the article. If a study did not specify the plant part used, it is referred to as UD in this review.

\(^2\) Nettle leaf (Urtica dioica subsp. dioica leaf) and the nettle plant (no specified part) were nominated with no statement of proposed use. The supporting reference cites use of nettle leaf in improving glycemic control in patients with advanced type 2 diabetes mellitus. FDA evaluated nettle’s use in improving glycemic control.

\(^3\) Urtica dioica L. is the Latin binomial name used to identify this specific plant species, commonly called stinging nettle or nettle leaf, in the genus Urtica, family Urticaceae. The species Urtica dioica L. is divided into six subspecies, which are specified by their Latin binomial names where appropriate in this review. The Latin binomial Urtica dioica L. is used to distinguish this species from other species in the genus Urtica; therefore, Latin binomial names will be used in this review when context requires differentiation between species or subspecies within the genus Urtica.
Urtica dioica L., the herbaceous perennial flowering plant known as common nettle or stinging nettle, is a species in the family Urticaceae. The species is divided into six subspecies and five of these have hollow needle-like stinging hairs, known as trichomes, which release mainly histamine and other chemicals that produce the stinging sensation and inflammatory response (e.g., itching, bumps, and redness) upon contact with skin (Per Brodal, 2010). Commercially available dried Urtica dioica and its extracts may be adulterated with minor amounts of Urtica urens L., which is known commercially as dwarf nettle, a common adulterant found in dried nettle leaf (Tyler, 1994). The entire Urtica dioica plant, both the fresh and dried plant material of the above and below ground parts, is a traditional herbal medicine used to treat a variety of illnesses and related symptoms. DNA fingerprinting, microscopic analysis, and chemical analysis of the botanical samples with authenticated reference materials may be used to correctly identify and differentiate Urtica dioica from some of the common adulterants, including Urtica urens. However, the chemical profiles of the botanical raw material (i.e., nettle leaf) from correctly identified Urtica dioica sources may still vary significantly under different growing/cultivation conditions and collection practices.

It is a difficult task to accurately analyze and quantify all of the various compounds found in a botanical, such as nettle leaf. Nevertheless, different classes of compounds have been isolated from Urtica dioica L., such as fatty acids, terpenoids (including triterpenoids), phenylpropanes, lignans, coumarins, polysaccharides, ceramides, sterols, and lectins. Within those classes, some of the major isolated compounds include oxalic acid, linoleic acid, β-sitosterol, scopoletin, p-hydroxybenzaldehyde, homovanillyl alcohol, neoolivil, oleanolic acid, α-dimorphemic acid (9-hydroxy-10-trans,12-cis-octadecadienoic acid), 14-octacosanol, 13-hydroxy-9-cis,11-trans-octadecadienoic acid, stigmasterol, 6α-diol, campesterol, daucosterol (and related glycosides), secoisolariciresinol-9-O-β-D-glucoside, ursolic acid, Urtica dioica agglutinin, and polysaccharides RP1-RP5 (Bombardelli et al. 1997; Blascheck et al. 1998; Farnsworth, 1998; Bruneton, 1995; European Society of Cognitive ESCOP Monographs, 1996; Gansser et al. 1995; Kraus et al. 1990; Schilcher et al. 1986; Wagner et al. 1994). Combined, the total percentages of the fully characterized and quantifiable compounds in nettle leaf are generally low and may also vary batch-to-batch. Some of these representative chemical marker compounds are depicted in Figure 1.

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4 As defined by WHO, the term traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. Accessed 03/21/2017: http://www.who.int/medicines/areas/traditional/definitions/en/
Stinging nettle is sold as a dietary supplement, sometimes in the form of capsules ranging from 250 to 500 mg, and sometimes in the form of a liquid extract with a nutritional label stating that 1 dropper (~1mL) is equivalent to 1 g of *Urtica dioica* leaf extract. Many of the dietary supplement products are not pure *Urtica dioica*; they usually have two or more other botanical or non-botanical ingredients.  

Several analytical methodologies for identifying *Urtica dioica* are referenced within the USP dietary supplement monographs for stinging nettle, powdered stinging nettle, and powdered stinging nettle extract, as well as the WHO monograph of Radix Urticae (the dried roots and rhizomes of *Urtica dioica* L. and *Urtica urens* L.). Total amino acids, β-sitosterol and scopoletin, are the focus of quantitation within the USP dietary supplement monographs (*Stinging Nettle, USP; Powdered Stinging Nettle, USP; Powdered Stinging Nettle Extract, USP*) and the WHO monograph (*Radix Urticae*). However, because there are so many different chemical components in each of the various classes within *Urtica dioica*, it is difficult to fully characterize and quantify them all accurately, especially the fraction of polysaccharides. There are various methodologies cited throughout the stinging nettle USP dietary supplement and WHO monographs to quantify some of the minor, albeit representative, marker compounds, and

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3 Dietary supplement dosages and routes of administration were found by a Google search for shopping “Stinging Nettle Supplements,” [https://www.google.com/search?q=stinging+nettle&ie=utf-8&oe=utf-8&gfe_rd=1:s,org.mozilla.en-US.official&client=firefox-a#q=stinging+nettle+supplements&tbs=shop Accessed 03/08/2017.](https://www.google.com/search?q=stinging+nettle&ie=utf-8&oe=utf-8&gfe_rd=1:s,org.mozilla.en-US.official&client=firefox-a#q=stinging+nettle+supplements&tbs=shop Accessed 03/08/2017.)
those methods (and data) are not considered adequate to fully characterize nettle as a whole for quality control purposes.

Only limited preliminary studies have suggested the possible active ingredient(s) in nettle leaves and the possible mechanisms of action. One study suggests that *Urtica dioica* may have an effect on the circulation of free (active) testosterone in the blood or potentially inhibit the enzyme aromatase, which is responsible for testosterone synthesis (Tyler, 1994). Others suggest that the plant lectin *Urtica dioica* agglutin (UDA), which is a monomeric lectin mixture inside the UD plant, in combination with various polysaccharides in the body, may be responsible for the effect on the amount of free testosterone activity in the blood. UDA has demonstrated high stability to acids and heat, which would allow it to maintain its proposed activity during oral administration (Willer et al. 1991 & Tyler, 1994).

1. **Stability of the API and likely dosage forms**

Generally speaking, botanicals like nettle leaves and the dried extracts made from those botanicals are considered stable for two years under favorable storage conditions. However, there are no stability data for nettle leaves and its various extracts available for review.

The nominator did not specify the dosage form(s), strength(s) or anticipated route(s) of administration of the compounded drug product. Because the supporting reference included in the nomination concerned its use for improving glycemic control in patients with diabetes, we reviewed available information on the dosing and dosage forms of nettle when used in the treatment of diabetes. The literature describing the use of nettle to treat diabetes suggests that the likely intended dosage forms for nettle leaves are dried powders of the leaves and leaf extracts (in capsules/tablets) for oral use. For example, PDR for Herbal Medicines suggests consuming a hot water infusion of 6 g of powdered dry nettle leaves (from *Urtica dioica*) two or three times daily as an anti-glycemic agent to treat diabetes mellitus (Fleming, 1998). In a randomized, double-blind, placebo-controlled clinical trial that examined *Urtica dioica* as a treatment for type 2 diabetes mellitus, the patients were dosed with 500 mg capsules of nettle extract (with 12% of capsule composed of toast powder as an excipient) every 8 hours (i.e., 500 mg, tid) for 3 months based on the traditional herbal medicine use of 6 g/day dosage of stinging nettle leaves (Kianbakht et al. 2013).6

2. **Probable routes of API synthesis**

The nominated substance, nettle, is a botanical-derived natural product, which is not synthesized.

3. **Likely impurities**

Based on the information from the marketed dietary supplements, the likely impurities of nettle (*Urtica dioica*) include other *Urtica* species, such as *Urtica urens* L. (known commercially as dwarf nettle), and other botanicals (e.g., *Lamium album*, commonly known as white dead nettle). There are several infraspecific taxa (e.g., subspecies) of the species *Urtica dioica* with stinging hairs that could potentially be misidentified as stinging nettle. Examples of the *Urtica dioica* subspecies are listed below.

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6 There is literature describing different dosage forms and/or different dosing for other uses of nettle. For instance, the literature describes topical application of nettle for osteoarthritis and the use of oral nettle for allergic rhinitis. (See e.g., Randall et al., 2000 and Mittman, 1990)
1. *Afghanica* Chrtek (Southwest/Central Asia, sometimes has stinging hairs or sometimes hairless)
2. *Gansuensis* C. J. Chen (Gansu Yizhu stinging nettle, China, has stinging hairs)
3. *Gracilis* (Ait.) Selander (American stinging nettle)
4. *Holosericea* (Nutt.) Thorne (Hoary stinging nettle, native to California)

Some common botanical impurities are listed below.

- Amino acids, other dipeptides, and polypeptides
- Residual organic solvents and reagents used in the manufacturing and purification process
- Heavy metal impurities and pesticides linked to the source of the starting material and the reagents used in the process
- Bioburden (such as microbial content, yeast, or mold)
- Inorganic impurities, including heavy metal and arsenic

The USP provides general chapters for dietary supplement products and dietary supplement ingredients, such as USP <231>, which includes limit testing for heavy metals. The USP also includes separate dietary supplement monographs for powdered stinging nettle and powdered stinging nettle extract (USP 39-NF34). Table 1 provides additional details on the specific methods from USP.

**Table 1: USP compendial analytical methodologies for stinging nettle impurities (powdered stinging nettle, USP 39-nf34)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Cited Method and Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy Metals (Inorganic Impurities)</td>
<td>Method II &lt;231&gt;: NMT 20 ppm</td>
</tr>
<tr>
<td>Articles of Botanical Origin (Organic Impurities)</td>
<td><em>Method for Pesticide Residue Analysis</em> &lt;561&gt;: Meets the requirements</td>
</tr>
<tr>
<td>Articles of Botanical Origin (Foreign Organic Matter)</td>
<td>&lt;561&gt; NMT 2.0%</td>
</tr>
<tr>
<td>Articles of Botanical Origin (Total Ash)</td>
<td>&lt;561&gt; NMT 10%</td>
</tr>
<tr>
<td>Microbial Enumeration Tests</td>
<td>&lt;2021&gt;: Total aerobic bacterial count does not exceed $10^6$ cfu/g, and the total combined molds and yeast count does not exceed $10^4$ cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed $10^3$ cfu/g</td>
</tr>
<tr>
<td>Absence of Specified Microorganisms</td>
<td>&lt;2022&gt;: Meets the requirements of the tests for absence of <em>Salmonella</em> species and <em>Escherichia coli</em></td>
</tr>
<tr>
<td>Loss on Drying</td>
<td>&lt;731&gt;: Dry 1.0 g of Stinging Nettle, finely powdered, at 105°C for 2 h; it loses NMT 12.0% of its weight</td>
</tr>
</tbody>
</table>

4. **Toxicity of those likely impurities**

Regarding the toxicity of the various *Urtica dioica* subspecies, there would likely be a very
minimal threat of toxicity to patients if the nettle leaf were to be contaminated by one or more of the above listed *Urtica dioica* subspecies.

Additional likely impurities include amino acids, residual organic solvents, heavy metal impurities, pesticides, bioburden, and inorganic impurities. In comparison to contamination with other *Urtica dioica* subspecies, these impurities have a higher risk of toxicity to patients if found to be present. According to Tchounwou, et al. (2012), heavy metals such as arsenic, cadmium, chromium, lead, and mercury are considered systematic toxicants and are known to induce multiple organ damage even at low levels of exposure. Their toxicity is dependent on a variety of factors including the dose, route of exposure, and chemical species, along with the age, gender, genetics, and nutritional status of exposed individuals. Many different types of pesticides are used in agriculture, and their toxicities range from acute to chronic symptoms. Acute toxicity is commonly referred to as the LD50, which refers to a dose that is acutely lethal for 50 percent of the animals to whom the chemical is administered under controlled laboratory conditions (Kard et al. 2013). There is no standard measure for chronic toxicity like the LD50; instead, the adverse effects are studied, such as carcinogenesis (oncogenesis), teratogenesis (i.e., production of birth defects), mutagenesis (i.e., production of changes to genetic structure), and reproductive toxicity (i.e., effects on fertility or reproduction rates of animals). Bioburden, such as microbial content, yeast, and mold, could be detrimental to immunocompromised patients if counts are too high. The American Herbal Products Association provides some recommended microbial limits for “finished” botanical products (AHPA, 2014).

5. Physicochemical characteristics pertinent to product performance, such as particle size and polymorphism

No information is available.

6. Any other information about the substance that may be relevant, such as whether the API is poorly characterized or difficult to characterize

Nettle leaf is not well characterized because only small percentages of the chemical components in nettle leaves are known and quantifiable. Furthermore, the active component(s) in nettle leaves are yet to be identified (Tyler, 1994).

Several known chemical marker compounds in nettle have been quantified for quality control purposes. For example, β-sitosterol (NLT 0.05%, by gas chromatography), scopoletin (NLT 3 μg/g, by high-performance liquid chromatography/HPLC), and total amino acids (NLT 0.8%, by spectrophotometry) are reported on a dry basis for nettle. In addition, *Urtica dioica* agglutin in Radix Urticae was quantitatively analyzed by HPLC and enzyme linked immunoabsorbent assay methods. The quantification of the marker compounds mentioned above may or may not correlate to the performance of nettle.

**Conclusions:** Based on its physicochemical characteristics, nettle (*Urtica dioica*) is not well characterized. Major and/or active components of nettle are unknown. The lack of characterization presents challenges for quality control.

B. Are there concerns about the safety of the substance for use in compounding?

1. Nonclinical assessment

The following databases were consulted in the preparation of this portion of this review: PubMed, TOXNET/HSDB, Google, Web of Science, and EMBASE.
a. Pharmacology of the drug substance and its likely impurities

The pharmacology of nettle has been investigated using various preparations of the nettle plant (e.g., aqueous and oil extracts of the whole plant, aerial parts, leaf, and root), generally referred to hereafter as nettle or *Urtica dioica* (UD).

In vitro assays, in vivo animal models, and human trials using various formulations of nettle have contributed to the available pharmacology information.

In vitro assays have been conducted to investigate the claimed mechanism of action for UD as an anti-inflammatory, anti-oxidative, immunomodulating, anti-nociceptive, muscular relaxation, and anti-glucosidase activity agent. Potential mechanisms of action for nettle’s antiproliferative effect (stinging nettle root extract and aqueous UD leaf extract) have also been investigated. When nettle was added to a human prostate cancer cell line (LNCaP) in vitro, a dose-dependent increase in cytotoxicity was seen in LNCaP cells which correlated with oxidative stress, mitochondrial depolarization, and apoptosis (Konrad et al. 2000; Levy et al. 2014).

The pharmacologic effect of UD with regard to glycemic control has been evaluated in multiple nonclinical studies, cited in Appendix 1. In normal animals, hypoglycemic effects observed following nettle ingestion after an oral glucose tolerance test in normal rats were hypothesized to be due in part to the reduction of intestinal glucose absorption (Bnouham et al. 2003). In other studies, aqueous or hydroalcoholic UD extract administration had no significant effect on blood glucose levels in normal rats (Swanston-Flatt et al. 1989; Roman Ramos et al. 1992).

In studies with animal models, in which diabetes is induced by streptozotocin (STZ) or alloxan, the effects of UD also appear to be associated with varied results. In a study of STZ-induced diabetic rats, nettle administration was associated with increased basal glucose concentrations and enhanced fluid intake and body weight loss compared with control animals, suggesting a more severe diabetic state (Swanston-Flatt et al. 1989). However, parameters such as insulin-induced hypoglycemia were not affected. Another study of the STZ-induced diabetic mouse model, in which animals also demonstrated an associative and special memory deficit mediated via the muscarinic cholinergic neuronal circuit, showed hydroalcoholic UD extract to be effective at ameliorating the cognitive impairment and reducing blood glucose (Patel et al. 2015). However, aqueous or hydroalcoholic UD extract administration had no significant effect on blood glucose levels in other studies of diabetic animals (Bnouham et al. 2003; Golalipour et al. 2006; Bnouham et al. 2010; Ozkol et al. 2013).

In vivo animal models have been used to assess UD for other potential therapeutic uses, such as diuretic, anti-depressant, anti-convulsive, hypo/hyperglycemic, analgesic, local anesthetic, blood pressure lowering agent, and lipid lowering agent, as described in various reviews, including the European Medicines Agency assessment report (European Medicines Agency, 2008), Natural Medicines review (Natural Medicines, 2015), and elsewhere (Mithril and Dragsted, 2012). Pharmacologic effects that have been explored for nettle include its potential analgesic and anti-inflammatory properties. In a study by Tekin et al. (2009), the analgesic effect of a 24% UD preparation was investigated in mice and the anti-inflammatory effect was investigated in rats.

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7 UD fixed oil was prepared by finely grinding the seeds of UD, macerating them with diethyl ether for 2 hours, followed by evaporation of the solution to yield a fixed oil concentration of UD at 24%.
In mice, the UD preparation did not show an analgesic effect when evaluated by tail-flick response comparing isotonic saline (negative control) and morphine hydrochloride (positive control). In rats, the anti-inflammatory effect of nettle was studied in a carrageenan-induced paw edema model where the effects of two different doses of UD were compared with isotonic saline and ethyl alcohol (two negative controls), and indomethacin (a positive control). A statistically significant, dose-dependent, anti-inflammatory effect was seen when nettle was administered intraperitoneally at a dose of 0.05 mL/kg resulting in a 47% decrease in inflammation from the rat paw edema model, whereas 0.15 mL/kg resulted in a 56% decrease. UD was considered to be mildly effective, resulting in a 95% reduction in inflammation, compared to the positive control, indomethacin (3 mg/kg IP).

Constituents of the nettle herb representing a number of chemical categories have been identified, including flavonoids, fatty acids, several caffeoyl esters and minerals (Chrubasik et al. 2007b). Limited data are available regarding the component(s) of nettle that are responsible for specific pharmacologic effects, and it is unknown how the quantities of these chemicals may differ among various UD plants and formulations (e.g., dried UD powder, UD extracted with ethanol or methanol). As an example, Obertreis et al. (1996) reported that the pharmacodynamic effect of caffeic malic acid could be represented by an inhibition of leukotriene B4 synthesis, which may be responsible for the claimed immunomodulatory activity of ethanolic nettle extract. Another study identified flavonoids isolated from UD leaves as responsible for the antiplatelet action of nettle (El Haouari et al. 2006). A third study attributed the effects observed in the treatment of benign prostatic hyperplasia to plant lignans isolated from UD root (Schottner et al. 1997). Tita et al. (1993) studied a formulation of UD extracted with ethanol and found that it had high potassium content (0.225 M). At higher intravenous doses in rats (500 mg/kg and above), ventricular and supraventricular arrhythmias were observed and attributed to the high potassium content. Further research is needed to characterize the pharmacologically active components of nettle.

b. Pharmacokinetics/toxicokinetics

No standard pharmacokinetic or toxicokinetic data were found for nettle, likely due to the components of nettle being largely uncharacterized. Nonclinical pharmacokinetic data were reported for one component of nettle root extract, 3,4-divanillyl tetrahydrofurane (DVTF) (Shan et al. 2016). In this study, male Sprague-Dawley rats (n = 6) received DVTF as a single oral or intravenous dose (oral: 50 mg/kg, IV: 25 mg/kg). For oral administration, DVTF reached maximum plasma concentration levels within 2 hours and was undetectable after 24 hours of dosing. For the oral route, the half-life, maximum concentration, time of maximum concentration, clearance, and the area under the curve (AUC) were calculated as follows: 162.78±25.46 min, 2351.5±149.57 ng/ml, 120±0.00 min, 49.23±4.69 L/min/kg, and 1023.20±94.93 μg·min/L (mean ± standard deviation), respectively. The calculated bioavailability for DVTF was 59.5%.

Ozen and Korkmaz (2003) reported the effect of nettle treatment on the level of activity of a number of drug metabolizing enzymes, antioxidant enzymes, and sulfhydryl groups. Mice were

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8 The exact number of rats used for oral and IV dosing was not provided. Whether the authors used the same rats to do both dosing regimens is not clear.
either fed a normal diet, a diet containing 0.75% butylated hydroxyanisole (an antioxidant used as a control), or orally gavaged a solution of UD extract (50 or 100 mg/kg). All four groups of mice were treated daily for 14 days (n = 8/group). No change in body weight was observed within each treatment group between the initial and final dosing days. No change for body or liver weight was observed between control and treatment groups at the conclusion of the experiment. Liver microsomes from mice treated with UD had 0.5-fold less total cytochrome P450 protein levels compared to control, untreated mice. Cytochrome P450 reductase, which is required for many P450 reactions, was also decreased in the nettle-treated groups compared to the untreated control group. Total cytochrome b5 levels also increased in UD-treated mice compared to control mice. The activity of several antioxidant enzymes found in the liver, kidney, forestomach, and lung showed variable responses to nettle. For example, catalase activity increased in the forestomach after UD treatment, but there was no change in catalase activity in the kidney. It is unknown how these changes in enzymatic expression or activity may affect the metabolism of UD extract in animals or humans, or whether there may be an impact on the metabolism of other substances (e.g., concomitant drugs) that share the affected metabolic pathways.

Pharmacokinetic data for other compounds from nettle were not found in the literature.

c. Acute toxicity

The lethal dose for 50% of animals (LD50) has been investigated with a number of nettle preparations via different routes of administration:

- Intraperitoneal injection of an aqueous extract of nettle herb (containing the stem, leaves, and flowers) in mice produced an LD50 of 3.625 g/kg body weight (BW) (Lasheras et al. 1986).
- Intraperitoneal injection of an aqueous UD extract in mice resulted in an LD50 of 3.5 g/kg (Bnouham et al. 2003). Doses above 750 mg/kg were associated with a decrease in spontaneous activity, loss of muscle tone, and hypothermia (Basaran et al. 1996).
- IV injection of nettle herb (infusion of 100 mg/ml for 72 h) in mice produced an LD50 of 1.9 g/kg (Chrubasik et al. 2007b).
- Oral administration of aqueous nettle extract (3:1) in mice produced an LD50 of 1.7 g/kg.
- An ethanolic extract of UD herb showed low toxicity in both rats and mice after oral and intraperitoneal administration up to 2 g of dried herbal substance/kg BW (Tita et al. 1993).
- An intragastric probe infusion of nettle administered to rats produced an LD50 of 1.310 g/kg BW (Baraibar et al. 1983; Bombardelli and Morazzoni, 1997). Affected rats exhibited nasal, oral, and orbital bleeding. When animals underwent autopsy, pulmonary edema and blood in the intestinal lumen were detected. The cause of death was identified as a delay in coagulation time in treated rats.

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9 Acute toxicity refers to adverse effects observed following administration of a single dose of a substance, or multiple doses given within a short period (approximately 24 hours). Endpoints captured in acute toxicity studies usually include mortality and gross clinical observations. Acute toxicity data are usually superseded by data obtained from longer term toxicity studies.
d. Repeat dose toxicity\textsuperscript{10}

A 14-day toxicity study was conducted in male Wistar rats (n=6/group) where animals were fed nettle extract at doses of 0, 100, 200, 400, and 800 mg/kg BW via oral gavage for 14 consecutive days. The behavior and body weight of treated rats were observed daily. At the end of the study, blood samples were collected. None of the treated rats showed any signs of toxicity up to 800 mg/kg BW.

Measurement of hematological and biochemical markers showed a significant decrease in lymphocytes in rats treated with 800 mg/kg nettle extract, a significant increase in packed cell volume at ≥400 mg/kg, and a significant increase in mean corpuscular hemoglobin at ≥200 mg/kg. A decrease in alkaline phosphatase was seen at ≥200 mg/kg, but no changes in the lipid profiles of treated animals were noted in this study (Singh et al. 2012).

No data were found for nettle use in chronic toxicity studies to characterize the potential long-term effects of administering nettle to treat diabetes as per the nominated use for this substance.

e. Genotoxicity\textsuperscript{11}

A number of studies using different genotoxicity assays were found in the literature. However, none of these studies was deemed adequate to reach a conclusion on whether nettle is considered a potential genetic toxicant.

Turkish medicinal herbs were tested for their genotoxic potential using the \textit{Salmonella typhimurium} microsomal activation assay and the alkaline single-cell gel electrophoresis (COMET) assay (Basaran et al. 1996). The species UD was examined. None of the plant extracts and fractions investigated produced a positive dose-related increase in mutations in TA98 and TA100 strains in the presence or absence of metabolic activation. An increase above negative control values was noted in the COMET assay when using the aerial parts, but not the seed of UD. The authors explained the positive findings in the COMET assay as due to nonspecific detection of strand breaks in this assay. This study was not considered adequate because the Ames assay only used two strains, which is not sufficient for evaluating the mutagenic potential of nettle. Furthermore, the COMET assay has not been validated as an assay that can be used to differentiate between genotoxic and non-genotoxic chemicals.

The potential genotoxicity of UDHL\textsubscript{30} (the protein fraction extracted from the aerial part of UD) was studied in human hepatoma HepG2 cells at doses up to 2 mg/mL (Di Sotto et al. 2015). No cytotoxicity was seen in the UDHL\textsubscript{30} extract up to 800 mg per plate. This was compared to the positive control, 2-aminoanthracene (2AA), where a strong antimutagenic activity was seen for all strains tested with a maximum inhibition of 56%, 78%, and 61% in TA98, TA100, and WP2uvrA strains, respectively. UDHL\textsubscript{30} also showed an ability to scavenge the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical and the superoxide anion at 0.1-640 μg/mL. The authors suggest that UDHL\textsubscript{30} may play a role in chemoprevention, particularly

\textsuperscript{10} Repeat-dose toxicity studies consist of in vivo animal studies that seek to evaluate the toxicity of the test substance by observing the changes that emerge in clinical observations, clinical chemistry, gross pathology, and histology endpoints when the test substance is repetitively administered daily for a predetermined period of time.

\textsuperscript{11} The genotoxicity assessment battery usually consists of a gene mutagenicity assay (for single dose trials) and a variety of clastogenicity/genotoxicity assays. To support multiple dose administration in humans, additional genotoxicity testing assessment is usually conducted to detect chromosomal damage in mammalian systems.
against DNA damage induced by environmental and dietary carcinogens.

The cytotoxic and genotoxic effects of essential oils extracted from UD were tested using the micronucleus assay and the chromosomal aberration assay in human lymphocyte culture in vitro (Gul et al. 2012). Mass spectroscopy (GC & GC–MS) analysis of the essential oils of UD identified 43 compounds, representing 95.8% of the oil, including the main components; the most abundant components are carvacrol (38.2%), carvone (9.0%), naphthalene (8.9%), (E)-anethol (4.7%), hexahydrofarnesyl acetone (3.0%), (E)-geranyl acetone (2.9%), (E)-b-ionone (2.8%), and phytol (2.7%). Under the conditions of the study, a significant correlation was found between the increase in the concentration of essential oil and the increase in aberrations, micronuclei frequency, apoptotic cells, necrotic cells, and binucleated cells. The paper did not specify whether the experiments were conducted a single time or whether the data represent the mean number obtained from a number of assay replicates. It is also unknown which of the 43 compounds found in nettle may be responsible for the increase in the genetic aberrations seen for either the micronucleus or chromosomal aberration assays.

f. Developmental and reproductive toxicity

Limited developmental and reproductive toxicity data were found in the literature describing the effects of UD on developmental and reproductive endpoints.

A preparation of nettle (250 mg/kg) was administered to pregnant albino rats (n=9) on gestational days (GD) 1 through 7. The number of rats with implantation sites on GD 10 showed a decrease in implantation sites (n=6 of 9), a 33.3% decrease in implantation (Sharma et al. 1983). This study was not considered adequate to reach a conclusion regarding the role of abortifacient effects of nettle. No data on embryofetal or pre/postnatal toxicity were reported in the literature.

UD extract was investigated for inhibiting the adverse effects of nicotine on sperm cells viability, count, motility, testicular histology, and testosterone levels in a mouse model (Jalili et al. 2014). An intraperitoneal injection of UD extract (10, 20, or 50 mg/kg) in the presence of nicotine (0.5 ml/kg) for 28 consecutive days in male mice showed a significant improvement in all of the reproductive indices tested among mice treated with UD and nicotine compared to nicotine alone.

UD was investigated for its potential benefit against mercury-induced toxicity in rats. UD was able to ameliorate the testicular histological structure and increase the number of sperm found in the lumen. In addition, a clear stabilization of organized seminiferous tubules and an increase in sperm numbers was noted in the UD-supplemented rats compared to mercury-exposed rats without UD supplementation (Siouda and Abdennour, 2015).

12 Developmental and reproductive toxicity studies are usually designed to assess the potential adverse effects in humans of both sexes and include females from various age groups that will be exposed to the proposed substance. Developmental toxicity or teratogenicity refers to adverse effects (can include embryo-fetal mortality, structural abnormalities, functional impairment, or alterations to growth) and can occur in pups either as a result of the exposure of their parents to the substance, prior to the pups’ birth, or by direct exposure of the pups to the substance after birth.
g. Carcinogenicity

No carcinogenicity studies were found in the literature.

**Conclusions:** UD has been investigated for a number of potential therapeutic uses with a variety of in vitro assay and in vivo animal models. Effects on glycemic control in normal and diabetic animal models have differed among various studies and no overall conclusion can be established. Limited evidence is available to identify which components of the nettle plant are responsible for any glycemic or other pharmacological effects.

The effect of long-term oral exposure to UD in animal models, which is necessary for establishing safety in the treatment of a chronic disease such as diabetes, has not been demonstrated. The available data for UD are not sufficient to characterize its toxicity profile. Key studies, including chronic toxicity, genotoxicity, carcinogenicity, and reproductive/developmental toxicity testing, were not found in the literature. The pharmacokinetic and toxicokinetic data that were found for nettle are minimal, but suggest a potential effect on metabolic enzymes and possible drug interactions.

Overall, the nonclinical data found in the literature are not adequate to make a balanced safety assessment about the use of nettle for treatment of diabetic patients.

2. Human safety

The following databases were consulted in the preparation of this section: PubMed, EMBASE, Google Scholar, ClinicalTrials.gov, ToxNet, and Google [Nettle or Stinging Nettle or Urtica dioica; all dates in database].

a. Reported adverse reactions (FAERS, CAERS)

The Office of Surveillance and Epidemiology (OSE) conducted a search of the FDA Adverse Events Reporting System (FAERS) database for reports of adverse events for *Urtica dioica* through September 27, 2016, and retrieved 48 reports for UD. Of these, 6 reported that a form of nettle or UD was a suspect product. It was concluded after additional review that, despite a temporal relationship in each case, causation cannot be established in any of these cases due to use of concomitant medication or multi-ingredient products.

The Center for Food Safety and Nutrition (CFSAN) collects reports of adverse events involving food, cosmetics, and dietary supplements in the CFSAN Adverse Event Reporting System (CAERS). A search of CAERS was conducted for adverse events associated with nettle on September 27, 2016, and retrieved 117 reports of adverse events in individuals using products containing nettle. Of these, 113 reports involved the use of products containing multiple herbal ingredients, which made causation difficult to establish.

Although 4 of these cases reported UD as the primary active ingredient in the product ingested, 2 cases attributed the reported events (renal failure, hypersensitivity) to inactive ingredients in the

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13 Studies that assess cancer risk in animals are used as predictive tools to evaluate the potential for drugs to result in tumors when used by humans on a chronic basis. Carcinogenicity studies are conducted if the clinical use is expected to be continuous for a minimum of 6 months of life, or if intermittent clinical use is expected to total 6 months or more of life.
product. A third report was of a burning tongue sensation, reported to be a result of chewing, rather than swallowing, the capsule formulation. The fourth case reported a myocardial infarction event that was assessed by the reporting physician as probably not the result of UD ingestion, although UD was started 3 days before the event.

- Recalls related to product safety

According to a publication (Upton 2013), in June of 2002, a major commercial supplier of U.S. herbal dietary supplements recalled four (4) lots of its nettle capsules because they contained excessive amounts of lead. The lots were traced to a single batch of raw material. No adverse events were reported in the publication.

b. Clinical trials assessing safety

Adverse event data were not reported in any of the seven clinical trials discussed in Section II.C. The following are some representative reports of adverse reactions described in the literature. Clinical safety data are available in the published literature from trials of UD in the treatment of diseases other than diabetes.

Chrubasik et al. (2007b) reviewed the safety data from seven open-label and uncontrolled studies, one open-label and placebo controlled study, and two double-blind studies of the use of aerial UD oral formulations for diuresis, osteoarthritis, and allergic rhinitis. Over 10,000 patients were included in these studies; however, adverse event data were not collected or reported in each study. The authors reported the risk for adverse events was “very low” at the doses studied and included mostly mild gastrointestinal or allergic skin reactions.

Chrubasik et al. (2007a) reported that 699 adverse events were documented among 34 clinical studies using “nettle root” in the treatment of benign prostatic hypertrophy (BPH). These studies included a total of approximately 40,000 patients, but adverse event data were not collected in each and it was not reported whether patients who experienced adverse events were receiving UD or placebo. The most common adverse events reported in these clinical studies were impotence and decreased libido. Other reported adverse events were mild gastrointestinal and allergic skin reactions.

Mittman et al. (1990) studied “freeze-dried” UD (600 mg total dose; 300 mg per capsule) treatment of allergy symptoms over 7 days. Of 31 UD patients, 5 reported mild gastric discomfort when the capsules were orally administered on an empty stomach and 2 withdrew from the study due to intensification of their allergy symptoms while on therapy.

Sonnenschein et al. (1987) conducted a 6-month study of Urtica-Plus capsules for the treatment of BPH. Dosing for the 4078 patients was two capsules twice daily for 12 weeks, then one capsule twice daily for a total of 26 weeks. Thirty-three patients experienced gastrointestinal adverse events, 9 patients’ experienced cutaneous allergic reactions, and 2 patients experienced hyperhidrosis. Nineteen patients dropped out due to unspecified adverse events.

Kaldewey et al. (1995) conducted a 6-month study using Urtica-Plus-N 270 mg capsules for the treatment of BPH. Dosing for the 1319 subjects was two capsules twice daily for the first 12 weeks, then one capsule two or three times daily, based on the discretion of their provider, for a total of 26 weeks. Thirteen patients developed “light” gastrointestinal symptoms and three discontinued the trial due to unspecified adverse events.
Engelmann et al. (1996) evaluated BPH therapy with three milliliters of Bazoton Liquid (n=20) administered twice daily versus similar colored liquid placebo (n=21). One patient in the active treatment group discontinued after having three bouts of vertigo.

Schneider et al. (2004) evaluated BPH therapy with Bazoton-uno 459 mg dry root extract (n=114) versus placebo (n=112) in a randomized, double-blind multicenter study. In the Bazoton-uno arm, subjects developed 29 adverse events versus 38 for placebo. The number of adverse events related to infection was reported as three for the active arm versus 10 in the trial’s placebo arm.

c. Published case reports

Bossuyt and Dooms-Goossens (1994) reported a case of diffuse edematous gingivostomatitis associated with regular ingestion of UD tea. The patient’s UD allergy was confirmed by a positive patch test to “common nettles leaf.”

Sahin et al. (2007) reported that a 33-year-old male who was taking no other medications developed unilateral gynecomastia after drinking two cups of nettle tea for about one month. After an extensive workup, no treatment other than cessation of nettle ingestion was prescribed. A decrease in gynecomastia was noted approximately one month later, but the condition was reported not to have totally abated.

Sahin et al. (2007) reported that a 33-year-old woman, with a prior history of menstrual irregularity, developed galactorrhea about one and a half years before presenting to the author’s clinic. She reported drinking nettle tea (amount not stated) for one month before her clinic presentation and taking no other medications. Her physical exam did not show any abnormalities. Her liver, thyroid, and renal laboratory evaluations were normal. However, her early follicular phase estradiol was high at 543 pg/ml and her FSH and LH levels were low at 1.2 mIU/mL and 1.7 mIU/mL, respectively. Her total and free testosterone levels were normal at 45.1 ng/dL and 3.20 ng/dL, respectively. Her prolactin level was measured at 27 ng/ml. After stopping the nettle tea for six weeks, her laboratory evaluated blood levels were as follows: estradiol level of 45 pg/ml; FSH level of 5.9 mIU/ml; LH level of 2.9 mIU; total testosterone level of 51.2 ng/dL; free testosterone level of 3.20 ng/dL; and a prolactin level of 32.6 ng/ml. The galactorrhea decreased but did not totally abate.

Uslu et al. (2011) reported that a general urticarial rash developed in a 17-day-old neonate of a mother applying water boiled with stinging nettle to her breasts for nipple cracks twice a day, before and after each breastfeeding, for the prior two days. The specific IgE levels for stinging nettle were also elevated in both the mother and child to 12.5 kU/L and 60.0 kU/L, respectively. The mother was advised to stop using the nettle solution on her breast, and the child’s rash completely disappeared two days after discontinuation. Two months later, the child reacted to a skin prick test of nettle boiled in water solution, although the mother and father (tested as a control) did not react.

d. Pharmacokinetic data

Because the chemicals responsible for any activity of nettle have not been identified, there are no pharmacokinetic data to report.

e. Availability of alternative approved therapies that may be as safe or safer
A summary of FDA approved products, which have been found by FDA to be safe and effective for the treatment of diabetes, is in Table 2 below.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Approved Drug Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulins and insulin analogs</td>
<td>Many different products and formulations</td>
</tr>
<tr>
<td>Sulfonylureas (SU)</td>
<td>Acetohexamide, chlorpropamide, tobutamide, glipizide, gliclazide, glyburide, glimepiride</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Metformin</td>
</tr>
<tr>
<td>Thiazolidinediones (TZDs)</td>
<td>Rosiglitazone, pioglitazone</td>
</tr>
<tr>
<td>Analogues of glucagon-like peptide 1 (GLP-1)</td>
<td>Exenatide, lixisenatide, liraglutide, albiglutide, dulaglutide, alogliptin</td>
</tr>
<tr>
<td>Dipeptidyl peptidase 4 (DPP-4) inhibitors</td>
<td>Sitagliptin, saxagliptin, linagliptin, alogliptin</td>
</tr>
<tr>
<td>SGLT2 inhibitors</td>
<td>Empagliflozin, dapagliflozin, canagliflozin</td>
</tr>
<tr>
<td>Alpha-glucosidase inhibitors</td>
<td>Acarbose, miglitol, voglibose</td>
</tr>
<tr>
<td>Meglitinides</td>
<td>Repaglinide; nateglinide</td>
</tr>
<tr>
<td>Synthetic analogues of human amylin</td>
<td>Pramlintide</td>
</tr>
<tr>
<td>Bile acid sequestrants</td>
<td>Colesevelam</td>
</tr>
<tr>
<td>Dopamine agonists</td>
<td>Bromocriptine</td>
</tr>
</tbody>
</table>

**Conclusions:** There is a dearth of systematically collected safety information regarding UD despite the large number of patients who were observed in clinical studies. Although the most frequent adverse effects appear to be mild gastrointestinal irritation and allergic reactions, the available information is based on formulations of UD with an uncharacterized composition. Furthermore, it is unclear how the formulations employed in the existing literature might compare qualitatively or quantitatively to a bulk substance or UD formulation used in the compounding realm. The safety profile of UD is not considered to be adequately established.

**C. Are there concerns about whether a substance is effective for a particular use?**

The following databases were consulted in the preparation of this section: PubMed, EMBASE, Google Scholar, ClinicalTrials.gov, ToxNet, and Google.

1. **Reports of trials, clinical evidence, and anecdotal reports of effectiveness, or lack of effectiveness, of the bulk drug substance**

Various formulations of UD were used in the clinical studies of glycemic control, and these will be further described with the individual studies. In general, either a powder of the dried nettle leaf or an ethanol extract of the plant were used (Namazi et al. 2011a; Namazi et al. 2011b; Namazi et al. 2012). Insufficient information was provided to calculate the total daily dose of ethanol in doses of extracts ingested by patients. Extracts prepared with 60% ethanol were associated with administration of 45% ethanol to patients (Namazi et al. 2011a). None of the trials provided pharmacokinetic data for any nettle components; therefore, no conclusions can be made regarding systemic exposure or activity of the individual components.

A total of seven trials having outcomes associated with glycemic control were included in this efficacy review, including six blinded, randomized, placebo-controlled trials, and one open-label trial. The outcomes of these trials are summarized below. All seven trials were conducted...
outside of the United States (US). Two additional clinical trials were identified, but were not evaluated. First, an abstract was found for Tarighat et al. (2012), but the full article was not published in English, and the methods and results could not be adequately evaluated. Second, a search of the website www.clinicaltrials.gov found a trial (Identifier: NCT00422357) for which no outcomes were reported and no published results could be identified.

Namazi et al. 2011a, Namazi et al. 2011b, and Namazi et al. 2012 appear, based on the trial design and patient descriptors, to be three publications derived from the same trial. These publications focused on different sets of endpoints: “cardiovascular risk factors,” “insulin sensitivity and some inflammatory indicators,” and “oxidative stress,” respectively. The trials used UD extracts of the arial parts of the plant prepared with 60% ethanol and were associated with levels of 45% ethanol in the final extraction solutions that were administered to patients. Adult Type II patients were “adjusted” into two treatment groups based on age, sex, pre- or post-menopausal status, duration of diabetes, dose of metformin or glibenclamide (also known as glyburide in the US) per day, and body mass index. Each patient continued on their pre-trial oral antidiabetic medication, adding either the UD formulation at doses of 100 mg/kg/day divided (n = 24), or identically colored placebo (water and ethanol mix) (n = 21) in a glass of water after each of three main meals for eight weeks. The authors state that “no biochemical parameters showed significant differences at the baseline in two study groups.”

The results for Namazi et al. 2011a and Namazi et al. 2011b are combined in Table 3. It is unclear from the publications whether changes from “beginning” to “end” of treatment for each treatment group were statistically compared or “end” values were statistically compared between the two treatment groups. No provision for multiple comparisons (e.g., adjustment of the p-value) was reported to have been made.
Table 3. Results of Namazi et al, (2011a and 2011b) trials

<table>
<thead>
<tr>
<th>“Variable”</th>
<th>UD</th>
<th>Placebo</th>
<th>p-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Beginning”</td>
<td>“End”</td>
<td>“Beginning”</td>
<td>“End”</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>129.65±31.16</td>
<td>112.56±39.57</td>
<td>142.52±37.77</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.30±1.40</td>
<td>6.11±1.19</td>
<td>7.44±1.35</td>
</tr>
<tr>
<td>Fasting Insulin Concent. (microU/mL)</td>
<td>6.20±0.52</td>
<td>4.90±0.40</td>
<td>5.00±0.51</td>
</tr>
<tr>
<td>Calculated Insulin Resistance</td>
<td>3.00±0.30</td>
<td>1.90±0.21</td>
<td>2.50±0.30</td>
</tr>
<tr>
<td>TG Level (mg/dL)</td>
<td>143.68±74.11</td>
<td>129.42±61.98</td>
<td>146.35±62.48</td>
</tr>
<tr>
<td>Total Chol. (mg/dL)</td>
<td>136.17±29.81</td>
<td>134.04±30.30</td>
<td>138.38±26.82</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>58.24±42.18</td>
<td>52.00±16.56</td>
<td>59.71±14.78</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>45.29±9.96</td>
<td>53.92±12.37</td>
<td>45.86±9.18</td>
</tr>
<tr>
<td>TC/ HCL-C</td>
<td>3.00±0.51</td>
<td>2.55±0.70</td>
<td>3.06±0.62</td>
</tr>
<tr>
<td>LDL-C/ HDL-C</td>
<td>1.28±0.41</td>
<td>0.98±0.31</td>
<td>1.32±0.34</td>
</tr>
<tr>
<td>Log(TG/ HDL-C)</td>
<td>0.42±0.35</td>
<td>0.34±0.15</td>
<td>0.44±0.34</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>116.9±13.30</td>
<td>100.0±61.98</td>
<td>124.2±14.9</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79.1±10.1</td>
<td>78.7±10.3</td>
<td>86.3±7.80</td>
</tr>
<tr>
<td>Calculated Insulin Sensitivity</td>
<td>0.32±0.03</td>
<td>0.31±0.02</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>3.52±2.11</td>
<td>1.19±0.271</td>
<td>4.11±1.07</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>11.50±0.62</td>
<td>10.00±0.83</td>
<td>10.50±0.50</td>
</tr>
<tr>
<td>hs-CRP (mg/dL)</td>
<td>2.52±1.06</td>
<td>1.37±0.11</td>
<td>3.01±1.07</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are presented as mean±SD

<sup>b</sup>No adjustment was made for multiple comparisons (“p<0.05 was considered significant for all comparisons”).

<sup>c</sup>There was a significant difference between the groups (P<0.05), either in change between “beginning” and “end,” or between “end” values for the two groups; publication is unclear in this regard.

*Abbreviations: FBS – Fasting blood sugar; HbA1c – hemoglobin A1c, TG – triglyceride; TC – total cholesterol; LDL-C – low density lipoprotein C; HDL-C – high density lipoprotein C; SBP – systolic blood pressure; DBP – diastolic blood pressure; IL-6 – interleukin 6; TNF-α – tissue necrosis factor alpha; hs-CRP – highly sensitive C-reactive protein*
Kianbakht et al. (2012 and 2013) employed a formulation of the aerial parts of UD extracted with 70% ethanol that was subsequently evaporated to create a powder. Adult type II diabetic patients continued on their pre-trial antidiabetic medication and added UD treatment for a period of 12 weeks. These trials appear to have data reporting errors. Figure 2 below is replicated from the Kianbakht et al. 2013 publication, providing the outcomes purportedly from the treatment of 92 patients. The earlier Kianbakht et al. 2012 publication, reporting the results following the treatment of 22 patients, shows identical results for the circled values in Figure 2. It is unclear which, if either, publication reports correct results.

Of potential interest from the Kianbakht publications was that the UD extraction process was reportedly performed on separate occasions, using the same methods, resulting in extractions comprising different amounts of known UD components. Total flavonoid content, total phenolic content, percent rutin, and percent gallic acid were reported to be three to six times higher in the 2013 extraction compared to the 2012 extraction. There was no quercetin found as a component of either formulation. The publications did not provide any context regarding the importance of these differences in formulation component levels or information about the differences’ potential impact on glycemic control. However, this information, if reported correctly, may provide a sense of the possible variability associated with UD extracted formulations.

Khajeh-Mehrizie et al. (2014) evaluated changes in fasting blood glucose (FBG) that occurred after eight weeks use of UD “extract” 100 mg/kg/day (n=24) or placebo (n=25) administered in divided doses after each of the patients’ three daily main meals. Placebo or UD extract was administered in combination with patients’ pre-trial oral antidiabetic medications in a randomized, double-blinded manner. Although the change in mean FBG (± standard deviation) was numerically greater for UD than for placebo (20.16 ± 52.6 mg/dL vs. -0.7 ± 45.29 mg/dl respectively) at the end of the 8-week treatment period, this study failed to show a statistical difference between the two groups.

Dabagh and Nikbakht (2016) evaluated changes in fasting blood sugar (FBS) that occurred after 8-weeks’ exposure to one of four treatments: aerobic exercise (n=10); 10 grams of UD leaves...
dried and ground to a powder (no extraction) and mixed in yogurt for daily administration before breakfast (n=10); a combination of both aerobic exercise and daily UD powder administration (n=10), or yogurt administered (placebo; n=10) daily in an unblinded, parallel group clinical trial. All participants in the trial were men who had either never taken any antidiabetic medication or had their antidiabetic medicine discontinued, followed by a washout period before the intervention period. A one-way analysis of variance established that there were statistically significant differences among the groups with respect to change in FBS, as reported in Table 4. The results of a Tukey Post Hoc Test show that the mean change in FBS was statistically significantly greater for all active treatments than for placebo. In addition, the mean FBS change of the combination of exercise and UD was statistically significantly greater than for UD alone.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ae</th>
<th>UD</th>
<th>Ae + UD</th>
<th>Placebo</th>
<th>f</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS, mg/dL</td>
<td>-9.50±6.96</td>
<td>-7.6±6.04</td>
<td>-18.30±6.63</td>
<td>+0.50±9.02</td>
<td>9.30</td>
<td>0.000b</td>
</tr>
</tbody>
</table>

aData are presented as mean±SD
bThere was a significant difference between the groups (P<0.05)

Abbreviations: Ae – exercise group; UD – Urtica dioica group; Ae + UD – exercise + Urtica dioica administration group; FBS – fasting blood sugar.

2. Whether the product compounded with this bulk drug substance is intended to be used in a serious or life-threatening disease

Nettle was nominated for glycemic control in diabetes mellitus. Diabetes mellitus is a serious disease affecting more than 29 million Americans. Diabetes complications are life-threatening and include heart disease, stroke, kidney disease, blindness and other eye problems, and peripheral vascular disease.

3. Whether there are any alternative approved therapies that may be as effective or more effective

See section II. B. 2. e. above.

Conclusions: The seven studies that were identified as evaluable from the published literature suggest that although UD may have some effect on reducing FBS and other parameters related to diabetes, they do not provide sufficient evidence of UD’s efficacy in providing glycemic control. FDA considers the reduction in hemoglobin A1c (HbA1c) to be an important outcome in demonstrating glycemic control efficacy in the treatment of diabetes. However, statistical confirmation of the numerical trends favoring UD for several assessed outcome variables, including fasting blood sugar and HbA1c, in (Namazi et al. 2011a, 2011b) cannot be verified based on the published statistical analysis, which failed to pre-specify primary endpoints or to control for the myriad of statistical comparisons. The outcomes reported in Kianbakht et al. (2012 and 2013) cannot be relied on because of the potential errors in data reporting, as

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discussed above. Khajeh-Mehrizie et al. (2014) failed to show statistically significant differences between UD and placebo treatments. Dabagh and Nikbakht (2016) demonstrated a statistically significant greater improvement in FBS for UD compared to placebo, but this was an open label study in which only 20 male patients with disparate diabetes treatment histories received UD. Among all the studies, there is lack of information regarding the composition of the UD formulations; Kianbakht (2013), which potentially suffers from data reporting errors, suggests that the variability is considerable.

D. Has the substance been used historically as a drug in compounding?
The following databases were consulted in preparation of this section: PubMed, Natural Medicines, European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, USP/NF, and Google.

1. Length of time the substance has been used in pharmacy compounding
Stinging nettle is a perennial plant that has been used as a medicinal agent since ancient times (Natural Database, 2011; Upton, 2013). According to the American Botanical Council, Greek physicians Pedanius Dioscorides and Galen reported that nettle leaf had diuretic and laxative properties in the first century (Blumenthal, 2000). From the literature, it appears that nettle has been used in pharmacy compounding for at least seven years (Rayburn et al., 2009).

2. The medical condition(s) it has been used to treat
Per the Natural Medicines Database, the above ground parts of nettle are used orally for allergies, allergic rhinitis, osteoarthritis, urinary tract infections, urinary tract inflammation, kidney stones, internal bleeding, anemia, poor circulation, splenomegaly, diabetes, gastric hyperacidity, biliary complaints, diarrhea and dysentery, asthma, pulmonary congestion, heart failure, rash and eczema, cancer, prevention of signs of aging, blood purification, wound healing, and as a general tonic. The above ground parts of the plant are used topically for musculoskeletal aches and pains, scalp seborrhea, oily hair, and hair loss (Natural Database, 2011).

Results from a Google search using the terms nettle, nettle leaf compounding pharmacy, and urtica dioica compounding pharmacy indicate that nettle leaf is compounded as a capsule in combination with other ingredients for hair loss.

3. How widespread its use has been
Insufficient data are available from which to draw conclusions about the extent of use of nettle in compounded drug products.

4. Recognition of the substance in other countries or foreign pharmacopeias

15 The monographs do not include sufficient information to characterize nettle leaf, nettle root, or Urtica dioica.
Conclusions: There is evidence that nettle has been used for centuries for a multitude of different conditions and there is evidence of its use in pharmacy compounding for at least 7 years.

III. RECOMMENDATION

We have balanced the criteria described in section II above to evaluate nettle for the 503A Bulks List. After considering the information currently available, a balancing of the criteria weighs against nettle being placed on that list based on the following:

1. Nettle is not well characterized chemically or physically. Consequently, absent strict controls on the manufacture and testing of the nettle-containing drug product, there can be no assurance that its properties and toxicities would be consistent.

2. The safety of nettle has not been adequately assessed with well-characterized formulations. Available safety data suggest that UD may be associated with mild gastrointestinal irritation and allergic responses, but the data do not allow for a thorough evaluation of all of the potential adverse effects of nettle. Nonclinical information is also incomplete and does not allow for an adequate assessment of the safety of UD, particularly for use in a chronic disease such as diabetes.

3. The effectiveness of nettle has not been adequately assessed with well-characterized formulations of nettle. A small number of clinical efficacy investigations of UD and some nonclinical data in animal models for diabetes suggest that UD may have some effect in reducing fasting blood sugar and other parameters related to diabetes. However, they do not provide sufficient evidence of nettle efficacy in providing glycemic control.

4. Nettle has been used for centuries as an herbal treatment for a variety of conditions and for at least 7 years in pharmacy compounding.

Based on this information the Agency has considered, a balancing of the four evaluation criteria weighs against nettle being added to the 503A Bulks List.
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Vogl, S; Picker, P; Mihaly-Bison, J; Fakhrudin, N; Atanasov, AG; Heiss, EH; Wawrosch, C; Reznicek, G; Dirsch, VM; Saukel, J; Kopp, B. Ethnopharmacological in vitro studies on Austria's folk medicine - An unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. J Ethnopharmacol, 2013, 149, 750–771.


APPENDIX 1: EFFECTS OF NETTLE EXTRACT ON GLYCEMIC CONTROL IN ANIMALS

The effects of nettle extract on glycemic control in animals are summarized from 16 articles obtained from the scientific literature. Administration of nettle extract had no effect on blood glucose in seven experiments that used normal mice, normal rabbits, streptozotocin (STZ)-induced diabetic rats, or alloxan-induced diabetic rats. A decrease in blood glucose was observed in 12 experiments that used normal rats, STZ-induced diabetic rats, alloxan-induced diabetic rats, fructose-induced diabetic rats, or STZ-induced diabetic mice. One study reported an increase in blood glucose in STZ-induced diabetic mice (Swanston-Flatt et al. 1989).

<table>
<thead>
<tr>
<th>Title/Author</th>
<th>Extract/Dose/Duration</th>
<th>In vivo model/Measurement</th>
<th>Observed effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Glycaemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice”. Swanston-Flatt et al. (1989)</td>
<td>6.25% UD leaf powder by weight in diet + aqueous UD plant extract, given instead of drinking water (1 g leaves + 400 ml water). Continuous treatment on day 0-43.</td>
<td>Treatment of normal mice on day 0-28, then STZ injection on day 28 to induce diabetes. Glycemic endpoints monitored until day 43.</td>
<td>No effect on plasma glucose in normal mice. Exacerbated diabetic state in STZ-treated mice by day 42, with increased glucose concentration, fluid intake, and rate of weight loss.</td>
</tr>
<tr>
<td>“Hypoglycemic Effect of Plants Used in Mexico as Antidiabetics.” Roman Ramos et al. (1992)</td>
<td>Aqueous UD leaf extract (132 g dried leaves + 1 L boiling water) at 4 ml/kg orally by gastric tube. Single dose immediately before glucose tolerance test.</td>
<td>Normal rabbits. Glucose tolerance test by subcutaneous infusion.</td>
<td>No effect on blood glucose (increased by 1.3%, not significant compared to water-treated control group).</td>
</tr>
<tr>
<td>“Antihyperglycemic activity of the aqueous extract of <em>Urtica dioica</em>.” Bouhaim et al. (2003)</td>
<td>Aqueous UD aerial material extract (500 mg/kg, oral). Single dose 1 h before initial glucose measurement.</td>
<td>Alloxan-induced diabetic rats. Animals with &gt;1.5 g/L FBG used 3 days after alloxan injection.</td>
<td>No effect on blood glucose in diabetic rats.</td>
</tr>
<tr>
<td>“Induction of insulin secretion by a component of <em>Urtica dioica</em> leaves in perfused Islets of Langerhans and in vivo effects in normal and streptozotocin diabetic rats.” Farzami et al. (2003)</td>
<td>Aqueous UD aerial material extract (250 mg/kg, oral). Single dose 30 min after glucose loading, hourly blood glucose measured from 1 h after loading.</td>
<td>Normal rats. Oral glucose tolerance test.</td>
<td>In normal rats, significant decrease in blood glucose level to ~33% of control, 1 h after glucose loading (compared to control group).</td>
</tr>
<tr>
<td>“Chronic effect of the hydroalcoholic extract of <em>Urtica dioica</em> leaves on regeneration of β-cells of hyperglycemic rats.” Golalipour and Khorii (2007)</td>
<td>Aqueous UD leaf powder extract (10 g + 200 ml boiling water). Fraction 1 obtained by thin layer chromatography was delivered by IP.</td>
<td>STZ-induced diabetic rats. Animals with ~250 mg/dl FBG used. Intraperitoneal glucose tolerance test.</td>
<td>Decrease in blood glucose and increase in serum insulin in both normal and diabetic rats (all statistically significant, compared to saline-treated mice).</td>
</tr>
<tr>
<td>“The protective activity of <em>Urtica dioica</em> leaves on blood glucose concentration and beta-cells in streptozotocin-diabetic rats.” Golalipour and Khori (2007)</td>
<td>Hydroalcoholic UD leaf powder extract (100 mg/kg/day, IP). 1 week after STZ injection, received daily dose for 4 weeks.</td>
<td>STZ-induced diabetic rats. Intraperitoneal glucose tolerance test.</td>
<td>No effect on blood glucose levels during week 5. No effect on loss of β-cells in pancreas due to STZ.</td>
</tr>
<tr>
<td>“The protective activity of <em>Urtica dioica</em> leaves on blood glucose concentration and beta-cells in streptozotocin-diabetic rats.” Golalipour and Khori (2007)</td>
<td>Hydroalcoholic UD leaf powder extract (100 mg/kg/day, IP). Daily dose for 5 days, followed by STZ injection, then monitoring for 5 weeks.</td>
<td>STZ-induced diabetic rats. Intraperitoneal glucose tolerance test.</td>
<td>Significant decrease in blood glucose levels by week 5. Significant increase in %β-cells (by area) for UD-treated rats (compared to diabetic group).</td>
</tr>
<tr>
<td>“The antidiabetic and antilipidemic activity of aqueous extract of <em>Urtica dioica</em> L. on type2 diabetic model rats.” Das et al. (2009)</td>
<td>Aqueous UD leaf powder extract (1.25 g/kg, oral). Fed for 14 days.</td>
<td>STZ-induced diabetic rats. Injected at 2-3 days old, used for experiment at 3 months old. FBG.</td>
<td>Significant decrease in FBG (compared to diabetic, water-treated control group).</td>
</tr>
<tr>
<td>“Antidiabetic effect of some Aqueous UD extract (likely)</td>
<td>STZ-induced diabetic rats.</td>
<td>No effect on plasma glucose</td>
<td></td>
</tr>
<tr>
<td>Medicinal plants of Oriental Morocco in neonatal non-insulin-dependent diabetes mellitus rats.</td>
<td></td>
<td></td>
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<tr>
<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Antidiabetic and anti-inflammatory activity of <em>Urtica dioica</em> leaves on STZ induced type 1 diabetic model rats.&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous UD (likely aerial parts, 150 mg/kg, oral or IP). Single dose 30 min before glucose loading, hourly glucose measured from 1 h after loading.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STZ-induced diabetic rats. Animals with &gt;1.25 g/L fasting plasma glucose used at 12-14 weeks after STZ. Oral glucose tolerance test.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant decrease in plasma glucose to ~50% of control, 3 h after glucose loading (compared to water-treated diabetic control group).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fasting blood glucose (FBG); intraperitoneal (IP); streptozotocin (STZ); <em>Urtica dioica</em> (UD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Urtica dioica** | "In vivo and in vitro evaluation of the effects of *Urtica dioica* and swimming activity on diabetic factors and pancreatic beta cells." |
|---|
| "Effects of a triplex mixture of *Peganum harmala*, *Rhus coriaria*, and *Urtica dioica* aqueous extracts on metabolic and histological parameters in diabetic rats." |
| Aqueous UD leaf powder extract (1.25 g/kg/10 ml of water, oral). Continuous feeding for 8 days. |
| STZ-induced diabetic rats. Injected as adults, unclear when treatment began. FBG. |
| Significant decrease in fasting serum glucose and body weight (compared to untreated diabetic control group). |

<table>
<thead>
<tr>
<th><strong>Pharmacological and toxicological evaluation of <em>Urtica dioica</em>.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety of extracts tested: hexane, chloroform, ethyl acetate, methanol, aqueous. All from UD leaf powder (all at 300 mg/kg, oral gavage). Single dose 90 min before glucose loading.</td>
</tr>
<tr>
<td>Aqueous extract most effective in reducing blood glucose in normal and STZ rats (claims to be significant difference between control and treated groups at 1 h after glucose loading).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Therapeutic Potential of Some Plant Extracts Used in Turkish Traditional Medicine on Streptozocin-Induced Type 1 Diabetes Mellitus in Rats.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic UD aerial parts extract (100 mg/kg/day, oral) for 28 days.</td>
</tr>
<tr>
<td>STZ-induced diabetic rats. Animals with &gt;200 mg/dl plasma glucose used at 1 week after STZ. FBG.</td>
</tr>
<tr>
<td>No effect on FBG on treatment days 7, 14, 21, 28 and 35 (compared to initial day).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>&quot;Urtica dioica&quot; modulates hippocampal insulin signaling and recognition memory deficit in streptozotocin induced diabetic mice.&quot;</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic UD leaf extract (50 mg/kg, oral gavage) for 8 weeks. STZ injected for 5 days, then once daily on day 6-60.</td>
</tr>
<tr>
<td>STZ-induced diabetic mice. Oral glucose tolerance test on day 60.</td>
</tr>
<tr>
<td>Chronic UD treatment showed significantly reduced glucose levels at 0, 0.5, 1, 1.5 and 2 h (compared to diabetic group).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>&quot;Antidiabetic effect of hydroalcoholic <em>Urtica dioica</em> leaf extract in male rats with fructose-induced insulin resistance.&quot;</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic UD leaf extract (50, 100, 200 mg/kg/day, IP). Administered on week 6-8.</td>
</tr>
<tr>
<td>Fructose-induced diabetic rats. 10% fructose in drinking water on week 1-8. Serum measurements.</td>
</tr>
<tr>
<td>Significant, dose-dependent decrease in serum glucose, significant decrease in serum insulin concentration (compared to untreated diabetic group).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>&quot;Effect of <em>Urtica dioica</em> leaf extract on activities of nucleoside diphosphate kinase and acetyl coenzyme, a carboxylase, in normal and hyperglycemic rats.&quot;</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous or ethanolic UD leaf powder extract (50 mg/kg/day, oral) for 14 days. Daily doses initiated 4 days after alloxan injection.</td>
</tr>
<tr>
<td>Significant decrease in blood glucose and increase in insulin for ethanolic extract-treated, diabetic group (compared to untreated diabetic group). No significant differences between aqueous extract-treated groups.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>&quot;In vivo and in vitro evaluation of the effects of <em>Urtica dioica</em> and swimming activity on diabetic factors and pancreatic beta cells.&quot;</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous UD leaf powder extract (625 mg/kg/day or 1.25 g/kg/day, oral) for 4 weeks.</td>
</tr>
<tr>
<td>STZ-induced diabetic rats. Animals with &gt;250 mg/dl FBG used in study. Glycemic endpoints.</td>
</tr>
<tr>
<td>Significant decrease in blood glucose, significant increase in insulin for both UD doses (compared to untreated diabetic group).</td>
</tr>
</tbody>
</table>
Tab 4

Ubiquinol
Tab 4a

Ubiquinol Nominations
<table>
<thead>
<tr>
<th>What is the name of the nominated ingredient?</th>
<th>Ubiquinol 30% Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is the ingredient an active ingredient that meets the definition of &quot;bulk drug substance&quot; in § 207.3(a)(4)?</td>
<td>Yes, Ubiquinol 30% powder is an active ingredient as defined in 207.3(a)(4) because when added to a pharmacologic dosage form it produces a pharmacological effect. References for Ubiquinol 30% powder pharmacological actions are provided. Mezawa, M., Takemoto, M., Onishi, S., Ishibashi, R., Ishikawa, T., Yamaga, M., . . . Yokote, K. (2012). The reduced form of coenzyme Q10 improves glycemic control in patients with type 2 diabetes: An open label pilot study. BioFactors (Oxford, England), 38(6), 416-21. doi:10.1002/biof.1038</td>
</tr>
<tr>
<td>Is the ingredient listed in any of the three sections of the Orange Book?</td>
<td>The nominated substance was searched for in all three sections of the Orange Book located at <a href="http://www.accessdata.fda.gov/scripts/cder/ob/docs/queryai.cfm">http://www.accessdata.fda.gov/scripts/cder/ob/docs/queryai.cfm</a>. The nominated substance does not appear in any section searches of the Orange Book.</td>
</tr>
<tr>
<td>Were any monographs for the ingredient found in the USP or NF monographs?</td>
<td>The nominated substance was searched for at <a href="http://www.uspnf.com">http://www.uspnf.com</a>. The nominated substance is not the subject of a USP or NF monograph.</td>
</tr>
<tr>
<td>What is the chemical name of the substance?</td>
<td>[(2E,6E,10E,14E,18E,22E,26E,30E,34E)-3,7,11,15,19,23,27,31,35,39-decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaenyl]-5,6-dimethoxy-3-methylcyclohexa-2,5-diene-1,4-diol</td>
</tr>
<tr>
<td>What is the common name of the substance?</td>
<td>Reduced CoQ10</td>
</tr>
<tr>
<td>Question</td>
<td>Answer</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Does the substance have a UNII Code?</td>
<td>M9NL0C577Y</td>
</tr>
<tr>
<td>What is the chemical grade of the substance?</td>
<td>no grade</td>
</tr>
<tr>
<td>What is the strength, quality, stability, and purity of the ingredient?</td>
<td>Appearance: Pale yellow to yellow solid</td>
</tr>
<tr>
<td></td>
<td>Ubiquinol: ≥ 30%</td>
</tr>
<tr>
<td></td>
<td>Aerobic Plate Count: ≤ 300 cfu/g</td>
</tr>
<tr>
<td></td>
<td>Coliforms: Negative</td>
</tr>
<tr>
<td>How is the ingredient supplied?</td>
<td>Powder</td>
</tr>
<tr>
<td>Is the substance recognized in foreign pharmacopeias or registered in</td>
<td>No foreign pharmacopeia monographs or registrations found.</td>
</tr>
<tr>
<td>other countries?</td>
<td></td>
</tr>
<tr>
<td>Has information been submitted about the substance to the USP for</td>
<td>No USP Monograph submission found.</td>
</tr>
<tr>
<td>consideration of monograph development?</td>
<td></td>
</tr>
<tr>
<td>What dosage form(s) will be compounded using the bulk drug substance?</td>
<td>Capsules</td>
</tr>
<tr>
<td>What strength(s) will be compounded from the nominated substance?</td>
<td>25-300mg</td>
</tr>
<tr>
<td>What are the anticipated route(s) of administration of the compounded</td>
<td>Oral</td>
</tr>
<tr>
<td>drug product(s)?</td>
<td></td>
</tr>
<tr>
<td>Has the bulk drug substance been used previously to compound drug product(s)?</td>
<td>Capsules</td>
</tr>
<tr>
<td>What is the proposed use for the drug product(s) to be compounded with the nominated substance?</td>
<td>Adjunctive therapy for glycemic control and oxidative stress</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Is there any other relevant information?</td>
<td>All relevant information was expressed in the above questions</td>
</tr>
</tbody>
</table>
Tab 4b

FDA Review of Ubiquinol
DATE: April 4, 2017

FROM: Ben Zhang, PhD
ORISE Fellow, Office of New Drug Products, Office of Pharmaceutical Quality (OPQ)

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Elizabeth Marek, PharmD
Consumer Safety Officer, Office of Compliance, Office of Unapproved Drugs and Labeling Compliance (OUDLC)

THROUGH: Ramesh K. Sood, PhD
Senior Scientific Advisor (Acting), Office of New Drug Products, OPQ

Charles Ganley, MD
Director, ODE 4, OND

Frances Gail Bormel, RPh, JD
Director, Division of Prescription Drugs, OUDLC

TO: Pharmacy Compounding Advisory Committee
SUBJECT:  Review of Ubiquinol for Inclusion on the 503A Bulk Drug Substances List

I. INTRODUCTION

Ubiquinol, 30% powder, has been nominated for inclusion on the list of bulk drug substances for use in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for use in adjunctive therapy for glycemic control\(^1\) as orally administered capsules.\(^2\)

We have reviewed available data on the physicochemical characteristics, safety, effectiveness, and historical use in compounding of this substance. For the reasons discussed below, we believe the evaluation criteria weigh against placing ubiquinol on the list of bulk drug substances that can be used to compound drug products in accordance with section 503A of the FD&C Act (503A Bulks List).

II. EVALUATION CRITERIA

A. Is the substance well-characterized, physically and chemically, such that it is appropriate for use in compounding?

Ubiquinol is the fully reduced form of coenzyme Q10 (ubiquinone), with greater bioavailability than oxidized coenzyme Q10 (Hosoe et al. 2007). It is currently marketed as a dietary supplement in soft gelatin capsule of 30 mg, 100, and 200 mg.

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\(^1\) Diabetes causes high blood sugar levels in the body either due to lack of insulin, as is the case with type 1 diabetes, or because the body doesn't respond properly to insulin which regulates sugar levels, as in type 2 diabetes. FDA considers the reduction in hemoglobin A1c (HbA1c) to be an important outcome in demonstrating efficacy for glycemic control in the treatment of diabetes.

\(^2\) The nominated proposed use for ubiquinol is “adjunctive therapy for glycemic control and oxidative stress”. The “adjunctive therapy” nomination is understood to mean that the nominator proposes to use ubiquinol in addition to other diabetes treatment options. Because of lack of data on ubiquinol as an adjunctive therapy for diabetes, we have focused on its use as a primary therapy. The nomination also includes “oxidative stress.” Oxidative stress refers to elevated intracellular levels of reactive oxygen species that cause damage to lipids, proteins and DNA, thus interfering with the normal functioning of the cellular metabolism. The FDA considers alleviation of oxidative stress as a mechanism of action, rather than a treatment option for a disease condition. In this review, oxidative stress is considered to the extent it was found to be relevant as a mechanism of action for glycemic control.
Databases searched for information on ubiquinol in regard to Section A of this consultation included PubMed, SciFinder, Analytical Profiles of Drug Substances, the European Pharmacopoeia, British Pharmacopoeia, and Japanese Pharmacopoeia, and USP/NF.

1. Stability of the API and likely dosage forms

Ubiquinol is sensitive to oxidants and is easily oxidized into ubiquinone. Its solutions are unstable at room temperature, but stable when kept in hexanes under – 80 °C (Yamamoto et al. 2002). This reference also reports that a hexane solution is 50% decomposed in two hours at room temperature. This suggests that significant degradation will occur under the ordinary storage conditions. Formulations that can enhance the stability of ubiquinol have been reported in literature (Ueda et al. 2005; Ueda et al. 2009; Kitamura and Ueda 2010). For example, Ueda and Kitamura report a patented formulation technique that involved mixing ubiquinol with propylene glycol fatty acid esters. Only minor oxidation (about 3 % by weight) was observed after preserving the mixture in the air at 40 °C for two weeks (Ueda et al. 2009).

2. Probable routes of API synthesis

Ubiquinol is usually prepared from the reduction of ubiquinone, which can be obtained from yeast fermentation. This synthetic strategy to produce ubiquinol has been reported in the literature, differing in the choice of reducing agents and reaction conditions. The synthetic procedure usually involves three major steps: 1) dissolving ubiquinone-10 in organic solvents (i.e. hexanes); 2) performing the reduction reaction under inert atmosphere with different reducing agents (i.e., sodium hydrosulfite, sodium borohydride) and catalysts; and 3) separation of the desired product from by-product, starting materials and catalysts with extractions and chromatographic techniques (Mukai et al. 1990; Wang et al. 2014; Dadali et al. 2014).

![Image of chemical structure](attachment:image.png)

3. Likely impurities

The likely impurities may include:

- Trace amount of starting material, ubiquinone; and
- Trace amount of catalysts involved in the reduction reaction, such as enzymes or residual heavy metals.

4. Toxicity of those likely impurities
The starting material, ubiquinone-10, is unlikely to be significantly toxic. However, depending on the different reduction conditions or separation techniques, some impurities with higher toxicity, such as copper, may be present in the product (Dadali et al. 2014).

5. **Physicochemical characteristics pertinent to product performance, such as particle size and polymorphism**

Ubiquinol is a pale yellow solid, practically insoluble in water, but highly soluble in organic solvents like acetone, hexanes, and diethyl ether. No further information on the influence of particle size and polymorphism on bioavailability was found in the literature.

6. **Any other information about the substance that may be relevant, such as whether the API is poorly characterized or difficult to characterize**

Ubiquinol is a simple small molecule and the substance has been well characterized with proton nuclear magnetic resonance (\(^1\)H NMR), Carbon-13 nuclear magnetic resonance (\(^{13}\)C NMR) and mass spectroscopy. The compound can also be easily identified by chromatography.

**Conclusions:** Ubiquinol is unlikely to be stable when stored under ordinary storage conditions, in the absence of special formulations, unless it is protected with inert atmosphere. However, stability-enhancement techniques have been reported in literature. Ubiquinol is a simple small molecule and the substance has been well characterized with \(^1\)H NMR, \(^{13}\)C NMR, and mass spectroscopy. The compound can also be easily identified by chromatography.

B. **Are there concerns about the safety of the substance for use in compounding?**

The following database(s) were consulted in the preparation of the nonclinical portion of this review: Embase, PubMed, and TOXNET.

1. **Pharmacology of the drug substance**

Ubiquinol is a small, lipophilic molecule and is the nonoxidized, reduced form of coenzyme Q10 (CoQ10H2). The fully oxidized form is known as ubiquinone (also referred to as CoQ10, CoQ10 coenzyme, or ubidecarenone). For clarity in this review, the term “ubiquinol” will be used to refer to the reduced form and the term “CoQ10” will be used to refer to the oxidized form.\(^3\) There is a third oxidative state among this group, the radical semiquinone intermediate (CoQ10H), which will not be discussed further in this review.

A more general term, coenzyme Q (CoQ), refers to a class of homologous benzoquinones (e.g., CoQ1, CoQ7, CoQ8, CoQ9 and CoQ10) that have been identified in all plants and animals, as well as in a majority of microorganisms. In humans and most mammals, the predominant form

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\(^{3}\) While we have considered studies of CoQ10 in this review whenever they were relevant for our evaluation of ubiquinol, this review evaluates only ubiquinol and does not address the eligibility of CoQ for use in compounded drug products.
of CoQ, is CoQ10 (which includes the oxidized CoQ10, and reduced ubiquinol forms) (Yamashita and Yamamoto 1997). In humans, CoQ10 represents more than 95% of total CoQ, which is detected in most tissues, including the pancreas, kidney, liver, and heart. CoQ10 has 10 isoprenoid units in its side chain. In rats and mice, the primary CoQ form is CoQ9, which contains 9 isoprenoid units (Ramasarma 1985; Battino et al. 1990). There are also differences between rodents and humans in the metabolism of CoQ (Takahashi et al. 1993; Kitano et al. 2008). The differences in CoQ among species may be important to consider when extrapolating animal data to humans and will be addressed further in this review.

Both ubiquinol and CoQ10 are endogenously synthesized and ingested from foods such as fish, meat, fruits, and vegetables (Kubo et al. 2008). Ubiquinol acts as an antioxidant in the cell, where it is found endogenously in the lipid membranes of cells and mitochondria and plays a key role in mitochondrial energy production. It serves as both an electron carrier and a proton translocator during cellular respiration and production of adenosine triphosphate (ATP). Ubiquinol serves to protect both cellular membranes and serum low density lipoprotein from lipid peroxidation (Stocker et al. 1991).

The mechanism of action for ubiquinol in glycemic control in humans has been the subject of several publications (Ceriello and Motz 2004). A state of persistent hyperglycemia has been shown to be associated with overproduction of reactive oxygen species in an ex-vivo study using human pancreatic beta-cells, which leads to oxidative stress (Kaneto et al. 1996). Based on these findings, it has been proposed that using supplements such as ubiquinol and CoQ10 may help to alleviate oxidative stress and improve glycemic control in diabetics (Shen and Pierce 2015).

The potential difference between rodents and humans in the pharmacology of CoQ, including ubiquinol and CoQ10, is unknown. Therefore, it is unclear how to interpret the outcomes of studies conducted in rodents. The effect of ubiquinol and CoQ10 on blood glucose levels was tested in a rat model of streptozotocin (STZ)-induced diabetes (Prangthip et al. 2016). In this study, normal and STZ-induced diabetic rats (n=6 per group) received either ubiquinol (5mg/kg body weight) or CoQ10 (5 mg/kg body weight) in drinking water for 1 or 4 weeks. Levels of nonfasting blood glucose levels, blood pressure and CoQ10 were measured. STZ-induced diabetic rats receiving ubiquinol showed significantly lower blood glucose levels than STZ-induced diabetic rats not receiving ubiquinol when measured after the second week of the study. However, this decrease was not statistically significant after the third or fourth weeks of the study. Treatment with CoQ10 decreased glucose levels after the second, third and fourth weeks of the study. In addition, treatment of STZ-induced diabetic rats with either ubiquinol or CoQ10 showed improvement in the measured oxidative stress parameters (derivatives of reactive oxygen species and malondialdehyde levels).

2. Pharmacokinetic and toxicokinetic data

Because of the close relationship between ubiquinol and CoQ10, as explained in section B.1, above, this review will contain pharmacokinetics/toxicokinetics data for both substances.

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4 Malondialdehyde is a reactive species which occurs naturally and is used as a marker for oxidative stress.
Ubiquinol is quickly oxidized at room temperature in plasma and tissue samples to CoQ10 (Kommuru et al. 1999; Kitano et al. 2008). Therefore, concentrations of “CoQ10” are commonly reported as outcomes in studies, representing the total of oxidized and reduced forms of CoQ10, (i.e., the levels of ubiquinol (reduced) and ubiquinone (oxidized) are not distinguished). The only exception was the study conducted by Prangthip et al. (2016), discussed in Appendix 1, in which all CoQ10 in test samples was reduced by the investigator to ubiquinol; as such the amount of ubiquinol represents the total CoQ10 level (oxidized plus reduced forms) in blood and tissue samples.

As mentioned in section B.1, above, it is unclear how to interpret the rodent pharmacokinetics/toxicokinetics outcomes given the differences between rodent and human CoQ. Therefore, this section includes pharmacokinetic data from human and dog studies. Rodent pharmacokinetics data can be found in Appendix 1.

a. Animal Data

Because ubiquinol and CoQ10 exist endogenously, some studies tested whether administration of exogenous ubiquinol affects the endogenous levels of ubiquinol and CoQ10. Available literature suggests that exogenous CoQ10 administration does not influence its endogenous biosynthesis and it does not accumulate after supplementation is stopped (Hidaka et al. 2008).

The pharmacokinetic profile of ubiquinol was investigated in dogs using a randomized, crossover design in which the bioavailability of CoQ10 was compared between powder-filled capsules and soft gelatin formulations (Zaghloul et al. 2002). Dogs (n=5) were dosed orally twice a day for four consecutive days with a dose of 30 mg ubiquinol formulated in hydrosoluble soft gelatin capsules (Q-Nol).5 On the fifth day of treatment, a serial blood sample collection was initiated, lasting 72 hours post dosing. Ubiquinol results were as follows: mean area under the curve (AUC)0-72h was 10.5 ± 0.1 μg.hr/ml, maximum plasma concentration (Cmax) was 0.4 ± 0.1 μg/ml, and time to reach maximum concentration (Tmax) was 4.5 ± 0.6 hours. Dogs were crossed over and received treatment with powder filled capsules of CoQ10 and hydrosoluble soft gelatin capsules of CoQ10, respectively, in the remaining two treatment periods. The AUC0-72h of ubiquinol in hydrosoluble soft gelatin capsules was approximately 4-fold higher than that of the powder filled capsules of CoQ10. Although this study did not directly test whether the nominated substance, a 30% powdered form of ubiquinol, is less bioavailable than a formulation in which the substance has been dissolved, the results may suggest such a finding.

In another pharmacokinetic dog study, ubiquinol (Kaneka QH)6 was administered by gavage to dogs (n=3/sex/group) at dose levels of 150, 300, and 600 mg/kg for 13 weeks. Blood samples were collected following the first dose, at week 7 and week 13 of the study. At each sampling

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point, serial blood samples were collected over a period of 24 hours. $C_{\text{max}}$ was approximately 4-5 $\mu$g/ml in all dose groups and $T_{\text{max}}$ was reached within 4-8 hours after the first dose. The $C_{\text{max}}$ value was increased to approximately 8-9 $\mu$g/ml in all dose groups when measured during weeks 7 and 13, suggesting some accumulation over time. Similarly, $\text{AUC}_{0-24h}$ levels for CoQ10 (oxidized form only) increased between the 150 and 300 mg/kg dose groups but seemed to reach steady state at doses higher than 300 mg/kg and longer duration of exposure (increased $\text{AUC}_{0-24h}$ levels between the first dose and week 7 but no changes seen between week 7 and 13 for all dose groups). No differences were noted between males and females for any of data points described above. Administration of ubiquinol (600 mg/kg) led to a greater $\text{AUC}_{0-24h}$ level of CoQ10 compared to CoQ10 (600 mg/kg), suggesting greater bioavailability of ubiquinol under the conditions of the study (Kitano et al. 2008).

Orally administered ubiquinol is more highly bioavailable (20-100%) than CoQ10 in rats and dogs after a single dose exposure or after a treatment period that is shorter than 7 weeks. The bioavailability of ubiquinol and CoQ10 did not show a difference following exposure for 13 weeks of daily repeat dosing in rats and dogs (Zaghloul et al. 2002; Kitano et al. 2008; Prangthip et al. 2016).

Liver CoQ10 concentrations were measured after 13 weeks of administration of ubiquinol (Kaneka QH) (150, 300, and 600 mg/kg) to dogs (Kitano et al. 2008). In female dogs, CoQ10 liver concentrations were approximately 1, 3, and 2 mg/g after administration of ubiquinol at 150, 300, and 600 mg/kg, respectively. In male dogs, dosing with ubiquinol (150, 300, and 600 mg/kg) resulted in CoQ10 levels of 1, 2, and 7 mg/g in the liver. Dosing with 600 mg/kg ubiquinol led to a higher level of liver CoQ10 concentrations in male dogs than those seen in females (See Table 1). Not shown in Table 1, CoQ10 concentrations in the liver of ubiquinol-treated dogs were greater than those of CoQ10-treated dogs.

Table 1. Liver CoQ10 concentrations in dogs following 13 weeks of oral dosing with ubiquinol (Kitano et al. 2008)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Doses (mg/kg)</th>
<th>Concentrations (mg/g liver wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>150</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>Male</td>
<td>150</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>2.2 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>6.9 ± 1.8</td>
</tr>
</tbody>
</table>

No elimination data following administration of ubiquinol were found in the literature.

In summary, ubiquinol appears to be more highly bioavailable than CoQ10 in dogs after a single dose exposure and up to seven weeks of dosing. No difference in bioavailability of ubiquinol and CoQ10 was seen after 13 weeks of daily repeat oral dosing in dogs. Plasma concentrations of CoQ10 increase in a dose dependent manner in the dog. Levels of plasma CoQ10 increased from day 1 to week 7 and remained at steady levels afterwards when measured up to 13 weeks. The dose dependent increase in plasma exposure seems to reach a plateau at $\geq$300 mg/kg in dogs.
CoQ10 appears to accumulate in the liver of dogs with a significantly greater accumulation in males compared to females after 13 weeks of exposure.

b. Human Data

A number of studies have evaluated the pharmacokinetic profile of ubiquinol in healthy volunteers. In all cited clinical studies, both plasma ubiquinol and CoQ10 levels were separately measured. However, since ubiquinol represents more than 90% of the total CoQ10 in vivo, some investigators only reported levels of ubiquinol while others reported “total CoQ10” (the reduced ubiquinol form plus the oxidized CoQ10 form) to characterize the pharmacokinetic profile of ubiquinol.

In one study, subjects (n=10) were orally given a single dose of 100mg of ubiquinol (CoQH-CF)\(^7\) (Evans et al. 2009). The \(T_{\text{max}}\) was approximately 6 hours after oral supplementation of ubiquinol. The \(C_{\text{max}}\) was 0.07 ± 0.05, 0.97± 0.63 and 1.0 ± 0.7\(\mu\)g/ml for CoQ10, ubiquinol and total CoQ10 (ubiquinol and CoQ10), respectively. The \(\text{AUC}_{0-72h}\) was 41.7 ± 25.9 \(\mu\)g.hr/ml of total CoQ10 after single exposure of 100mg of ubiquinol. These data suggest that orally administered ubiquinol was readily absorbed in the gastrointestinal tract in humans.

In a single dose study, administration of ubiquinol (Q-Nol) (180 mg) produced a \(C_{\text{max}}\) of 1.27 ± 0.7 \(\mu\)g/ml, \(\text{AUC}_{0-144h}\) of 41.1 ± 21.8 \(\mu\)g.hr/ml and \(\text{AUC}_{0-\infty}\) of 55.3 ± 35.0 \(\mu\)g.hr/ml in total CoQ10 concentrations (Miles et al., 2002). These data also confirm that the bioavailability of exogenously administered ubiquinol was much higher than that obtained with CoQ10 dosing (Miles et al. 2002; Evans et al. 2009).

Also in a single-dose study, healthy subjects received single oral doses of 150 or 300 mg of ubiquinol (Kaneka QH) (n=5/sex/group) (Hosoe et al. 2007). The \(C_{\text{max}}\) of ubiquinol increased from 1.88 to 3.19 \(\mu\)g/ml following single oral doses of 150 and 300 mg, respectively. Similarly, the \(\text{AUC}_{0-48h}\) increased from 74.61 to 91.76 \(\mu\)g.hr/ml following dosing of ubiquinol at 150 and 300 mg, respectively. The half-life of ubiquinol was estimated to be around 48 hours.

In a multiple dose study, ubiquinol (Kaneka QH) (200mg/day) was administered to healthy subjects (n=12) for 4 weeks, and an increase in total CoQ10 levels, which ranged between 0.88 ± 0.30 and 4.34 ± 1.97 \(\mu\)g/ml, was seen (Langsjoen and Langsjoen 2013).

Also in a multiple dose study, subjects (n=10/sex/group) were assigned to receive one of the following doses of ubiquinol (Kaneka QH): placebo, 90, 150 or 300 mg/day for four weeks (Hosoe et al. 2007). Blood samples were collected before administration on the first day of treatment (day 0), day 1, day 14, day 28 (through), as well as 2 weeks and 6 months after completion of treatment. The baseline values of plasma ubiquinol concentrations ranged from 0.57 ± 0.04 to 0.66 ± 0.04 \(\mu\)g/ml. An increase in plasma ubiquinol was seen in all dose groups (see Table 2 below). Mean trough concentration increased dose-dependently for all sampling

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points. The mean trough concentrations at day 14 and day 28 were 2.61 and 2.84 μg/ml for 90mg, 3.66 and 3.84 μg/ml for 150mg, and 6.53 and 7.28 μg/ml for 300mg, respectively. The ratios of the mean concentrations on day 28 and day 14 did not show a difference (ranged from 1.09 to 1.110).

Table 2. Plasma Ubiquinol Levels (μg/ml)

<table>
<thead>
<tr>
<th>Treatment Day</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 mg/kg</td>
</tr>
<tr>
<td>Day 0</td>
<td>0.57</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.21</td>
</tr>
<tr>
<td>Day 14</td>
<td>2.61</td>
</tr>
<tr>
<td>Day 28</td>
<td>2.84</td>
</tr>
</tbody>
</table>

The data above show a dose-dependent increase in plasma ubiquinol levels which had reached steady-state within 2 weeks of treatment. Ubiquinol levels returned to baseline about 2 weeks after termination of the treatment for all dose levels, suggesting that under the conditions of this study, ubiquinol did not accumulate over time.

In summary, ubiquinol is readily absorbed in the gastrointestinal tract in humans when orally ingested. The T_{max} appears to be around six hours. The C_{max} and AUC of total CoQ10 or ubiquinol increased with increasing doses of ubiquinol up to the maximum human dose studied, 300 mg/day. Plasma ubiquinol concentrations reached steady-state within two weeks of treatment and returned to baseline two weeks after the end of dosing, suggesting that accumulation of ubiquinol in plasma over a long term period is unlikely.

3. Nonclinical safety

Because of the close relationship between ubiquinol and CoQ10, as explained in section B.1, above, this review will contain nonclinical toxicity data for both substances.

a. Acute toxicity

No adverse effects were seen in male and female rats (n=5/sex) when administered a single dose of ubiquinol (Kaneka QH) up to 5 g/kg (Hosoe et al. 2007). No acute toxicity studies were reported in the dog or other species.

b. Repeat dose toxicity

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8 Acute toxicity refers to adverse effects observed following administration of a single dose of a substance, or multiple doses given within a short period (approximately 24 hours). Endpoints captured in acute toxicity studies usually include mortality and gross clinical observations. Acute toxicity data are usually superseded by data obtained from longer term toxicity studies.

9 Repeated-dose toxicity studies consist of in vivo animal studies that seek to evaluate the toxicity of the test substance by observing the changes that emerge in clinical observations, clinical chemistry, gross pathology, and histology endpoints when the test substance is repetitively administered daily for a predetermined period of time.
Three subchronic toxicity studies were identified in the literature, each consisting of 13 weeks of daily exposure regimen in rats and dogs. These studies were reported to have been conducted by the manufacturer of the ubiquinol dietary supplement product.

In an oral gavage study, ubiquinol (Kaneka QH) was administered to male and female rats (n = 5/sex/group) for 13 weeks at dose levels of 0, 300, 600, or 1200 mg/kg (Kitano et al. 2008). A subset group of rats (n= 5/sex) was included in the study which was administered a single dose of CoQ10 (1200 mg/kg) for 13 weeks. No deaths were seen in this study. No treatment related changes were noted for body weight, ophthalmology, urinalysis, or hematology. Histopathological examinations revealed dose dependent adverse effects in the lungs, liver, spleen, and mesenteric lymph node in female rats, but not in male rats. Liver changes consisted of increases in blood chemistry enzymes (approximately 2-fold increases in aspartate aminotransferase [AST], alanine aminotransferase [ALT], and lactate dehydrogenase [LDH]), increase in liver weight accompanied by a substantial accumulation of ubiquinol in the liver of treated females (males accumulated less ubiquinol in the liver), and dose dependent increases in the incidence of fine vacuolation of hepatocytes among rats treated with ubiquinol at 200 mg/kg and above. Other hepatic findings included increased incidence of liver microgranuloma, vacuolation of Kupffer cells and hepatocytes, and focal necrosis with accumulation of macrophages starting from the 300 mg/kg ubiquinol dose. Other histopathological changes seen in treated females (which were not observed in males administered the same doses) included accumulation of macrophages in the spleen and mesenteric lymph nodes. The only clinical sign seen among treated male rats was a slight prolongation of blood clotting time as measured by prothrombin and activated partial thromboplastin time among males treated with 1200 mg/kg ubiquinol.

To further understand the findings seen in treated females in the first 13 week rat study, another 13 week oral gavage study was conducted in female rats only (n=10/group) where the ubiquinol (Kaneka QH) doses were limited to a maximum of 300 mg/kg/day (lower doses tested included 75, 150, and 200 mg/kg/day) (Kitano et al. 2008). An additional group of female rats (n = 10) was administered CoQ10 at a dose of 600 mg/kg. None of the toxicological findings documented in the first 13 week study was observed in the second study, suggesting a no observed adverse effect level of 300 mg/kg/day was established for female rats.

In a 13 week oral gavage study, male and female dogs (n=3/sex/group) were administered either ubiquinol (Kaneka QH) at dose levels of 150, 300, and 600 mg/kg or CoQ10 at 600 mg/kg (Kitano et al. 2008). There were no deaths, changes in body weight, food consumption, ophthalmology, electrocardiogram, urinalysis, hematology, or blood chemistry in ubiquinol treated dogs. The only change noted was a 15 to 21% reduction in heart rate after treatment with 150, 300, and 600 mg/kg ubiquinol. Histopathological examination revealed no treatment-related effects in any of the examined organs in either males or females. Based on the absence of toxicity under the conditions of this study, a no observed adverse effect level for ubiquinol in male and female dogs was estimated to be greater than 600 mg/kg/day.

In summary, ubiquinol was well tolerated when administered in acute oral toxicity studies (rat) and in 13 week repeat dose toxicity studies (rat, dog). The rat model showed more toxicity than
the dog model, potentially related to the difference in the makeup and metabolism of the CoQ10 in the two species. Repeat dose toxicity studies in the rat showed dose-dependent adverse effects (up to 1200 mg/kg/day ubiquinol) in treated females and included increases in liver weight, accumulation of ubiquinol in the liver, increases in liver enzyme activity (approximately 2-fold increases in AST, ALT, and LDH), increased incidence of liver microgranulomas, vacuolation of Kupffer cells and hepatocytes, and accumulation of macrophages in the spleen and mesenteric lymph nodes. None of these findings was seen in a separate study conducted at doses up to 300 mg/kg ubiquinol. The 13 week dog study did not show any toxicological findings, except for a slight change in heart rate which was not accompanied by other changes in cardiac parameters (e.g., heart weight, histopathology evaluation of the cardiac tissue).

c. Genotoxicity

Ubiquinol (Kaneka QH) was tested in a standard panel of genotoxicity assays (bacterial reverse mutation, in vivo micronucleus, and chromosomal aberration assays) (Kitano et al. 2007). Ubiquinol did not induce reverse mutations in *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537 and *Escherichia coli* WP2uvrA at concentrations up to 5000 μg/plate, in the absence or presence of exogenous metabolic activation by rat liver S9. Ubiquinol did not induce chromosome aberrations in Chinese hamster lung fibroblast cells in short-term (6 hours) tests in the presence or absence of rat liver S9 at concentrations up to 5000 μg/ml or in a continuous (24 hours) treatment test at concentrations up to 1201 μg/ml. Ubiquinol was also found to be negative in the in vivo micronucleus test (no chromosomal damage) in rats (n=5/group) administered oral doses of up to 2000 mg/kg/day ubiquinol.

No evidence of genetic toxicity activity was seen for CoQ10 in a standard panel of genotoxicity assays (Kitano et al. 2006).

d. Developmental and reproductive toxicity

There are no reproductive toxicity studies available for ubiquinol. A review describing reproductive toxicity studies for CoQ10 in rats and mice did not show any adverse effects when dams were given doses up to 600 mg/kg/day during the organogenesis

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10 Genotoxicity assessment battery usually consists of a gene mutagenicity assay (for single dose trials). To support multiple dose administration in humans, additional genotoxicity testing assessment is usually conducted to detect chromosomal damage in mammalian systems.

11 The S9 fraction is a homogenate of organ tissues which contains cytochrome P450 isoforms (phase I metabolism) and other enzyme activities. The S9 fraction is used to mimic the presence of metabolic activity in in vitro assays such as the Ames Assay, where such activity may be required for a given drug to become mutagenic.

12 Developmental and reproductive toxicity studies are usually designed to assess the potential adverse effects in humans of both sexes and include females from various age groups that will be exposed to the proposed substance. Developmental toxicity or teratogenicity refers to adverse effects (can include embryo-fetal mortality, structural abnormalities, functional impairment, or alterations to growth) and can occur in pups either as a result of the exposure of their parents to the substance, prior to the pups’ birth, or by direct exposure of the pups to the substance after birth.
stage of development (reviewed in Hidaka et al. 2008). However, these findings cannot be taken into consideration in this review because the original published data were not available in the literature for an independent analysis (Notake et al. 1972).

e. Carcinogenicity

Long-term rodent carcinogenicity studies have not been conducted with ubiquinol. The data suggesting an absence of genotoxicity (described above) reduce the likelihood of carcinogenic risk via a genotoxic mechanism of action. However, since tumors can develop via nongenotoxic pathways, it is possible that exposure to ubiquinol over a life time, as would be needed for a therapeutic intervention targeting a chronic disease such as type 2 diabetes, can result in tumor formation. The liver toxicity findings seen in the 13 week rat study could progress to a preneoplastic or neoplastic response at clinically relevant doses of ubiquinol if chronic studies were to be conducted over a life time exposure to ubiquinol.

Overall, there are insufficient data to make a definitive conclusion regarding the carcinogenic risk of long-term exposure to ubiquinol.

f. Toxicokinetics

No toxicokinetic data were found for ubiquinol (see section II.B.2 above for more details).

Conclusions: Ubiquinol is an endogenously synthesized antioxidant which is essential for mitochondrial energy production. Orally ingested ubiquinol is readily absorbed in the gastrointestinal tract in humans. The T\text{max} appears to be approximately six hours. Plasma ubiquinol concentrations reach steady-state within two weeks of treatment and returned rapidly to baseline after dosing, suggesting no accumulation.

Subchronic dosing at levels producing exposures near those encountered by clinical subjects caused vacuolation in the liver and lymph node in female rats, slightly increased clotting times in male rats, and a slightly decreased heart rates in dogs. The significance of the findings in the rat studies is unknown due to the differences in the forms of CoQ10 and the metabolism between rodents and humans. Ubiquinol does not appear to present a genotoxic risk under the conditions of the conducted studies. The literature lacks data on reproductive and developmental effects, chronic toxicity, and carcinogenicity testing for ubiquinol. These data would be needed to provide a safety assessment of ubiquinol when used chronically for the nominated use of glycemic control in diabetics.

4. Human Safety

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\textsuperscript{13} Studies that assess cancer risk in animals are used as predictive tools to evaluate the potential for drugs to result in tumors when used by humans on a chronic basis. Carcinogenicity studies are conducted if the clinical use is expected to be continuous for a minimum of 6 months of life, or if intermittent clinical use is expected to total 6 months or more of life.
The following databases were consulted in the preparation of this section: Embase, PubMed, and ClinicalTrials.gov.

a. Reported adverse reactions

The Office of Surveillance and Epidemiology conducted a search of the FDA Adverse Events Reporting System (FAERS) database for reports of adverse events for ubiquinol and CoQ10 through February 8, 2017, and retrieved 39 serious cases in which ubiquinol is either possibly (n=29) or unlikely (n=10) associated with the reported adverse event. The most common type of reports for ubiquinol were potential drug-drug interactions in which concurrent use of ubiquinol led to a change in efficacy or toxicity of a co-suspect drug. However, the changes in drug effect and toxicity were inconsistent and the data are insufficient to truly confirm if a drug interaction exists. Increased hepatic enzymes were also reported in four cases; however, all four cases reported confounders (i.e., concomitant medications labeled for elevated hepatic enzymes). One case reported abnormal blood glucose in a patient with diabetes mellitus after initiating ubiquinol; however, the dose of ubiquinol, blood glucose values, and latency to the event were not reported.

The Center for Food Safety and Nutrition (CFSAN) collects reports of adverse events involving food, cosmetics, and dietary supplements in the CFSAN Adverse Event Reporting System (CAERS). A search of CAERS that was conducted for adverse events associated with ubiquinol on February 10, 2017, retrieved 112 cases. Most of these reports involved multiple ingredient supplement products. Eight reports named a single ingredient ubiquinol product. In five of these reports, assessments of causation were confounded by the reporter’s use of multiple other dietary supplements or the reporter’s failure to provide information about potential causality (e.g., relevant time sequence).

A 69 year old white female reported that about two days after beginning to take ubiquinol 100 mg daily, she experienced shortness of breath with any exertion, tightness in her chest as she experiences with a “chest cold,” and felt like falling asleep when she was inactive. She has a medical history of chronic obstructive pulmonary disease, hypertension and unspecified allergies. She used her albuterol inhaler to treat shortness of breath and discontinued the use of ubiquinol. No medical intervention was reported, but the patient reported that she “felt a lot better” at an unspecified time after discontinuing ubiquinol.

A 49 year old female reported taking 100 mg ubiquinol orally after breakfast with water for three consecutive days. After dosing on the third day, the reporter experienced pain on the lower right side near her rib cage and palpitations. Later that day she was “not able to breathe.” No medical intervention was reported, the product was discontinued, and symptoms resolved on the fifth day after beginning dosing. A history of allergy to multiple medications was reported.

According to 21 C.F.R. 314.80, a serious adverse drug experience includes any adverse drug experience occurring at any dose that results in any of the following outcomes: a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.
A female consumer of unspecified age reported having been diagnosed with multiple myeloma. She was discontinuing and returning a ubiquinol product that she had used for approximately three and a half years prior to the diagnosis, because she had been told that “antioxidants block the performance of chemotherapy drugs” and her doctor had prescribed other vitamins for her.

b. Clinical trials assessing safety

The single study of ubiquinol (Kaneka) in type 2 diabetes, discussed below, did not report safety information (Mezawa et al. 2012).

Twenty three additional clinical studies were identified in which ubiquinol use was investigated.


Few adverse events were observed in association with ubiquinol dosing in four studies of healthy subjects. Miles et al. (2002) gave single doses of 180 mg ubiquinol (Q-Nol) to nine healthy subjects. Langsjoen and Langsjoen (2013) studied doses of 200 mg ubiquinol (Kaneka QH) in 12 healthy subjects for four weeks. Kizaki et al. (2015) studied doses of 600 mg ubiquinol (Kaneka QH) in 18 healthy subjects for 22 days. Hosoe et al. (2007) conducted a study in fifteen healthy subjects, giving single oral doses of 150 (n=10) or 300 mg (n=5) of ubiquinol (Kaneka QH). No clinically significant changes in standard laboratory parameters were found. One subject had tachycardia on day 2, which was judged to be unrelated to the drug by the study director because symptoms were transient and occurred the day after treatment. A second subject had soft stool recorded as an adverse event, but this was also judged to be not clinically significant.

In addition, Hosoe et al. (2007) reported that during a four-week safety and bioavailability study in which healthy subjects received placebo or 90, 150, or 300 mg per day of ubiquinol (Kaneka QH) (n = 20 per dosing group), a total of 10, 19, 12, and 13 adverse events were reported in each dosing group, respectively. Events in both active and placebo treatment groups were predominantly associated with the gastrointestinal system, were not dose-related, and were described as slight with one exception: a subject who had received ubiquinol 300 mg dropped out of the study on day 1 with enterocolitis. The investigators considered the event to be a serious event but unrelated to treatment because the patient had leukocytosis at screening, suggesting a pre-existing condition. Safety laboratory tests were performed at screening, on treatment at weeks 2 and 4, and 2 weeks post-treatment. There were two slight laboratory abnormalities detected upon treatment: one subject in the ubiquinol 90 mg arm developed eosinophilia and one subject in the ubiquinol 150 mg arm had an increase in LDL cholesterol. Causes other than ubiquinol therapy could not be ruled out. Although the study was not designed to evaluate glycemic control, glucose, hemoglobin A1c (HbA1c), and insulin were in the panel of laboratory safety tests; there were no clinically significant changes in these laboratory tests in healthy subjects.
Four studies in patients with various disease states reported that no safety issues occurred. Miles et al. (2007) studied doses of 10 mg/kg ubiquinol (LiQ-NOL) in 14 individuals with Down syndrome for three months. There were no significant adverse effects reported and no significant changes in blood counts or chemistry profiles. Langsjoen and Langsjoen (2008) reported on seven individuals with congestive heart failure who received ubiquinol (Kaneka QH) therapy (450 mg/day) for 3 to 20 months. No adverse events were observed. Yoritaka et al. (2015) studied 64 patients with Parkinson disease taking 300 mg of ubiquinol (Kaneka) for up to 96 weeks. It was reported that the substance was well tolerated and no patients discontinued the study due to adverse effects. Mitsui et al. (2017) reported on a 3 year follow-up of ubiquinol (Kaneka QH) dosing at 1200 mg/day in a 60 year old male patient with familial multiple system atrophy. Doses at this high level were reported not to have caused any adverse events.

Patients experienced adverse events in several studies. Evans et al. (2009) treated ten patients age 60 or older with a single dose of 106.25 mg ubiquinol (CoQH-CF) and followed each for 72 hours after dosing. Two adverse events were reported: nosebleed and nausea/cramping. Gvozdjakova et al. (2014) studied 24 children with autism between the ages of 3 and 6 years, giving doses of 100 mg ubiquinol (Li-QH) per day for up to two months. Six children discontinued treatment during that time due to: asthma (1), broken leg (1), increased activity (1), aggression (2), and sleep disorder (1). Donnino et al. (2015) assessed 19 patients with severe sepsis or septic shock who received 200 mg enteral ubiquinol (Kaneka) for up to seven days. Four deaths occurred in the ubiquinol treatment group; each was ruled unrelated to ubiquinol by an independent safety review board. One patient was reported to have had a gastrointestinal disturbance possibly related to ubiquinol. Fukuda et al. (2016) assessed a total of 21 patients with chronic fatigue syndrome taking doses of 150 mg ubiquinol (Kaneka) daily after a meal for up to 12 weeks. One patient on ubiquinol dropped out of the study due to deconditioning.15

Clinical safety data regarding the adverse events associated with ubiquinol are derived from its use in diverse health conditions. The available information does not suggest substantive safety concerns. However, little safety information is available about the use of ubiquinol in diabetes.

c. The availability of alternative approved therapies that may be as safe or safer

A summary of FDA approved products for the treatment of diabetes is captured in Table 3 (see below).

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Approved Drug Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulins and insulin analogs</td>
<td>Many different products and formulations</td>
</tr>
<tr>
<td>Sulfonylureas (SU)</td>
<td>Acetohexamide, chlorpropamide, tobutamide, glipizide, gliclazide, glyburide,</td>
</tr>
</tbody>
</table>

15 Deconditioning can be defined as a general weakening and includes multiple, potentially reversible changes in body systems brought about by physical inactivity and disuse. Such changes often have significant functional and clinical consequences in older people.
<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Approved Drug Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biguanides</td>
<td>glimepiride</td>
</tr>
<tr>
<td>Thiazolidinediones (TZDs)</td>
<td>Metformin, Rosiglitazone, pioglitazone</td>
</tr>
<tr>
<td>Analogues of glucagon-like peptide 1 (GLP-1)</td>
<td>Exenatide, lixisenatide, liraglutide,</td>
</tr>
<tr>
<td></td>
<td>albiglutide, dulaglutide</td>
</tr>
<tr>
<td>Dipeptidyl peptidase 4 (DPP-4) inhibitors</td>
<td>Sitagliptin, saxagliptin, linagliptin, alogliptin</td>
</tr>
<tr>
<td>SGLT2 inhibitors</td>
<td>Empagliflozin, dapagliflozin, canagliflozin</td>
</tr>
<tr>
<td>Alpha-glucosidase inhibitors</td>
<td>Acarbose, miglitol, voglibose</td>
</tr>
<tr>
<td>Meglitinides</td>
<td>Repaglinide; nateglinide</td>
</tr>
<tr>
<td>Synthetic analogues of human amylin</td>
<td>Pramlintide</td>
</tr>
<tr>
<td>Bile acid sequestrants</td>
<td>Colesevelam</td>
</tr>
<tr>
<td>Dopamine agonists</td>
<td>Bromocriptine</td>
</tr>
</tbody>
</table>

**Conclusions:** Clinical safety data regarding the adverse events associated with ubiquinol are derived from its use in diverse health conditions. The available information does not suggest substantive safety concerns. However, little safety information is available about the use of ubiquinol in diabetes.

**C. Are there concerns about whether a substance is effective for a particular use?**

1. *Reports of trials, clinical evidence, and anecdotal reports of effectiveness, or lack of effectiveness, of the bulk drug substance*

The following database(s) were consulted in the preparation of this section: Embase, PubMed, and ClinicalTrials.gov. One trial of ubiquinol in the treatment of diabetes is listed on ClinicalTrials.gov. The study completion date is listed as March 1, 2017; there are no results posted to date.

In a review article discussing the efficacy of CoQ10 supplementation by Shen and Pierce (2015), a study evaluating the efficacy of ubiquinol in treating diabetes was identified (Mezawa et al. 2012). Mezawa et al. (2012) conducted an open-labeled uncontrolled pilot study evaluating nine type 2 diabetic patients taking 200 mg of ubiquinol daily for 12 weeks. Seven of the nine patients received therapy with one or more antidiabetic drugs during the trial. Mean hemoglobin A1c (HbA1c) was reduced in the treatment group (8.7 to 8.2 mmol/L). However, there was no diabetic patient control group and only intra-group statistical comparisons were made. Assessment of the potential effects of ubiquinol is confounded by the potential effects of other aspects of trial participation (e.g., regular blood glucose monitoring and interaction with concomitant antidiabetic medications).

Seven clinical studies of CoQ10 in Type 2 diabetics were identified. Of these, five studies failed to show an improvement in glycemic control compared to a placebo treated group of diabetic patients, as measured by HbA1c and/or fasting blood glucose (Ériksson et al. 1999; Playford et al. 2003; Lim et al. 2008; Akbari Fakhrabadi et al. 2014; Moazen et al. 2015).
Two studies described below were conducted in patients with Type 2 diabetes reported improvements in HbA1c and/or fasting plasma glucose.

Mohammed-Jawad et al. (2014) conducted a single-blinded placebo-controlled trial evaluating CoQ10 as add-on therapy in type 2 diabetic patients already taking oral antidiabetic medications. Nineteen patients received CoQ10 (75 mg twice daily) or placebo for 8 weeks. In these patients, there was a 20% decrease in mean fasting blood glucose and a lowering in HbA1c from 8.4 to 7.3. The mean decreases in both of these parameters were statistically significantly different from changes in the placebo group.

In a randomized, double-blind, placebo-controlled trial, patients with type 2 diabetes were randomly assigned to receive 200 mg per day of CoQ10 or placebo for 12 weeks (Kolahdouz Mohammadi et al. 2013). The study report does not specify whether patients were taking antidiabetic medications. A decrease in HbA1c was observed in the CoQ10 group; however, only intra-group statistical analyses were conducted.

Two clinical studies of CoQ10 administration in Type 1 diabetics were identified that had been conducted in patients with type 1 diabetes (Andersen et al. 1997; Henriksen et al. 1999). Neither showed an improvement in glycemic control as measured by HbA1c and/or fasting blood glucose.

2. **Whether the product compounded with this bulk drug substance is intended to be used in a serious or life-threatening disease**

Ubiquinol was nominated for adjunctive therapy in glycemic control. Diabetes mellitus is a serious disease affecting more than 29 million Americans. Diabetes complications are life-threatening and include heart disease, stroke, kidney disease, blindness and other eye problems, and peripheral vascular disease.

3. **Whether there are any alternative approved therapies that may be as effective or more effective.**

A summary of FDA approved products for the treatment of diabetes can be found in Section II. 4.C above.

**Conclusions:** Overall, the single study of ubiquinol and trials of CoQ10 in diabetes are insufficient to draw any conclusions regarding the effect of ubiquinol on glycemic control as either a single treatment or an adjunct treatment.

The single published ubiquinol study cannot be relied upon to assess the potential benefit of ubiquinol in diabetic patients as the study was not well controlled and was confounded by several other factors.

Each of the CoQ10 studies had a small number of patients, was of short duration, and did not provide adequate assessment, analyses or results on which to base a conclusion that CoQ10
provides a beneficial effect in type 1 or type 2 diabetes. Further, it is unclear whether the effects of CoQ10 supplementation could be generalized to ubiquinol supplementation.

There are FDA-approved drug products available that are indicated for the treatment of diabetes, which is a serious condition.

**D. Has the substance been used historically in compounding?**

The following databases were consulted in preparation of this section: PubMed, Natural Medicines, European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, USP/NF, and Google.

1. **Length of time the substance has been used in pharmacy compounding**

Insufficient information is available to determine how long ubiquinol has been used in pharmacy compounding.

2. **The medical condition(s) it has been used to treat**

Results from a Google search using the terms *ubiquinol compounding pharmacy* yielded multiple dietary supplements; we found no information on the treatment of medical conditions with compounded ubiquinol products.

3. **How widespread its use has been**

McPherson et al. (2016) conducted a retrospective claims analysis from January 1, 2012, through December 31, 2013, of commercially insured patients in the United States and found that ubiquinol was used as an ingredient in compounded medications. Insufficient data are available from which to draw conclusions about the extent of use of ubiquinol in compounded drug products.

4. **Recognition of the substance in other countries or foreign pharmacopeias**


On October 14, 2016, ubiquinol was granted orphan designation by the European Commission to Centro de Investigación Biomédica en Red (CIBER), Spain, for the treatment of primary coenzyme Q10 deficiency syndrome (European Medicines Agency, 2016). We note that an orphan designation is not a marketing authorization; rather, a demonstration of quality, safety and efficacy is necessary before a product can be granted a marketing authorization.

**Conclusions:** Information is insufficient to determine the historical use of ubiquinol in compounded drug products. Based on a single retrospective claims study, it appears that ubiquinol has been used as an ingredient in compounded medications.
III. RECOMMENDATION

We have balanced the criteria described in section II above to evaluate ubiquinol for the 503A Bulks List. After considering the information currently available, a balancing of the criteria weighs against ubiquinol being placed on that list based on the following:

1. Ubiquinol is a well characterized small molecule. The synthesis of this compound has been well developed. Ubiquinol itself is sensitive to oxidants like oxygen, and is unlikely to be stable when stored under ambient conditions, in the absence of special formulations. However, with reasonable formulation techniques, such as addition of antioxidants (e.g. propylene glycol fatty acid esters), enhancement in stability of ubiquinol can be achieved.

2. We have reviewed available data on the safety of ubiquinol 30% powder as an adjunctive therapy in glycemic control. We found one clinical study of ubiquinol in type 2 diabetic patients. Substantive safety concerns have not been identified; however, minimal clinical data are available regarding use of ubiquinol in diabetes. Nonclinical data found in the literature are inadequate to characterize the potential toxicity profile of ubiquinol, particularly for use in a chronic disease such as diabetes.

3. The single study of ubiquinol and trials of CoQ10 in diabetes are insufficient to conclude that ubiquinol improves glycemic control as a single treatment or as an adjunct treatment. Further, it is unclear whether the effects of CoQ10 supplementation can be generalized to ubiquinol supplementation.

4. There is insufficient information to determine the historical use of ubiquinol in pharmacy compounding.

Based on this information the Agency has considered, a balancing of the four evaluation criteria weighs against ubiquinol being added to the 503A Bulks List.
REFERENCES


Dadali Y, Dadali V, Makarov V. Rapid catalytic method for obtaining the reduced form of coenzyme Q10 for use in pharmaceutical and food compositions, RU 2013125386 (A), Dec. 10, 2014


Failla ML, Chitchumroonchokchai C and Aoki F. 2014. Increased bioavailability of ubiquinol compared to that of ubiquinone is due to more efficient micellarization during digestion and greater GSH-dependent uptake and basolateral secretion by Caco-2 cells. J Agric Food Chem 62:7174-7182.


Mohammed-Jawad NK, Sabbagh MA- and AL-Jezaeri KA. 2014. Role of L-carnitine and Coenzyme Q10 as Adjuvant Therapy in Patients with Type 2 Diabetes Mellitus. American Journal of Pharmacological Sciences 2:82-86.

Mohr D, Bowry VW and Stocker R. 1992. Dietary supplementation with coenzyme Q10 results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low-density lipoprotein to the initiation of lipid peroxidation. Biochim Biophys Acta 1126:247-254.


Ueda, T., Ono, T., Moro, M., Kitamura, S., and Ueda, Y., Method of stabilizing reduced coenzyme q10. US 20050008630 A1, Jan 13, 2005.


Appendix 1. Pharmacokinetic data from studies using the rat model

The pharmacokinetics/toxicokinetic profile of ubiquinol (Kaneka QH) was examined in rats where animals (n=10/sex/group) were orally gavaged with ubiquinol at 300, 600, or 1200 mg/kg for 13 weeks. Blood samples were collected after the first dose and after 13 weeks of daily dosing of ubiquinol over a period of 24 hours. Similar to the data obtained in dogs, Cmax was reached within 4-8 hours in all sampling points. The Cmax was 1-2, 2-3 and 3-4 μg/ml following the first dose of ubiquinol at levels of 300, 600 and 1200 mg/kg, respectively. After 13 weeks of dosing, Cmax was 2-3, 4-5 and 5-6 μg/ml in response to dosing with ubiquinol at levels of 300, 600 and 1200 mg/kg, respectively. Increases in AUC0–24h levels of ubiquinone were dose-dependent, especially when measured after dosing for 13 weeks under the conditions of the study. A gender difference was noted in plasma kinetics profile where Cmax and AUC0–24h were greater in treated females compared to male rats. Dosing with oral ubiquinol (1200 mg/kg) resulted in a greater ubiquinol AUC0–24h levels compared to dosing with CoQ10 administration (1200 mg/kg) after a single dose exposure. Similarly after a 13-week treatment period with CoQ10, the AUC0–24h levels of ubiquinol were either similar (in females) or greater (in males) than levels of CoQ10 (Kitano et al., 2008) (See Table below).

Table A. Liver CoQ10 levels in rats following oral dosing with ubiquinol

<table>
<thead>
<tr>
<th>Author</th>
<th>Gender</th>
<th>Doses (mg/kg)</th>
<th>Duration</th>
<th>Concentrations (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prangthip et al 2016</td>
<td>Male</td>
<td>5</td>
<td>4 weeks</td>
<td>170.4 ± 4.3*</td>
</tr>
<tr>
<td>Kitano et al 2008</td>
<td>Female</td>
<td>300</td>
<td>13 weeks</td>
<td>8.5 ± 5.2 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td></td>
<td>12.8 ± 1.4 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1200</td>
<td></td>
<td>16.1 ± 2.2 **</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>300</td>
<td></td>
<td>1.4 ± 0.3 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td></td>
<td>3.3 ± 1.3 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1200</td>
<td></td>
<td>3.4 ± 1.3 **</td>
</tr>
</tbody>
</table>

*total CoQ10 in mg/g of protein; ** CoQ10 mg/g of liver wet weight

Liver CoQ10 concentrations were measured in female rats after dosing with ubiquinol. Administration of 300, 600, and 1200 mg/kg of ubiquinol resulted in CoQ10 liver concentrations of 8.5, 13, and 16 mg/g, respectively and were 4 to 6 fold higher than those in males. Uptake of CoQ10 by the liver decreased to baseline levels around 3 weeks after termination of supplementation of CoQ10 in rats (Zhang et al. 1996). Streptozotocin-induced diabetic rats (n=6/group) were dosed with ubiquinol in drinking water (5mg/kg body weight) for 4 weeks. Concentrations of total CoQ10 levels increased in the serum by about 0.26 μg/ml compared to control nondiabetic rats. Similar results were seen in animals receiving CoQ10 for 4 weeks compared to their control counterparts (Prangthip et al. 2016).

The bioavailability of ubiquinol in the central nervous system in rats was investigated. An increase in the concentrations of coenzyme Q10 and coenzyme Q9 was observed in various brain regions over a 120 minute observational period after intracerebroventricular administration of liposomally-enclosed ubiquinol (40 μl of 10% ubiquinol) to rats (Gvozdjakova et al. 2012).

In another study using diabetic rats, oral administration of ubiquinol for 4 weeks resulted in an increase in total CoQ10 levels in the liver by162 μg/g and in the pancreas by 3.6 μg/g, compared
to control nondiabetic rats. Similar results were seen after a four week treatment period in animals treated with CoQ10 (Prangthip et al. 2016).

In summary, ubiquinol showed dose dependent increases in systemic levels (AUC$_{0-24h}$) when measured after dosing for 13 weeks in rats. A gender difference was noted in plasma kinetics profile where C$_{max}$ and AUC$_{0-24h}$ were greater in treated females compared to male rats. Treatment for a 13-week period with CoQ10 resulted in ubiquinol AUC$_{0-24h}$ levels that were either similar (in females) or greater (in males) than levels of CoQ10. Ubiquinol showed accumulation in the liver of rats treated with ubiquinol in either normal or diabetic-induced rat models. Uptake of CoQ10 by the liver decreased to baseline levels around 3 weeks after termination of supplementation of CoQ10 in a nondiabetic rat model.
Tab 5

Vanadyl Sulfate
Tab 5a

Vanadyl Sulfate Nomination
Nominated by: McGuff Compound Pharmacy Services, Inc., Integrative Medical Consortium, Alliance for Natural Health USA, and American Association of Naturopathic Physicians

<table>
<thead>
<tr>
<th>Column A—What information is requested?</th>
<th>Column B—Put data specific to the nominated substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the name of the nominated ingredient?</td>
<td>Vanadyl sulfate, hydrate</td>
</tr>
<tr>
<td>Is the ingredient an active ingredient that meets the definition of “bulk drug substance” in § 207.3(a)(4)?</td>
<td>Yes. <a href="https://doi.org/10.1016/B978-0-12-800270-4.00002-X">Adv Food Nutr Res. 2014;71:55-100. doi: 10.1016/B978-0-12-800270-4.00002-X.</a> Micronutrient status in type 2 diabetes: a review. The less well-known micronutrients notably zinc, magnesium, chromium, copper, manganese, iron, selenium, <a href="https://doi.org/10.1016/B978-0-12-800270-4.00002-X">vanadium</a>, B-group vitamins, and certain antioxidants are assessed. Kaur B1, Henry J2. Or please see section “safety and efficacy data” below.</td>
</tr>
<tr>
<td>Is the ingredient listed in any of the three sections of the Orange Book?</td>
<td>No for Vanadium.</td>
</tr>
<tr>
<td>Were any monographs for the ingredient found in the USP or NF monographs?</td>
<td>There is a Dietary Supplements: Minerals Tablets monograph available for a combination product including Vanadium in USP37-NF32, Page 5503 Pharmacopeial Forum: Volume No. 38(5).</td>
</tr>
<tr>
<td>What is the chemical name of the substance?</td>
<td>Vanadyl sulfate, hydrate</td>
</tr>
<tr>
<td>What is the common name of the substance?</td>
<td>Vanadium</td>
</tr>
<tr>
<td>Does the substance have a UNII Code?</td>
<td>no UNII code found for Spectrum’s vanadyl sulfate hydrate</td>
</tr>
<tr>
<td>What is the chemical grade of the substance?</td>
<td>not graded</td>
</tr>
<tr>
<td>What is the strength, quality, stability, and purity of the ingredient?</td>
<td>Vanadyl sulfate, hydrate is not graded. A valid Certificate of analysis accompanies every lot of raw material received.</td>
</tr>
<tr>
<td>How is the ingredient supplied?</td>
<td>U.S. TSCA: Sulfate, hydrate (CAS no. 123334-20-3) is exempt from TSCA 8(b) Inventory listing since it is a hydrate. However, Vanadyl Sulfate (CAS no. 27774-13-6) is listed on the TSCA 8(b) Inventory. New Jersey (EHS) List: Present. Listed as Vanadium compounds New Jersey - Discharge Prevention - List of Hazardous Substances Present. Listed as Vanadium compounds Canada WHMIS hazard class: D1A Very toxic materials; D2B Toxic materials</td>
</tr>
<tr>
<td>Is the substance recognized in foreign pharmacopeias or registered in other countries?</td>
<td></td>
</tr>
<tr>
<td>Has information been submitted about the substance to the USP for consideration of monograph development?</td>
<td>There are Dietary monographs available for Mineral Tablets/Mineral Capsules containing Vanadium in the USP.</td>
</tr>
<tr>
<td>What dosage form(s) will be compounded using the bulk drug substance?</td>
<td>Injection</td>
</tr>
<tr>
<td>What strength(s) will be compounded from the nominated substance?</td>
<td>Compounded Vanadium products can be formulated in strengths of Vanadium ranging from 100 mcg/mL (3 mg/30 mL) to 100 mcg/mL (25 mg/250 mL)</td>
</tr>
</tbody>
</table>
What are the anticipated route(s) of administration of the compounded drug product(s)?

Slow intravenous

Are there safety and efficacy data on compounded drugs using the nominated substance?


Has the bulk drug substance been used previously to compound drug product(s)?

Yes. Compounded Vanadium products have been formulated in strengths of Vanadium ranging from 100 mcg/mL (3 mg/30 mL) to 100 mcg/mL (25 mg/250 mL).

What is the proposed use for the drug product(s) to be compounded with the nominated substance?

Orally, vanadium is used for diabetes, hypoglycemia, hyperlipidemia, heart disease, edema, improving a hletic performance in weight training, and preventing malignant cell development. Vanadium is also used for treating tuberculosis, diabetes, syphilis, and a form of microcytic anemia (chlorosis). Vanadium is also used as a source of trace mineral supplementation in vanadium-deficient cases.

What is the reason for use of a compounded drug product rather than an FDA-approved product?

There are no FDA-approved injectable drug products containing vanadium as an active ingredient in a combination or single product.
Is there any other relevant information?

Some examples of USP-verified products containing vanadium are:
- Advanced Formula Multi-Vitamins and Minerals for Seniors with Lycopene by Berkley & Jensen...
- Central Vite Select by Equaline...
- Central-Vitamin by YourLife...
- Daily Multi Vitamins & Minerals by Kirkland Signature...
- Equaline Central Vite by Equaline...
- Equaline One Daily Multi Vitamin by Equaline...
- Essen ial 50+ by Nature Made...
Tab 5b

FDA Review of Vanadyl Sulfate
DATE: April 3, 2017

FROM: Ben Zhang, PhD
ORISE Fellow, Office of New Drug Products, Office of Pharmaceutical Quality (OPQ)

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Charles Ganley, MD
Director, ODE4, OND

Frances Gail Bormel, RPh, JD
Director, Division of Prescription Drugs, OUDLC

TO: Pharmacy Compounding Advisory Committee

SUBJECT: Review of Vanadyl Sulfate for Inclusion on the 503A Bulk Drug Substances List
I. INTRODUCTION

Vanadyl sulfate\(^1\) has been nominated for inclusion on the list of bulk drug substances for use in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act). It has been nominated for use in the treatment of diabetes, hypoglycemia, hyperlipidemia, heart disease, edema, tuberculosis, syphilis, and microcytic anemia (chlorosis), and to improve athletic performance in weight training, and to prevent cancer, and for “supplementation in vanadium-deficient cases.” The nominated route of administration is injection (“slow intravenous”). This review focuses on only vanadyl sulfate’s use to treat diabetes, hyperlipidemia, and heart disease, and to prevent cancer because adequate support was not provided for the other nominated uses.

We have reviewed available data on the physicochemical characteristics, safety, effectiveness, and historical use in compounding of this substance. For the reasons discussed below, we believe the evaluation criteria \textit{weigh against} placing vanadyl sulfate on the list of bulk drug substances that can be used to compound drug products in accordance with section 503A of the FD&C Act (503A Bulks List).

II. EVALUATION CRITERIA

A. Is the substance well-characterized, physically and chemically, such that it is appropriate for use in compounding?

\begin{center}
\begin{tikzpicture}
\node (v) at (0,0) {$\text{O}_5\text{V}^{2+}\text{O}_3\text{S}\text{O}_4$};
\end{tikzpicture}
\end{center}

\textit{Vanadyl sulfate (VOSO}_4\textit{) is an inorganic salt of vanadium. This compound is currently marketed as a dietary supplement as capsules (5 mg) and tablets (1 mg, 2 mg, 5 mg, 10 mg, and 20 mg).}

The following databases were consulted in preparation of this section: PubMed, SciFinder, Analytical Profiles of Drug Substances, European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, and USP/NF.

1. Stability of the API and likely dosage forms

No issues concerning the stability of vanadyl sulfate have been reported in the literature. The vanadyl sulfate crystal is very hygroscopic, but when compounded into the injection solution, it is likely to be stable in a wide pH range under ordinary storage conditions.

\footnotesize\textit{1 In the nomination, the name of the nominated substance was listed as “vanadyl sulfate, hydrate.” We are referring to the substance as “vanadyl sulfate” because the nominated dosage form of the compounded bulk drug substance is injection; thus, “vanadyl sulfate” and “vanadyl sulfate, hydrate” result in the same entity when in solution.}
2. Probable routes of API synthesis

Vanadyl sulfate can be obtained from the reduction of vanadium pentoxide (shown below). Vanadium pentoxide is usually dissolved in sulfuric acid aqueous solution in the presence of sulfur dioxide as the reducing agent under vigorous agitation (Lewis 2001; Dormehl et al. 2006).

\[ V_2O_5 + SO_2 + H_2SO_4 + 7 H_2O \rightarrow 2 [V(O)(H_2O)_4]SO_4 \]

3. Likely impurities

Likely impurities may include:
- Residual starting materials
- Byproducts from the over reduction of \( V_2O_5 \), such as \( V_2SO_4 \). Excess amount of \( SO_2 \) will result in the formation of \( V_2SO_4 \).

4. Toxicity of those likely impurities

Impurities are unlikely to be significantly toxic. Further characterization of the toxicity of the impurities is not necessary.

5. Physicochemical characteristics pertinent to product performance, such as particle size and polymorphism

Vanadyl sulfate is a blue crystalline solid which is highly soluble in water. Vanadium containing compounds are able to bind to various ligands, such as citrate, lactate and amino acid residues on proteins. The formation of such complexes will potentially decrease its bioavailability (Reul et al. 2001; Scior et al. 2016). No further information on the influence of particle size and polymorphism on bioavailability was found in the literature.

6. Any other information about the substance that may be relevant, such as whether the API is poorly characterized or difficult to characterize

Vanadyl sulfate can be characterized with atomic absorption/emission spectroscopy and X-ray crystallography.

Conclusions: Vanadyl sulfate is an inorganic vanadium salt and it is very likely to be stable under ordinary storage conditions as an injection solution. The nominated compound is easily characterized and the preparation of this compound has been well developed.
B. Are there concerns about the safety of the substance for use in compounding?

The following databases were consulted in preparation of this section: Embase, PubMed, TOXNET, and Web of Science. This review focuses on the nonclinical studies conducted with the nominated substance, vanadyl sulfate.²

Toxicology studies conducted with other forms of vanadium containing compounds can be found in Appendix 1 of this review.

1. Pharmacology of the drug substance

Vanadium (V) is a naturally occurring element, constituting 0.015% of the earth’s crust (Scior et al. 2016). It is one of 38 elements called transition metals. It exists in oxidation states ranging from -1 to +5, with the most common valence states of +3, +4, and +5. The nominated substance, vanadyl sulfate (VS, VOSO₄), contains the most stable oxidation state (i.e., +4), the tetravalent form (VO²⁺, vanadyl). The tetravalent form is the most common intracellular form available, whereas the pentavalent form³ (VO₃⁻) predominates in extracellular body fluids (Barceloux 1999). Both tetravalent and pentavalent forms can be found in drinking water and various dietary supplements. Foods rich in vanadium include black pepper, mushrooms, shellfish, parsley, and dill seed. The average daily oral dietary intake of vanadium in adults is estimated to range between 10 to 60 μg, where vanadyl sulfate is present at <1 ng/g (Barceloux 1999; NRC 2005).

The Institute of Medicine (IOM) concludes that a biological or functional role for vanadium in humans has not been identified; thus, no estimated average requirement, recommended dietary allowance or adequate intake level has been established for vanadium (IOM 2001). The IOM has established the dietary reference intake which is considered by FDA’s Center for Food Safety and Nutrition (CFSAN) in setting the daily values in the Nutrition Facts label⁴ (National Academy of Sciences 2001). The tolerable upper intake level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of an adverse health outcome. The tolerable UL of vanadium established by the IOM is 1.8 mg/day of elemental vanadium for adults age 19 years and older. Due to the lack of clinical information, no UL has been established by IOM for infants or children age 18 years and younger, or for pregnant or lactating women. The Environmental Protection Agency (EPA) has set a reference dose for chronic oral exposure to vanadium pentoxide in drinking water at 0.009 mg/kg/day (equivalent to 0.62 mg/day for a 70 kg person). The EPA reference dose is reported with “low confidence” due to limited available data.⁵

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² Vanadyl sulfate (VO²⁺, vanadyl) is also referred to in various studies as VS or VOSO₄.

³ This evaluation is specific to vanadyl sulfate. Wherever it was found to be relevant to our evaluation of vanadyl sulfate, we have considered data from other vanadium valences. However, other vanadium substances would need to be evaluated separately for inclusion on the bulk list.


⁵ https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=125
Nonclinical studies have evaluated the mechanism of action of vanadium containing compounds in animal models to assess the potential therapeutic value of vanadium in the treatment of various diseases such as diabetes, cancer, and heart disease.

**Glucose and Lipid Lowering Effect / Diabetes**

Vanadium containing compounds have been studied extensively in vitro and in vivo for potential glucose lowering effect (Gruzewska et al. 2014). The mechanism of action of vanadium containing compounds likely involves multiple pathways including the following:

- Non-competitive inhibition of Na⁺/K⁺-ATPases that may increase the oxidation and transport of glucose in the cell
- Competitive inhibition of glucose-6-phosphatase, an enzyme associated with the homeostatic regulation of blood glucose levels and insulin resistance
- Interaction with phosphate-dependent enzymes; vanadium in certain forms (vanadate anion) can mimic phosphate and inhibit the activity of certain enzymes (e.g., protein tyrosine phosphatases)
- Increase in the uptake of glucose into the cell through the GLUT4 transporter that may be independent of insulin
- Increase in insulin sensitivity in skeletal muscle, liver, kidneys, and adipose tissue
- Increase in glycogen synthesis and gluconeogenesis; Just as insulin increases lipogenesis and inhibits lipolysis, vanadium containing compounds have insulin mimetic actions resulting in similar effects.

**Cardioprotective Effect / Heart Disease**

Various vanadium containing compounds have shown the potential for cardioprotective benefits (Gruzewska et al. 2014). Vanadium’s therapeutic effect may be mediated by a number of mechanisms which include:

- Increased activity of nitric oxide synthetase in heart vessels
- Increased activity of tyrosine kinase which leads to a decrease in mitochondrial apoptosis and decrease in the extent of myocardial infarction
- Increased activity of tyrosine phosphorylation
- Decreased activity of tyrosine phosphatase which may lead to protection against ischemia.

Exaggerated pharmacology can occur from exposure to vanadium containing compounds through their inhibitory action on Na⁺/K⁺-ATPases, which can lead to an increase in intracellular calcium and vasoconstriction. This latter effect, rather than being cardioprotective, may lead to high blood pressure which cascades into adverse cardiac effects.

Certain forms of heart disease are treated with anticoagulant therapy. Sodium orthovanadate was shown in vitro to prolong the kaolin-induced clotting time of normal human plasma in a dose-
dependent manner via inhibition of clotting factor Xa and thrombin (Funakoshi et al. 1992). Orthovanadate seems to suppress platelet aggregation induced by platelet aggregating factor in vitro (Suenaga and Ueki 2004). No in vivo investigations of the anticoagulant effects of vanadium containing compounds were identified in the literature.

**Antitumor Effect / Cancer Prevention**

The potential for cancer prevention and antitumor activity of vanadium containing compounds have been extensively studied over the last three decades using several in vitro and in vivo animal models. The anticancer properties of vanadium containing compounds are hypothesized to involve several molecular pathways including reduction of cell growth (antiproliferation), activation of cell death by apoptosis or necrosis, and reduction of invasive/metastatic potential of tumor cells (Evangelou 2002).

A variety of organometallic vanadium containing compounds and complexes have been reported to exhibit antitumor activity in a number of in vivo cancer models, including vanadocene dichloride (Murthy et al. 1988), orthovanadate (Cruz et al. 1995), bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium (IV) (metvan) (Narla et al. 2001; D'Cruz and Uckun 2002), and bisperoxovanadium[4,7-dimethyl-1,10-phenanthroline-bisperoxo-oxo-vanadium] (bpV(Me2Phen)) (Scrivens et al. 2003). The rate of tumor incidence was used as an endpoint to identify the chemotherapeutic effects of vanadium containing compounds in chemically induced carcinogenicity murine models (Evangelou et al. 1997), allograft establishment from murine cancer cell lines (Cruz et al. 1995), or xenograft implantation from human cancer cell lines or primary tumors (D'Cruz and Uckun 2002).

Using animal models of chemical-induced carcinogenesis, supplementation of vanadium in drinking water, before or after administration of a given carcinogen, has been shown to protect against various phases of carcinogenesis (e.g., initiation, promotion, and progression) (Bishayee et al. 2010). In a seminal chemoprevention study, where methylnitrosourea was administered to rats to induce mammary carcinogenesis, vanadyl sulfate supplementation in the diet resulted in a decrease in the cancer incidence and in the average number of tumors detected per rat, as well as an increase in the median cancer-free time (Thompson et al. 1984). In subsequent studies, ad libitum supplementation of ammonium metavanadate or ammonium monovanadate in drinking water was associated with chemopreventive effects in multiple chemical-induced rat models of breast (Bishayee et al. 2000), colorectal (Kanna et al. 2003) and liver (Chakraborty and Selvaraj 2000) carcinogenicity models.

However, there are published reports where vanadium supplementation did not result in antiproliferative effects. In one study using 1,2-dimethylhydrazine as a chemical to induce colon carcinogenesis in a mouse model, supplementation of ammonium vanadate in drinking water did not ameliorate the incidence or histological type of tumors detected in 1,2-dimethylhydrazine treated mice (Kingsnorth et al. 1986).
2. Pharmacokinetic and toxicokinetic data

Absorption of vanadium salts from the gastrointestinal tract is generally low (less than 5%) (NRC 2005; NTP 2008). Out of the various vanadate salts, vanadyl sulfate is orally absorbed with an absolute bioavailability of about 16% (Azay et al. 2001). When taken with food, vanadyl sulfate or vanadate is absorbed from the gastrointestinal tract and transported in blood by albumin or transferrin to various organs and tissues, including the liver, spleen, kidneys, bones and testes (Zwolak 2014). Soluble vanadium compounds can be absorbed in the lungs as was demonstrated in both animals (inhalation carcinogenicity studies; see Appendix 1) and humans (metal workers exposed to dust containing vanadium), resulting in toxic outcomes (Barceloux 1999).

a. Animal data

In a single dose study using intravenous bolus injection, vanadyl sulfate pentahydrate (representing 3.025 mg elemental vanadium/kg body weight) was administered to male Wistar rats (n=6-7/group) (Azay et al. 2001). Vanadium levels decreased over time according to the three compartment linear model representing concentrations in highly perfused tissues, fast turnover pool and slow turnover pool. Mean half-lives were calculated as 1, 25, or 200 h, respectively, for the three phases observed. Exposure to a single intravenous dose of vanadyl sulfate pentahydrate resulted in plasma concentration (AUC\textsubscript{mod}) of 23.1 ± 6.04 mg·h/L, a large apparent volume of distribution of the central compartment (V\textsubscript{1}=2.43 ± 1.22 L/kg) and a volume of distribution at steady state (V\textsubscript{ss}=25.40 ± 3.90 L/kg), indicating a high uptake and retention of vanadium in tissues. Vanadyl sulfate pentahydrate’s blood clearance was 0.137 ± 0.0317 L/h/kg and ranged from 0.0885 to 0.173 L/h/kg, while mean residence time in tissues (MRT) was 199 ± 73 h (range between 116 to 315 h).

After a single oral gavage administration of vanadyl sulfate (either 7.56 or 15.12 mg elemental vanadium/kg body weight (BW)) to male Wistar rats (Azay et al. 2001), the absolute bioavailability was estimated to be around 16%. Although the corresponding AUC\textsubscript{mod}, maximum plasma concentration (C\textsubscript{max}) and MRT\textsubscript{mod} showed a trend toward an increase with the higher dose, the changes were not statistically different between the these two doses (see Table 1 below).

Table 1. Pharmacokinetic Parameters for Single Oral Dose of Vanadyl Sulfate in Male Rats

<table>
<thead>
<tr>
<th>Oral dose</th>
<th>7.56 elemental vanadium mg/kg BW</th>
<th>15.12 elemental vanadium mg/kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability</td>
<td>15.7%</td>
<td>16.4%</td>
</tr>
<tr>
<td>AUC\textsubscript{mod}</td>
<td>9.32 ± 1.45 mg·h/L</td>
<td>13.90 ± 6.60 mg·h/L</td>
</tr>
<tr>
<td>C\textsubscript{max}</td>
<td>0.227 ± 0.0629 mg/L</td>
<td>0.498 ± 0.150 mg/L</td>
</tr>
<tr>
<td>MRT\textsubscript{mod}</td>
<td>184 ± 37 h</td>
<td>134 ± 61 h</td>
</tr>
<tr>
<td>AUC\textsubscript{mod} = Area under the curve obtained after modeling; MRT\textsubscript{mod} = maximum resident time after modeling; Source: Azay et al. (2001)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The distribution of [\textsuperscript{48}V]vanadium was evaluated in five male Wistar rats using [\textsuperscript{48}V]vanadium tracer (H\textsubscript{2}VO\textsubscript{4}) by intraperitoneal injection (4 injections/5 days with a total dose of 5.7 pmol
vanadium anion) (De Cremer et al. 2002). Tissue distribution data show that $^{48}$V vanadium is taken up by several tissues, with the highest levels seen in the kidneys and bones followed by the spleen and liver (see Table 2 below). In blood, 86.4% of the $^{48}$V vanadium was detected in serum and 13.6% was detected in the packed cells where it was mainly bound to hemoglobin. $^{48}$V vanadium was bound to ferritin, transferrin, and hemoglobin in tissues.

**Table 2. Distribution of $^{48}$V Vanadium in Tissues Following Intraperitoneal Injection**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$[^{48}V]$vanadium activity/g tissue (% of total measured $[^{48}V]$vanadium activity, mean ± 3σ, n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>36.6 ± 8.7</td>
</tr>
<tr>
<td>Bone</td>
<td>27.8 ± 10.8</td>
</tr>
<tr>
<td>Spleen</td>
<td>11.1 ± 5.2</td>
</tr>
<tr>
<td>Liver</td>
<td>8.5 ± 2.6</td>
</tr>
<tr>
<td>Lung</td>
<td>4.5 ± 3.3</td>
</tr>
<tr>
<td>Testes</td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td>Heart</td>
<td>2.4 ± 1.1</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>Bladder</td>
<td>1.6 ± 2.0</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>Skin</td>
<td>1.1 ± 1.5</td>
</tr>
</tbody>
</table>

Source: De Cremer et al. (2002)

Biodistribution and excretion of vanadyl sulfate (VOSO$_4$·3H$_2$O) was evaluated in male Wistar rats (n=3/time point) using $^{48}$V vanadium as a tracer by either oral gavage (0.012 mmol/animal for the distribution study or 0.009 mmol/animal for the excretion study) or intraperitoneal injection (0.010 mmol/animal) (Setyawati et al. 1998). The highest $^{48}$V vanadium concentrations measured at 24 hours after gavage were detected in bones, followed by kidneys and liver with levels not exceeding 0.6 % of the administered dose (AD)/g tissue. After oral gavage, most tissues showed a gradual uptake of the radiolabeled substance with a peak uptake seen at 2–6 hours post dosing and a decline thereafter, except in bones, liver, and kidneys where levels remained high at 24 h, indicating either accumulation or slow clearance in these tissues. Intraperitoneal injection resulted in a higher uptake in the kidneys and bones (not exceeding 1.6 %AD/g tissue) than that seen in the oral gavage group. A slightly different uptake pattern was seen for most other tissues between 4 and 24 hours after intraperitoneal injection when compared to the oral route of administration; the shortest residence time was in blood (5 min) and the longest was in bones (11 days). The pattern of distribution of unexcreted $^{48}$V vanadium in tissues was as follows: kidney > bone > liver > intestine > muscle (in %AD/g tissue) and was unchanged when administration was done via the intraperitoneal or the oral gavage route. Most ingested $^{48}$V vanadium was eliminated unabsorbed via the feces 24 hours after an oral dose (75%).

As described in the toxicology section below, dose related accumulation of vanadium was observed in tissues with the highest levels in kidneys and bones followed by spleen and liver (Dai et al. 1994). Accumulation of vanadium was also reported in placentae and fetal tissues in mice (Paternain et al. 1990).
In summary, when vanadyl sulfate is administered orally, it is absorbed with an absolute bioavailability of about 16%. Peak uptake levels are attained 2–6 hours after a single oral or intraperitoneal dosing, followed by a decline thereafter except in bones, liver, and kidneys where levels remain high at 24 hours after dosing, indicating either accumulation or slow clearance in these tissues. Vanadium related compounds can pass through the placental barrier of pregnant mice and was detected in fetal tissues, resulting in maternal and fetal toxicity. When vanadium containing products are inhaled, vanadium can accumulate in the lungs of animals. Vanadium containing compounds are mainly excreted unchanged via the fecal route, with the bulk of the elimination seen at 24 hours after oral dosing. Accumulation of vanadium in various tissues is concerning especially for treatment of a chronic disease such as diabetes.

b. Human data

Limited pharmacokinetic data are available from studies of oral administration of vanadyl sulfate to humans. These are summarized in Table 3 below.

<table>
<thead>
<tr>
<th>Study and Population</th>
<th>Dose</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohen et al. (1995) n = 4 Type 2 diabetics</td>
<td>100 mg/d for 3 weeks</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>73.3 ± 22.4 μg/L</td>
<td>N/A</td>
</tr>
<tr>
<td>Boden et al. (1996) n = 4 Type 2 diabetics</td>
<td>100 mg/d for 4 weeks</td>
<td>8</td>
<td>18</td>
<td>167 ± 32 ng/mL</td>
<td>N/A</td>
</tr>
<tr>
<td>Goldfine et al. (2000) n = 11 Type 2 diabetics</td>
<td>75, 150 or 300 mg/d for 6 weeks</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>75 mg: 16.0 ± 5.1 150 mg: 83.6 ± 44.0 300 mg: 284.5 ± 146.3 (all in ng/mL)</td>
<td>300 mg dose at steady state: avg. amt. of vanadium excreted in the urine was 1%</td>
</tr>
<tr>
<td>Cusi et al. (2001) n = 11 Type 2 diabetics</td>
<td>150 mg/d for 6 weeks</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>not assessed</td>
<td>Pre-dose plasma level of vanadium at 6 weeks 104 ± 18 mcg/L</td>
</tr>
<tr>
<td>Thompson and Orvig (2006) n = 4 Healthy humans</td>
<td>50 mg single dose</td>
<td>6.0</td>
<td>59.2</td>
<td>21.6 ng/mL</td>
<td>See Table 4</td>
</tr>
</tbody>
</table>

Heinemann et al. (2003) studied intravenous administration of a single dose of a 20% albumin solution, containing 47.6 μg/90 mL vanadium as an impurity, which was given to five healthy male volunteers over a period of 20 minutes. Spectroscopic analysis indicated that the vanadium impurity was in the +5 oxidation state, which is the vanadate form. Based on their average weight, the mean vanadium dose administered was calculated to be 0.6 μg/kg. The subjects were studied for 31 days. The authors determined that the vanadium pharmacokinetics in these volunteers fit into a three compartment model. Initially, there was a rapid decrease in serum vanadium concentrations with half-lives of 1.2 and 26 hours followed by a long terminal half-life of 10 days. The terminal phase accounted for 80% of the total AUC. Mean volume of distribution of the central compartment was 10 L. The volume of distribution at steady state was
54 L, and total clearance was 0.15 L/h. Vanadium was mainly excreted by the kidneys. Fifty-two percent of the dose was recovered in the urine after 12 days. Fecal elimination was not assessed in this trial; however, the authors calculated, based on the subject’s vanadium blood level and volume of distribution, about 13% of the total dose was unaccounted for after 12 days. After 31 days, the mean blood level of the five subjects was about 0.1 μg/L, which was described by the authors as close to baseline.

Pharmacokinetic data for vanadium containing compounds in humans are also available from recent attempts by the pharmaceutical industry to develop a marketable vanadium drug product, primarily as an orally dosed alternative to injectable insulin for the treatment of diabetes. Thompson and Orvig (2006) outlined the pharmacokinetic analysis from the Phase I clinical trial of administration of oral bis(ethylmaltoïlato)oxovanadium (IV) (BEOV; KP102 Medeval Ltd, Manchester, UK), containing tetravalent vanadium. BEOV had been developed by adding a carrier/ligand to vanadium in an attempt to identify a non-toxic carrier that could improve oral bioavailability of vanadium compared to inorganic salts such as vanadyl sulfate, while at the same time allowing for dissociation of vanadium and the carrier following absorption, as necessary for vanadium pharmacologic effect as an insulin-mimetic. In this trial, BEOV’s pharmacokinetic data were compared to that of oral administration of vanadyl sulfate. Forty healthy subjects (non-diabetic) in total participated in the trial. Four subjects received a single dose of one of the escalating BEOV dose levels (there were also two placebo control subjects for each dose level) or vanadyl sulfate, and eight subjects received two doses of 75 mg BEOV. The pharmacokinetic assay results are provided in Table 4 below.

Table 4. Pharmacokinetic assay results for BEOV and vanadyl sulfate in a Phase I clinical trial of KP102

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BEOV</th>
<th>VOSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>Tₘₐₓ (h)</td>
<td>3.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Cₘₐₓ (μg/mL)</td>
<td>4.1</td>
<td>38.5</td>
</tr>
<tr>
<td>AUC (ng h/mL)</td>
<td>276</td>
<td>1392</td>
</tr>
<tr>
<td>T₁/₂ (h)</td>
<td>63.5</td>
<td>45.1</td>
</tr>
<tr>
<td>%ADₜₚₜ (0-72 h)</td>
<td>1.90</td>
<td>5.81</td>
</tr>
<tr>
<td>%ADₚₑₑₑₑₑ (0-72 h)</td>
<td>139.8</td>
<td>66.8</td>
</tr>
<tr>
<td>Clᵣ (L/h)</td>
<td>0.237</td>
<td>0.168</td>
</tr>
</tbody>
</table>

Abbreviations: Tₘₐₓ=time to maximal plasma concentration of vanadium; Cₘₐₓ=maximum plasma concentration of vanadium; AUC=area under the curve of plasma vanadium; t₁/₂=half-life of plasma vanadium; %AD=percentage of administered vanadium dose; Clᵣ=renal clearance of vanadium; *vanadium content similar to 50 mg VOSO₄

Source: Thompson and Orvig (2006)

The design of the study evaluated the effects of food on the bioavailability of BEOV, showing that it was much diminished when BEOV was administered immediately prior to a meal as
compared to during fasted conditions. However, the bioavailability of vanadium from the BEOV using 60 mg dose was approximately three times greater than from the vanadyl sulfate 50 mg dose, although the two doses contain approximately the same amount of vanadium.

BEOV was subsequently studied in a Phase IIa study, which will be summarized in the Clinical Efficacy section. The sponsor of BEOV’s development publicly stated that “based on the renal changes resulting from the doses used in our preclinical safety program, (the sponsor has) determined that the safety profile of (BEOV) makes it no longer viable as a drug candidate in a chronic disease setting.” Additional nonclinical information regarding renal toxicity associated with vanadium containing compounds is discussed in the nonclinical section of this review (section II.B.3.b.).

The existing human pharmacokinetic data demonstrate that vanadium is systemically available from oral dosing of vanadyl sulfate. However, there appears to be agreement in the literature that the pharmacokinetic profile of vanadium could be improved via the use of novel, synthesized vanadium-based drugs as compared to the pharmacokinetic profile of vanadyl sulfate. Scior et al. (2016) concludes that development of clinically useful vanadium compounds is dependent on finding organic ligands that will provide selectivity, facilitate bioavailability, minimize body accumulation, and have few side effects. Others suggest that the inherent toxicity of vanadium cannot be overcome via modification of vanadium compounds and improved pharmacokinetics (Domingo and Gomez 2016). We did not identify human pharmacokinetic data from injection of vanadium compounds, with the exception of vanadium as an impurity in albumin solutions.

3. Nonclinical Safety

Numerous studies have been conducted to evaluate the toxicity of vanadium compounds. The bulk of the toxicology studies found in the literature were conducted using the oral route of exposure. These studies indicate that the toxicity of vanadium increases with higher valences, where the pentavalent compounds appear to be the most toxic form of vanadium (Barceloux 1999). No nonclinical toxicology data were available for vanadium administered via the intravenous route. Vanadium toxicity is reported to be low via oral ingestion, moderate via inhalation, and high via injection (Barceloux 1999; NTP 2008).

The data captured in this review are obtained from publicly available studies that were conducted mostly to meet the regulatory requirements of the EPA for vanadyl sulfate in drinking water. However, the quality of the data and the conclusions that were drawn from these studies were not adequate to complete the EPA’s safety assessment of vanadium sulfate. As of the time this review was completed, vanadium was still listed on the EPA Drinking Water Contaminant Candidate List (CCL) as a priority contaminant with insufficient information to support a

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regulatory determination of its safety. Because of concerns for human health following chronic oral exposure to soluble vanadium salts from drinking water and from dietary supplements, the tetravalent and pentavalent forms of vanadium have been nominated by the National Institute of Environmental Health Sciences for a comprehensive toxicological characterization by the National Toxicology Program (NTP) via the oral route of administration (NTP 2008). Other reasons cited by the NTP nomination included the demonstrated positive carcinogenicity signal of the pentavalent form in experimental animals in inhalation studies. The NTP testing program includes a 2-year carcinogenicity bioassay as well as a 2-generation reproductive/developmental toxicity study of pentavalent vanadium compounds (e.g., sodium metavanadate or ammonium metavanadate), both conducted using drinking water as the route of exposure. NTP studies are currently ongoing.

a. Acute toxicity

Exposure to single doses of vanadium in acute toxicity studies is associated with a wide range of toxicities including mortality, neurotoxicity, cardiotoxicity, respiratory, and hematological findings.

Single dose toxicity studies with a 14 day follow up observation period were conducted for vanadyl sulfate pentahydrate (VOSO₄·⁵H₂O,) and sodium metavanadate (SMV, NaVO₃) in adult male Swiss mice and Sprague-Dawley rats (n=10/group) (Llobet and Domingo 1984). Dose related deaths were noted with no survivors among animals treated with the highest dose of both vanadyl sulfate pentahydrate and SMV. No deaths were observed in the lowest doses used. The majority of deaths occurred during the first 24 hours after dosing (80% for intraperitoneal and 60% for oral dosing). The rest of the mortality cases occurred by 48h post dose for the intraperitoneal group and by day 7 for the orally treated group (see Table 5 below). Under the conditions of this study, SMV was more potent than vanadyl sulfate pentahydrate and the intraperitoneal injection route was more toxic than the oral route, probably due to poor oral absorption. Nonlethal doses were associated with severe toxicities in both animal species tested, but were more severe for SMV compared to vanadyl sulfate pentahydrate. Clinical signs included irregular respiration, increased cardiac rhythm, increased ataxia, decreased locomotor activity, paralysis of the rear legs, and decreased sensitivity to pain. Severe diarrhea was noted in treated rats (but not in mice). Most signs disappeared by 48 hours post-dosing in surviving animals.

Table 5. Acute Toxicity Studies Conducted with Two Vanadium Salts

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9 Acute toxicity refers to adverse effects observed following administration of a single dose of a substance, or multiple doses given within a short period (approximately 24 hours). Endpoints captured in acute toxicity studies usually include mortality and gross clinical observations. Acute toxicity data are usually superseded by data obtained from longer term toxicity studies.
<table>
<thead>
<tr>
<th></th>
<th>Oral VOSO₄·5H₂O</th>
<th>Oral NaVO₃</th>
<th>Intraperitoneal VOSO₄·5H₂O</th>
<th>Intraperitoneal NaVO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose in male rats (n=10/group)</td>
<td>296-845 mg/kg</td>
<td>39-256 mg/kg</td>
<td>50-143 mg/kg</td>
<td>7-34 mg/kg</td>
</tr>
<tr>
<td>Dose in male mice (n=10/group)</td>
<td>186-714 mg/kg</td>
<td>41-157 mg/kg</td>
<td>45-178 mg/kg</td>
<td>18-91 mg/kg</td>
</tr>
<tr>
<td>LD₅₀ (oral), rats</td>
<td>448 mg/kg</td>
<td>98.0 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD₅₀ (oral), mice</td>
<td>467.2 mg/kg</td>
<td>74.6 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD₅₀ (intraperitoneal), rats</td>
<td></td>
<td></td>
<td>74.1 mg/kg</td>
<td>18.4 mg/kg</td>
</tr>
<tr>
<td>LD₅₀ (intraperitoneal), mice</td>
<td></td>
<td></td>
<td>113.0 mg/kg</td>
<td>35.9 mg/kg</td>
</tr>
</tbody>
</table>

Source: Llobet and Domingo (1984)

In a separate study, young adult female ICR mice (n=10/group/time point) were administered a single intraperitoneal dose of 28.3 mg/kg vanadium chloride (VC, V⁵⁺), 29.3 mg/kg vanadyl sulfate (V⁴⁺), or 33.1 mg/kg sodium orthovanadate (SOV, V³⁺), which delivered an equivalent vanadium dose of 9.2 mg elemental vanadium/kg (Hogan 2000). When compared to controls, transient but significant decreases in peripheral erythrocyte counts, and increases in reticulocyte percentages and erythrocyte iron uptake were noted in all vanadium groups, suggesting an adverse hemolytic effect of vanadium.

b. Repeat dose toxicity¹⁰

Findings from the repeat dose toxicity testing were similar to those reported for the acute dose testing discussed above. The majority of the studies were not adequately designed; studies had included only one sex (usually males), the species selected was limited to rats, the bulk of the studies were conducted via the oral (gavage or drinking water) route of administration, and only limited analysis was conducted.

Fourteen-day repeat dose toxicity study

The NTP conducted a 14-day comparative toxicity study between vanadyl sulfate and sodium metavanadate in drinking water in mice and rats (n=5/sex/group) at doses of 0, 125, 250, 500, 1000, or 2000 mg/L (Roberts et al. 2016). A decrease in water consumption was seen in all groups and was accompanied by a decrease in body weights at the highest concentrations tested for both compounds. Thinness was also observed in high dose treated animals exposed to either compound, while mortality, lethargy, abnormal breathing, and abnormal gait were only observed in the sodium metavanadate-treated group.

¹⁰ Repeated-dose toxicity studies consist of in vivo animal studies that seek to evaluate the toxicity of the test substance by observing the changes that emerge in clinical observations, clinical chemistry, gross pathology, and histology endpoints when the test substance is repetitively administered daily for a predetermined period of time.
Four-week toxicity studies

A four week study was conducted in streptozotocin-induced diabetic (STZ-diabetic) male Sprague-Dawley rats (n=10/group) where rats were allowed to drink ad libitum water containing sodium metavanadate (0.15 mg/ml), sodium orthovanadate (0.23 mg/ml), or vanadyl sulfate pentahydrate (0.31 mg/ml) for 28 days (Domingo et al. 1991).

Vanadium was detected in all organs/tissues analyzed (kidneys, bone, spleen, liver, pancreas, heart, and muscle) with the highest concentrations in kidneys followed by bone and spleen whereas the lowest concentration was detected in muscles. Significant toxicities associated with administration of vanadium containing compounds included mortality, decreases in body weight gain, decreases in kidney weights, and increases in serum concentrations of urea and creatinine (highest increase was seen in vanadyl sulfate group). No histopathology was conducted for this study.

Another four week oral toxicity study was conducted in non-diabetic and STZ-diabetic male Wistar rats (n=8/group) using vanadyl sulfate suspended in 0.5% sodium carboxy methyl cellulose solution (0, 0.2, or 0.5 mmol/kg/day) (Majithiya et al. 2005). Dose- and duration-dependent decreases in body weights were reported. Dose-related diarrhea (ranging from mild at 0.2 mmol/kg/day to severe at 0.5 mmol/kg/day) was reported in both STZ-diabetic and non-diabetic rats treated with vanadyl sulfate. Vanadyl sulfate toxicities were limited to significant reduction in glucose and lipid levels in the STZ-diabetic rats treated with 0.5 mmol/kg/day vanadyl sulfate. No other observations were reported in this study.

Three-month toxicity study

Wistar male rats were treated with 0.26 mg/mL vanadyl sulfate in drinking water ad libitum for 12 weeks (n=8/group, calculated average intake as 0.15 mmol elemental vanadium/kg/day) (Dai et al. 1995). No significant change was observed for body weight between the vanadyl sulfate and control groups. No significant changes in any hematological parameters were reported (hematocrit, hemoglobin, erythrocytes, leukocytes, platelets, reticulocytes, and erythrocyte osmotic fragility).

One-year repeat dose toxicity study

STZ-diabetic and non-diabetic male Wistar rats (n=8-10/group) were treated with vanadyl sulfate in drinking water at concentrations of 0.5-1.5 mg/mL for 52 weeks (Dai et al. 1994). A 16 week recovery period was included in the study.

The key findings of the study included:

- Death in one non-diabetic rat (1.25 mg/mL) after 18 weeks of treatment with vanadyl sulfate (cause of death was unknown). An increased incidence of mortality was seen in control STZ-diabetic rats (6/10) compared to the vanadyl sulfate-treated group (6/32).
Gross pathology evaluation in both groups showed hemorrhages (gastrointestinal and intracranial), pulmonary edema and renal tumors in STZ-diabetic rats.

- Vanadyl sulfate was detected in plasma and tissues in a dose-related manner with the highest levels detected in bones followed by kidneys, testes, liver, pancreas, plasma, and brain.
- Although vanadyl sulfate was undetectable in plasma after 16 weeks of recovery, it was still retained in organs.
- Significantly higher levels of plasma alanine aminotransferase (ALT) and urea were seen in the non-diabetic rats after 3 months of vanadyl sulfate treatment (but not at 6, 9, or 12 months). In STZ-diabetic rats, ALT levels were significantly lower in the vanadyl sulfate-treated group compared to the control group at 3, 6, and 9 months (but not at 12 months of treatment or after 16 week recovery period).
- A transient increase in urea levels was seen after 3 month of vanadyl sulfate dosing in the STZ-treated rats. No differences were seen in other dose groups or later time periods tested (6, 9 or 12 months).
- Increases in organ weights were noted for the vanadyl sulfate treated rats (brain, lung, heart, spleen and testis) (see Table 6 below).
- The incidence of glomerular and tubular degeneration, interstitial cell infiltration and fibrosis in kidneys were higher in the vanadyl sulfate-treated non-diabetic group compared to controls [100% (15/15) vs. 60% (3/5) at 12 months and 88% (7/8) vs. 66% (2/3) after 16 week recovery]. In the STZ-diabetic rats, the incidence of renal cell tumors were 86% (6/7) in the control group compared to 52% (11/21) in the vanadyl sulfate-treated group.
- Mucosal petechiae or ulceration in stomach were noted in the vanadyl sulfate-treated non-diabetic group only (27%).
- Urinary bladder stones were reported in 60% of control rats but only 7% in the vanadyl sulfate-treated non-diabetic group.
- Interstitial cell hyperplasia and Leydig cell tumor were noted in one rat in the vanadyl sulfate-treated non-diabetic group.

Table 6. Organ weight/body weight ratio (mg/g) of various organs in vanadyl-treated nondiabetic and STZ-diabetic rats at 16 weeks following the withdrawal of vanadyl sulfate treatment
In summary, mortality was noted in animals dosed with high doses of vanadyl sulfate. At lower doses, the main adverse effects observed included decreased water consumption, decreased body weight gain, diarrhea, and renal toxicity (increased serum concentrations of urea and creatinine, and increased incidences of glomerular and tubular degeneration and interstitial cell infiltration and fibrosis). The decrease in water consumption where vanadyl sulfate was dissolved must have resulted in a lower drug exposure; as a result, the toxicities reported in these studies may have underestimated the toxicological response in animals treated with vanadyl sulfate. The accumulation of vanadyl sulfate that was observed in several organs (kidneys, bones, spleen, liver, testes) was dose dependent and was still detected after 16 weeks of recovery where vanadyl sulfate was no longer detectable in the plasma.

c. Genotoxicity:

The genotoxicity of vanadyl sulfate has been evaluated in vitro using several cell lines (primary human lymphocytes, cultured mammalian cells), as well as in ex vivo and in vivo assays (mice). No reports on bacterial reverse mutation assay (Ames test) were found in the literature for vanadyl sulfate. Overall, vanadyl sulfate was shown to cause cytogenetic damage and exhibited clastogenic potential in both in vitro and in vivo assays.

*In vitro studies*

Vanadyl sulfate was found to be genotoxic in the in vitro micronucleus, satellite association, and sister chromatid exchange assays (Migliore et al. 1993). Primary human peripheral blood lymphocytes were treated with 5, 10, 20, 40, or 80 μM of vanadyl sulfate. For the micronucleus

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11 Genotoxicity assessment battery usually consists of a gene mutagenicity assay (for single dose trials). To support multiple dose administration in humans, additional genotoxicity testing assessment is usually conducted to detect chromosomal damage in mammalian systems.
assay, a significant increase in micronuclei frequency was observed at concentrations of 10 µM and greater (See Table 7 below). Significant increases in satellite associations per cell (in the 5-40 µM concentrations tested), and in the total chromosome aberrations for hypoploidy (20-80 µM) and hyperploidy (5 and 80 µM) were seen in the vanadyl sulfate group compared to control cells. No significant increase in the number of structural chromosomal aberrations was seen among cells treated with vanadyl sulfate.

Table 7. In vitro induction of micronuclei by vanadyl sulfate in human lymphocytes

<table>
<thead>
<tr>
<th>Vanadyl sulfate (µM)</th>
<th>Cell analyzed</th>
<th>Number of micronucleated or binucleated cells</th>
<th>Micronucleus (%)</th>
<th>Binucleate cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2000</td>
<td>7</td>
<td>3.50</td>
<td>37.90</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
<td>12</td>
<td>6.00</td>
<td>32.60</td>
</tr>
<tr>
<td>10</td>
<td>2000</td>
<td>16 *</td>
<td>8.00</td>
<td>25.80</td>
</tr>
<tr>
<td>20</td>
<td>2000</td>
<td>16 *</td>
<td>8.00</td>
<td>17.80</td>
</tr>
<tr>
<td>40</td>
<td>1050</td>
<td>10 *</td>
<td>9.50</td>
<td>17.60</td>
</tr>
<tr>
<td>80</td>
<td>2000</td>
<td>50 ***</td>
<td>25.00</td>
<td>17.70</td>
</tr>
</tbody>
</table>

* p < 0.05, *** p < 0.001 (Fisher's exact test). Adapted from Migliore et al. (1993)

Vanadyl sulfate was genotoxic in the in vitro comet assay testing human lymphocytes and the HeLa cancer cell line (Wozniak and Blasiak 2004). HeLa cells exhibited increased tail length and greater percentage of DNA fragments in the tail for all concentrations tested (0.05-1.0 mM), while human lymphocytes showed increases in tail length at 0.1-1.0 mM and % tail DNA at 0.5 and 1.0 mM.

Genotoxicity of vanadyl sulfate was also observed in cultured Chinese Hamster Ovary cells treated for 2 hours in the presence or absence of rat hepatic S9 mix (Owusu-Yaw et al. 1990). Sister chromatid exchange and chromosome aberrations were examined, as well as the cytotoxicity of vanadyl sulfate in the culture system (toxic concentration to 50% of the cells (TC_{50}) = 23.3 ± 4.2 µg vanadium/ml). In the absence of S9 mix, there was a significant increase in sister chromatid exchange frequency (0.5, 1.0, 6.0 µg vanadium/ml) and chromosomal aberrations (6, 12, 24 µg vanadium/ml) in the vanadyl sulfate treatment compared to controls. A concentration of 1/4 of the TC_{50} (i.e., 6 µg vanadium/ml) produced the highest sister chromatid exchange frequency. The presence of S9 mix decreased the clastogenic activity of vanadyl sulfate; which may have been caused by the reduced availability of vanadyl sulfate due to its binding to S9 in the culture medium.

In vivo studies

In a dietary intake study, male CD1 mice were administered vanadyl sulfate hydrate (VOSO\(_4\cdot5\)H\(_2\)O) in drinking water using doses of 2, 10, 100, 500, or 1000 mg/L for 5 weeks (n=8-10 per group) (Villani et al. 2007). Blood samples were collected at the beginning of treatment and 7, 14, 21, 28, and 35 days thereafter. At the end of the experiment, several tissues were harvested and total vanadyl sulfate concentration was measured in the samples. Daily water consumption was significantly reduced in the two highest dose groups (500 and 1000 mg/L), while no differences were observed in food consumption. A dose-related, linear increase in vanadium content of bone tissues was observed, but no genotoxic effects were observed.
There were no differences or dose-related increases in the frequency of micronucleated polychromatic erythrocytes, micronucleated blood reticulocytes, or in the mean tail moment values of bone marrow cells for any of the concentrations tested. Because no large differences in vanadyl sulfate content were observed in the soft tissues analyzed (liver, kidney, spleen, testis), the lack of genotoxic potential was attributed to limited oral bioavailability of vanadyl sulfate.

In a single dose oral study, vanadyl sulfate (100 mg/kg, equivalent to 0.60 mM of elemental vanadium) was administered to male CD1 mice via intragastric intubation (n=3 per experimental group) (Ciranni et al. 1995). For the micronucleus experiment, bone marrow cells were analyzed for micronuclei at 6 hour intervals starting at 6 hours and ending at 72 hours after treatment. For the chromosomal aberration experiments, bone marrow cells of treated mice were examined for structural and numerical chromosome aberrations at 24 hours and 36 hours after vanadyl sulfate administration. Vanadyl sulfate treatment resulted in a significant increase in micronuclei at the majority of sampling intervals (Table 8). A significant increase in structural chromosome aberrations was detected at 24 and 36 hours (i.e., % aberrant cells with exclusion gaps), while an increase in numerical chromosome aberrations was observed only at 24 hours (in both hyperploid and hypoploid cells) following dosing with vanadyl sulfate.

Table 8. Frequencies of micronuclei in bone marrow after a single dose of vanadyl sulfate in mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Time (h)</th>
<th>No. of animals*</th>
<th>PCEs/NCEs (% ± SD)</th>
<th>MNPCEs/PCEs (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−</td>
<td>4</td>
<td>0.85 ± 0.15</td>
<td>0.19 ± 0.07</td>
</tr>
<tr>
<td>Vanadyl sulfate</td>
<td>100</td>
<td>6</td>
<td>0.41 ± 0.27*</td>
<td>0.41 ± 0.20*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12</td>
<td>0.51 ± 0.04</td>
<td>0.50 ± 0.20*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>18</td>
<td>0.78 ± 0.24</td>
<td>0.60 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>24</td>
<td>0.72 ± 0.13</td>
<td>0.65 ± 0.20*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30</td>
<td>0.87 ± 0.35</td>
<td>0.54 ± 0.09*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>36</td>
<td>0.38 ± 0.05*</td>
<td>0.48 ± 0.19*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>42</td>
<td>0.48 ± 0.03</td>
<td>0.18 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>48</td>
<td>0.49 ± 0.16*</td>
<td>0.62 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>72</td>
<td>0.62 ± 0.20</td>
<td>0.15 ± 0.06</td>
</tr>
</tbody>
</table>

PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte; MNPCE=micronuclei induced in PCE; *3000 PCEs scored per animal; *p < 0.05; **p < 0.01; Adapted from Ciranni et al. (1995)

In summary, vanadyl sulfate was shown to be a genetic toxicant in a battery of in vitro studies, including micronucleus, COMET, satellite association, sister chromatid exchange, and chromosome aberration assays. Vanadyl sulfate was also found positive in the in vivo micronucleus and chromosome aberration assays. The effects were dose dependent where cells (in vitro) or animals (in vivo or ex vivo) exhibited cytogenetic and clastogenic effects with increasing doses of vanadyl sulfate.
d. Developmental and reproductive toxicity\textsuperscript{12}

Developmental and reproductive toxicities were reported for vanadyl sulfate where the majority of adverse reproductive effects consisted of adverse male fertility effects in rats, embryofetal toxicity in mice, and prenatal and postnatal developmental toxicity in rats. Furthermore, vanadium can accumulate in the developing fetus as well as in the placenta.

**Male fertility**

Male reproductive functions were evaluated for vanadyl sulfate in Wistar adult male rat (n=7/group) (Jain et al. 2007), where rats were orally administered 100 mg/kg vanadyl sulfate in distilled water for 60 days, a period that covers a full cycle of spermatogenesis in the rat. During the last 5 days of dosing, treated males were mated with untreated females. Following mating, male rats were terminated and necropsy was conducted. Treatment with vanadyl sulfate was associated with a significant decrease in testis weight (mean 1174 vs. 1435 mg/100 g body weight in controls, p < 0.05), epididymides, seminal vesicles, and ventral prostate weights. A reduction in caudal epididymal sperm density (mean 35 vs. 47 million/mm\textsuperscript{3}, p < 0.01), motility (42 vs. 74%, p < 0.001) and reduced fertility (mating index= 5/10 in treated vs. 10/10 in controls) and litter size (5 in treated vs. 8 in controls, p < 0.01) were also noted. Abnormal histopathological findings including degeneration of spermatocytes were also noted, indicating that treatment with vanadyl sulfate for 60 days impaired sperm cells at various stages of their growth and maturation into sperm cells.

**Embryofetal development**

Vanadyl sulfate was evaluated in a study (Paternain et al. 1990), where Swiss mice were orally gavaged with 0, 37.5, 75, or 150 mg vanadyl sulfate pentahydrate (\textit{VOSO}_4\cdot5\textit{H}_2\textit{O} in water)/kg/day (7.55, 15.10, or 30.19 mg elemental vanadium/kg/day) on gestation days (GD) 6-15. Pregnant dams were terminated on GD 18 (n=16-20/group) to assess the effects of vanadyl sulfate on the developing fetuses. A dose-related increase in embryonic loss (increased number of early resorptions per litter), reduced fetal body weight and body length per litter, external malformations (cleft palate and micrognathia), and/or skeletal defects (bipartite sternebrae, irregular shape of ribs, and delayed ossification) were seen. Maternal toxicity was evidenced by a dose-related decrease in body weight. In addition, vanadyl sulfate accumulated in the fetus as well as in the placenta, liver, kidney, and spleen of treated dams.

**Prenatal/postnatal development**

\textsuperscript{12} Developmental and reproductive toxicity studies are usually designed to assess the potential adverse effects in humans of both sexes and include females from various age groups that will be exposed to the proposed substance. Developmental toxicity or teratogenicity refers to adverse effects (can include embryo-fetal mortality, structural abnormalities, functional impairment, or alterations to growth) and can occur in pups either as a result of the exposure of their parents to the substance, prior to the pups’ birth, or by direct exposure of the pups to the substance after birth.
Vanadyl sulfate was evaluated in Wistar rats which were orally dosed with 300 mg/L (70 mg elemental vanadium/L) via drinking water (dissolved in 5 g/L NaCl, equal to an ingested dose of 10 mg elemental vanadium/kg/day according to the publication) to dams (n=5/group) during the last 3 days of pregnancy until pup weaning (25 days postnatally, PND). After weaning, pups (10/sex/group) continued to receive vanadyl sulfate until day 100 after birth (Poggioli et al. 2001). Decreased survival rate [61% (25/41) vs. 100% (48/48) in the NaCl controls] and decreased body weight at weaning (pooled mean: 53g vs. 64g) were noted in the vanadyl sulfate-treated group. Furthermore, decreased body weight was continuously observed in males until day 70 after birth but not in females. No significant body weight difference was seen on Day 100 after birth between the two groups. In the open-field test evaluated at 1 month of age, vanadyl sulfate treated rats had reduced outer ambulation, rearing posture and grooming activity (altered behavior). Other adverse effects included increased defecation in the vanadyl sulfate-treated group, which was probably linked to the fecal elimination of vanadyl sulfate. None of the findings was statically significant (p < 0.05) in females (but were in males). There were no differences in locomotor activity or memory tests in either sex. The reproductive competence of the exposed pups was not assessed at the end of the study.

e. Carcinogenicity

Carcinogenicity studies were conducted for vanadyl sulfate in mice by oral administration and for vanadium pentoxide (VP, V₂O₅) in rats and mice by inhalation (Kanisawa and Schroeder 1967; Schroeder and Mitchener 1975; NTP 2002; Ress et al. 2003). The study results for vanadium pentoxide can be found in Appendix 1.

In both studies conducted for vanadyl sulfate, male and female Swiss mice were provided vanadyl sulfate in drinking water (5 ppm elemental vanadium or 5 μg V/mL) until the end of their life. The authors estimated that the elemental vanadium intake was approximately 35 μg V/100 g body weight/day, or 13 mg/100 g/year. Vanadyl sulfate did not increase the overall incidence of tumors (approximately 32% in both groups: 15/47 in the vanadyl sulfate group vs 55/170 in the concurrent control group). However, a higher incidence of malignant tumors was noted in the vanadyl sulfate group (60% vs 27% in the concurrent control group). A statistically significant increase in the overall tumor incidence was seen mainly in vanadyl sulfate-treated females [37% (19/51) vs. 20% (9/45) in the control group, p < 0.05]. This effect was not seen in male mice in a subsequent study using a similar dose (Schroeder and Mitchener 1975). Interestingly, in this later study, mice in the vanadyl sulfate-treated group survived longer than mice in the control group and showed a higher body weight. However, the details of this latter study were not provided (the study only contained a small number of animals; the study addressed a large number of other trace minerals and was not dedicated to studying vanadyl sulfate). A conclusion regarding the tumorigenic effect of vanadyl sulfate cannot be reached based solely on these two publications.

13 Studies that assess cancer risk in animals are used as predictive tools to evaluate the potential for drugs to result in tumors when used by humans on a chronic basis. Carcinogenicity studies are conducted if the clinical use is expected to be continuous for a minimum of 6 months of life, or if intermittent clinical use is expected to total 6 months or more of life.
Based on the equivocal animal data discussed above, the International Agency for Research on Cancer (IARC) categorized vanadyl pentoxide as a possible human carcinogen (Group 2B) (IARC 2006).

No carcinogenicity studies were found in the literature where vanadyl sulfate was tested using the injection route of administration.

f. Toxicokinetics

No toxicokinetic data were found for vanadyl sulfate (see section II.B.2 above for more details).

Conclusions: The toxicity profile of vanadyl sulfate has not been adequately evaluated in animals by the nominated route of exposure (injection). The bulk of the available studies was conducted via the oral (gavage or drinking water) route of administration and had limited data analysis of toxicology endpoints.

Data obtained with various vanadium containing compounds other than vanadyl sulfate show a higher level of toxicity when the substances were administered via the intraperitoneal injection compared to the oral route of administration (oral gavage, drinking water). Toxicity studies ranging in duration from 4 weeks to 1 year showed a number of adverse effects ranging from decreased body weight gain, diarrhea, and renal toxicity (increased serum concentrations of urea and creatinine, and increased incidences of glomerular and tubular degeneration, interstitial cell infiltration, and fibrosis). Other effects included hematotoxicity (alterations of erythrocyte parameters) and neurotoxicity (for sodium metavanadate and vanadium pentoxide salts). Accumulation of vanadium was also noted in several organs (bone, kidney, spleen, and liver).

Genotoxicity was observed in multiple studies for vanadyl sulfate in both in vitro and in vivo assays. Increases in the frequency of micronuclei, chromosome aberrations, and sister chromatid exchanges were observed in the in vitro tests with human lymphocytes and cultured mammalian cells. Genotoxicity studies conducted in vivo, while inconsistent likely due to poor oral vanadyl sulfate bioavailability, also showed increased micronuclei and chromosome aberrations in bone marrow cells following intragastric administration of vanadyl sulfate.

Developmental and reproductive toxicities were reported for vanadyl sulfate in rats and mice. In rats, adverse reproductive effects seen in male rats included impaired spermatogenesis and reduced fertility. Pre/postnatal developmental toxicity was also seen and included fetal/pup deaths and reduced body weight at weaning. In mice, embryofetal effects included a dose-related increase in early resorptions, reduced fetal body weight and body length, external malformations including cleft palate, micrognathia, and skeletal defects. Accumulation of vanadium was reported in fetal tissues and in the placenta.

Carcinogenicity studies conducted for vanadyl sulfate in mice by oral administration showed inconsistent results and a conclusion cannot be drawn because sufficient details of the studies were not reported. Based on the equivocal animal findings, the IARC categorized vanadyl
pentoxide as a possible human carcinogen. Because of potential toxicity concerns for human safety and lack of pivotal toxicology data, the NTP is currently conducting an oral carcinogenicity study and a 2-generation study to address the safety concerns for vanadium salts in the water supply and in dietary supplements.

4. Human Safety

The following database(s) were consulted in preparation of this section: PubMed, ClinicalTrials.gov.

a. Reported adverse reactions

**FAERS**

The Office of Surveillance and Epidemiology (OSE) conducted a search of the FDA Adverse Events Reporting System (FAERS) database for reports of adverse events for vanadyl sulfate through December 15, 2016, and retrieved one report that the OSE reviewer determined was possibly related to vanadyl sulfate. The report described a patient with Crohn’s disease, treated with infliximab 300 mg for four years, who experienced dehydration, vomiting, diarrhea with bleeding, and abdominal pain after taking vanadyl sulfate for an unspecified period of time. The patient recovered after being hospitalized for 10 days; computerized tomography (CT) scans and abdominal ultrasound were unremarkable.

**CAERS**

The Center for Food Safety and Nutrition (CFSAN) collects reports of adverse events for dietary supplements in the CFSAN Adverse Event Reporting System (CAERS). A search of CAERS was conducted for adverse events associated with vanadium containing compounds on November 30, 2016, and retrieved 1,341 reports. These reports were generally associated with multi-ingredient dietary supplements, containing various vitamins, minerals, and botanical ingredients. Only two reports were associated with products that contained three or fewer ingredients in addition to vanadium containing compounds. In one report, a 57 year old female was hospitalized after developing ventricular fibrillation while exercising. She was reported to have been taking a product called Metabolic Research Phentratrim Plus, containing vanadium and “hoodia gordani,” levothyroxine, and lovastatin. No details were provided regarding the dose or duration of treatment with vanadium. In the second report, an anonymous complainant reported that the distributor firm described on the label of the product, Univera, Inc., did not properly record and report all adverse events. The associated product was Univera Dietary Supplements – Vanadium. No specific adverse event was reported in association with this product.

**Published clinical review of vanadium for treatment of human diabetes**

Domingo and Gomez (2016) reviewed 30 years of articles related to the use of vanadium containing compounds in diabetes. Noting the nonclinical toxicities and the chronicity of diabetes, the authors point out that, in the few studies on human patients with positive results,
vanadium containing compounds were administered during very short periods. The adverse effects in humans that were common to the studies were gastrointestinal including nausea, vomiting, diarrhea and bloating.

**Case reports or other literature**

A recent drug development program of bis(ethylmaltolato)oxovanadium (IV) was halted in light of nonclinical renal toxicity findings, although clinical toxicities were not reported (Akesis 2009). We were not able to find literature on the safety of intravenous injection vanadyl sulfate.

Heinemann et al. (2000) examined the possibility that vanadium, found as an impurity in albumin solutions, could cause renal damage in patients with existing reduced renal function. The details of this case can be found in Appendix 2. The authors found that proximal tubular function of the patient given albumin may have been adversely affected by the vanadium secondary to the patient’s reduced renal function as consequence of cardiac surgery.

**b. Clinical trials assessing safety**

We identified seven clinical reports of vanadyl sulfate that mentioned human safety in diabetics:

- Cusi et al. (2001) reported that of 11 type 2 diabetic patients given 150 mg/day of vanadyl sulfate orally for four to six weeks, four reported diarrhea and two reported abdominal discomfort. Diarrhea was persistent in one patient, requiring a dose reduction to 75 mg/day after three weeks of treatment. The authors concluded that a slow titration from 50 to 150 mg/day over two weeks was responsible for the relatively low rate of gastrointestinal effects relative to other studies.
- Goldfine et al. (2000) reported that vanadyl sulfate was “relatively well tolerated” at doses of 75 to 300 mg orally daily for 6 weeks in 16 type 2 diabetics. They also noted that the 150-mg and 300-mg doses cause some gastrointestinal intolerance. No additional adverse event information was provided.
- Boden et al. (1996) reported there were “transient gastrointestinal side effects” in a study of eight non-insulin dependent diabetes mellitus (NIDDM) patients taking 100 mg/day of vanadyl sulfate orally for four weeks.
- In a study of six NIDDM patients taking 100 mg/day of vanadyl sulfate orally for three weeks, Cohen et al. (1995) reports that the treatments were “well tolerated.”
- Halberstam et al. (1996) studied seven type 2 diabetic patients receiving oral doses of 200 mg vanadyl sulfate daily for three weeks. It was reported that “except for some minor gastrointestinal discomfort and stool discoloration, subjects were relatively asymptomatic, and no one withdrew from the study.” It was also reported that no anorexic effects were observed.
- Soveid et al. (2013) followed 14 type 1 diabetics on oral vanadyl sulfate 50-100 mg three times daily for 30 months and reported diarrhea and abdominal pain in 30% of patients. Patients who experienced these effects were given a lower dose for a short period; then the dose was again titrated upward. No patients discontinued the study due to adverse events. A greenish-blue discoloration of stool was reported by a number of patients.
Hematologic parameters and hepatic and renal function tests were reported to have been unchanged throughout the study.

- Jacques-Camarena et al. (2008) reported that only one of the 7 study patients who received 100 mg of vanadyl sulfate orally for four weeks had an adverse event. This patient, who had a previous history of intestinal disorders, complained of nausea, abdominal pain, and diarrhea, which were temporary and did not require treatment interruption.

  c. Pharmacokinetic data

See section II.B.2.b for human pharmacokinetic data.

  d. The availability of alternative FDA-approved therapies

- Diabetes

A summary of FDA-approved products that have been found to be safe and effective for the treatment of diabetes is listed in Table 9, below.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Approved Drug Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulins and insulin analogs</td>
<td>Many different products and formulations</td>
</tr>
<tr>
<td>Sulfonylureas (SU)</td>
<td>Acetohexamide, chlorpropanide, tolbutamide, glipizide, gliclazide, glyburide, glimepiride</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Metformin</td>
</tr>
<tr>
<td>Thiazolidinediones (TZDs)</td>
<td>Rosiglitazone, pioglitazone</td>
</tr>
<tr>
<td>Analogues of glucagon-like peptide 1 (GLP-1)</td>
<td>Exenatide, lixisenatide, liraglutide, albiglutide, dulaglutide</td>
</tr>
<tr>
<td>Dipeptidyl peptidase 4 (DPP-4) inhibitors</td>
<td>Sitagliptin, saxagliptin, linagliptin, alogliptin</td>
</tr>
<tr>
<td>SGLT2 inhibitors</td>
<td>Empagliflozin, dapagliflozin, canagliflozin</td>
</tr>
<tr>
<td>Alpha-glucosidase inhibitors</td>
<td>Acarbose, miglitol, voglibose</td>
</tr>
<tr>
<td>Meglinides</td>
<td>Repaglinide; nateglinide</td>
</tr>
<tr>
<td>Synthetic analogues of human amylin</td>
<td>Pramlintide</td>
</tr>
<tr>
<td>Bile acid sequestrants</td>
<td>Colesevelam</td>
</tr>
<tr>
<td>Dopamine agonists</td>
<td>Bromocriptine</td>
</tr>
</tbody>
</table>

- Other nominated uses

There are a number of FDA-approved drug products that have been established to be safe and effective for the treatment of hyperlipidemia, heart disease, and various types of cancer.
Conclusions: Adverse events associated with vanadyl sulfate observed in the clinical setting are predominantly gastrointestinal complaints. Limitations of the clinical safety data available in the literature for the proposed use include small numbers of subjects, lack of safety data for the intravenous route of administration, and paucity of long-term safety data. Discolored (blue or green) stool and saliva have also been reported. However, nonclinical toxicity studies for vanadium containing compounds have exposed extensive concerns that have been amplified in the published literature as a limitation of their clinical utility.

C. Are there concerns about whether a substance is effective for a particular use?

1. Reports of trials, clinical evidence, and anecdotal reports of effectiveness, or lack of effectiveness, of the bulk drug substance

As previously explained in this review, the IOM finds that there is no known functional role for elemental vanadium in humans. Use in the treatment of nominated diseases will be discussed in the following sections.

Diabetes/Hypoglycemia

Several recent review papers have been published examining the findings of the related studies, although no Cochrane reviews of vanadium use in the treatment of diabetes were identified. No clinical studies of injectable vanadium use in diabetes were identified. Smith et al. (2008) identified clinical studies from 14 databases, experts, published study authors, and manufacturers that compared treatment with an oral vanadium containing compound to placebo in adults with type 2 diabetes (at least 10 patients per treatment arm) for a minimum of two months. There were 150 studies identified, but none met the inclusion criteria for the review. Five studies were identified in which diabetic patients received oral doses of vanadyl sulfate between 30 and 150 mg daily (Cohen et al. 1995; Boden et al. 1996; Halberstam et al. 1996; Goldfine et al. 2000; Cusi et al. 2001). Kaur and Henry (2014) reviewed the clinical literature for the use of oral supplements in type 2 diabetes and identified the same five studies of vanadyl sulfate and one additional study of sodium metavanadate (Goldfine et al. 1995). In their literature review, Domingo and Gomez (2016) identified the same six clinical studies. In general, these studies suggest treatment related effects but evidence for the efficacy of vanadium containing compounds is limited, particularly given the small number of patients and relatively short durations (i.e., two to six weeks).

As an example of the studies of oral vanadyl sulfate in diabetes, Cusi et al. (2001) enrolled 11 type 2 diabetic patients; six had been treated with diet alone prior to the study and five had been treated with a sulfonylurea, which was continued during the study. There was a four week run-in phase to establish compliance with a weight maintaining dietary regimen, followed by a two week titration phase in which daily doses were titrated from 50 mg daily to 150 mg daily as three divided doses. Patients were treated for four weeks at full doses (150 mg daily) and followed for six weeks beyond discontinuation of treatment. Five non-diabetic subjects served as controls, but were not given vanadyl sulfate. Although mean fasting plasma glucose and hemoglobin A1c
decreased during the six weeks of treatment for diabetic patients, only intra-group comparisons were assessed so it is unclear that the changes observed were attributable to vanadyl sulfate.

One study of vanadyl sulfate treatment in type 1 diabetics was identified (Soveid et al. 2013). The 14 patients were between 8 and 36 years of age (mean: 17 years). All were using insulin and characterized as having poor glycemic control. After a two month run-in, patients took 80 to 120 mg of vanadyl sulfate pentahydrate for two to five weeks, and then doses were titrated up to between 225 and 300 mg/day for 30 months. Weekly assessment visits were required for the first two months and thereafter patients were generally seen every two months. Insulin use and fasting blood sugar were reported to have declined during the 30 month treatment period, but, as with studies in type 2 diabetes, no comparison was made between active treatment and a placebo or other control group. Therefore, it is not possible to conclude that the changes observed are attributable to vanadyl sulfate.

Jacques-Camarena et al. (2008) studied 14 overweight/obese patients diagnosed with impaired glucose tolerance based on insulin sensitivity assessment, giving half of the patients a placebo dose and the rest vanadyl sulfate (100 mg/day). After 30 days of treatment, no changes in insulin sensitivity were found.

Hyperlipidemia

The potential of vanadyl sulfate to affect hyperlipidemia, a common risk factor for cardiovascular disease particularly in diabetics, has been assessed in studies of diabetic patients and healthy adults.

Cusi et al. (2001) gave 150 mg/day of vanadyl sulfate to 11 type 2 diabetics for six weeks. Improvements in low-density lipoprotein (LDL) and total cholesterol were observed but there was no change reported in high-density lipoprotein (HDL) or triglycerides. Only intra-group comparisons were made, so it is not possible to conclude that the changes observed are attributable to vanadyl sulfate.

Boden et al. (1996) reported no changes in basal rate of lipolysis or plasma free fatty acids among four vanadyl sulfate (100 mg/day) type 2 diabetic recipients or six placebo recipients, after four weeks of treatment.

Soveid et al. (2013) reported that in type 1 diabetics treated for 30 months (see prior description of dosing), mean total cholesterol fell from $232 \pm 30$ to $180 \pm 22$ mg/dL, but triglycerides were described as not having significantly changed.

Jacques-Camarena et al. (2008) studied 14 overweight/obese patients diagnosed with impaired glucose tolerance, giving half of the patients placebo and the rest vanadyl sulfate (100 mg/day). After 30 days of treatment, the vanadyl sulfate group had increased triglycerides compared to placebo ($1.7 \pm 0.5$ versus $1.5 \pm 0.5$ mmol/L). Total cholesterol, HDL and LDL were not different between the vanadyl sulfate and placebo groups.
Fawcett et al. (1997) gave 0.5 mg/kg/day vanadyl sulfate (dose range 5 – 14 mg) (n=15) or placebo (n=16) to weight training athletes for 12 weeks. The investigators concluded that no changes in HDL, triglycerides, or total cholesterol occurred.

Heart disease

Heart disease is defined by the American Heart Association as a spectrum of conditions. Many are related to atherosclerosis, such as coronary artery disease, myocardial infarctions, or stroke. Other examples include congestive heart failure, arrhythmia, cardiomyopathy, and heart valve disease. No clinical studies of vanadyl sulfate, or other vanadium containing compounds, in heart disease have been identified.

Prevention of cancer

In vitro, nonclinical in vivo, and human ex vivo studies suggest that vanadium containing compounds could have a role in prevention or treatment of cancer. However, no clinical studies of vanadium containing compounds for these uses have been identified (Desoize 2004; Bishayee et al. 2010).

2. Whether the product compounded with this bulk drug substance is intended to be used in a serious or life-threatening disease

Diabetes, heart disease, and cancer are serious, chronic diseases that can result in life-threatening complications, particularly if untreated.

3. Whether there are any alternative approved therapies that may be as effective or more effective.

A summary of FDA-approved products was presented in section II.B.4.d above.

Conclusions: Despite the expectation based on mechanism of action theories that various vanadium containing compounds could play a therapeutic role in the treatment or prevention of diabetes/hyperglycemia, hyperlipidemia, heart disease, or cancer, there is insufficient evidence that vanadyl sulfate is effective in treating any of these diseases.

D. Has the substance been used historically in compounding?

The following databases were consulted in preparation of this section: PubMed, Natural Medicines, European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, USP/NF, and Google.

1. Length of time the substance has been used in pharmacy compounding

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14 https://www.goredforwomen.org/about-heart-disease/facts_about_heart_disease_in_women-sub-category/causes-prevention/
Vanadium containing compounds were used as early as the 19th century for treating anemia, tuberculosis, chronic rheumatism, and diabetes (Willsky 1990). From the literature, it appears that vanadyl sulfate has been used in compounded drug products (as a capsule) for at least 17 years (Loyd 2000). We were unable to find any information regarding the length of time it has been compounded as an injection.

2. The medical condition(s) it has been used to treat

The Natural Medicines Database reports that vanadium containing compounds are used orally for diabetes, hypoglycemia, hyperlipidemia, heart disease, edema, improving athletic performance in weight training, and preventing cancer. It also reports that vanadium containing compounds are used more generally for treating tuberculosis, diabetes, syphilis, and a form of microcytic anemia (chlorosis) (Natural Database 2013).

Results from a Google search using the terms vanadium compounding pharmacy and vanadyl sulfate compounding pharmacy indicate that vanadyl sulfate has been compounded as a capsule alone or in combination with other ingredients for treating diabetes (Harshbarger and Harshbarger 2000). In addition, at least one pharmacy advertised “vanadium” as a component of intravenous remineralization.

3. How widespread its use has been

Insufficient data are available from which to draw conclusions about the extent of use of vanadyl sulfate in compounded drug products.

4. Recognition of the substance in other countries or foreign pharmacopeias


Conclusions: Information is insufficient to determine the historical use of vanadyl sulfate in pharmacy compounding. Based on internet searches, it appears that compounding pharmacies have been preparing vanadyl sulfate in oral and intravenous forms.

III. RECOMMENDATION

We have reviewed the criteria described in section II above to evaluate vanadyl sulfate for the 503A Bulks List. After considering the information currently available, a balancing of the criteria weighs against vanadyl sulfate being placed on that list based on the following:

1. Vanadyl sulfate is an inorganic vanadium salt and it is very likely to be stable under ordinary storage conditions as an injection solution. The nominated compound is easily characterized and the preparation of this compound has been well developed.
2. Nonclinical safety data found in the literature suggest the potential for a high toxicity profile for vanadyl sulfate based on toxicokinetics (accumulation in several organs), chronic toxicity data (ranging from death at high doses to renal, hematotoxic, neurologic toxicities), reproductive data (impairment male fertility, embryotoxicity, pre/postnatal malformations), and carcinogenicity data (increased incidence of tumors in male and female mice, and in female rats). The nominated route of administration (injection) appears to be associated with greater toxicity than the oral route of administration. Human safety data are limited and do not reveal the same types or degrees of toxicity that are shown in nonclinical testing. The differences between nonclinical and clinical safety findings may be the result of gaps in the human safety data, including limited systemic exposure to vanadyl sulfate because the data come from oral dosing studies, limited safety assessments (e.g., for accumulation in organs), and limited duration of treatment.

3. Clinical efficacy data, from oral dosing studies, provide preliminary evidence that vanadyl sulfate or other vanadium containing compounds could have an effect in treating diabetes/hyperglycemia or hyperlipidemia. We identified no clinical efficacy data for cancer or heart disease. There is insufficient evidence to indicate that vanadyl sulfate has any efficacy in treating any of these conditions.

4. Information is insufficient to determine the historical use of vanadyl sulfate in compounding.

Based on the information the Agency has considered, a balancing of the four evaluation criteria weighs against vanadyl sulfate being added to the 503A Bulks List.
REFERENCES


Domingo JL, Gomez M, Llobet JM et al. 1991. Oral vanadium administration to streptozotocin-diabetic rats has marked negative side-effects which are independent of the form of vanadium used. Toxicology 66:279-287.


Narla RK, Chen CL, Dong Y et al. 2001. In vivo antitumor activity of bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV) (METVAN [VO(SO₄)(Me₂-Phen)₂]). Clin Cancer Res 7:2124-2133.


Appendix 1. Additional nonclinical toxicity studies conducted with various vanadium containing compounds other than vanadyl sulfate

One-month neurotoxicity study in mice

Male C57BL/6 mice were intranasally administered 182 μg vanadium pentoxide (VP, V₂O₅) three times a week for 1 month, and locomotor activity, neurochemical and biochemical tests of olfactory bulb were performed one week after the last dosing (Ngwa et al. 2014). When compared with controls, there were significant decreases in the locomotor activities (68% decrease in total vertical movement, 57% decrease in total distance travelled, and 54% decrease in total movement time) and olfaction (spending 63% less time sniffing during a 5-minute testing session) in the VP group. In addition, olfactory bulbs from the VP group had dopaminergic neuron loss; decreased levels (82-88% decreases) of tyrosine hydroxylase, and dopamine and its metabolite, 3, 4-dihydroxyphenylacetic acid; and increased astroglia, indicating degeneration of dopaminergic neurons and neuroinflammation. Dopaminergic neurotoxicity of VP was also demonstrated in dopaminergic neuronal cells.

Six-week hemotoxicity study in rats

Sodium metavanadate (SMV, NaVO₃) was orally administered in drinking water ad libitum to male Wistar rats (n=11-16/group) for 6 weeks. The main findings include alteration in red blood cell count (RBC) and hemoglobin (Hb) values for SMV treated rats (Scibior 2005; Scibior et al. 2006). When compared with concurrent controls, significant decreases in mean corpuscular hemoglobin (MCH) values were noted in both studies. A significant increase in RBC was noted in the study with lower SMV intake (8.35 ± 0.7 mg elemental vanadium/kg/day) while a decrease in the study with higher SMV intake (10.69 ± 0.79 mg V/kg/day). In addition, significant decreases in Hb concentration and mean corpuscular volume (MCV) values were noted in the study with higher SMV intake (10.69 ± 0.79 mg V/kg/day). As described in the review above, no significant changes in RBC parameters were noted for vanadyl sulfate in a 12 week study (Dai et al. 1995). The difference in homological findings may be related to differences in forms of vanadium, doses, durations of doing, and ages of rats.

Three-month repeat dose toxicity study in rats

Sprague-Dawley rats were treated with SMV (5, 10, or 50 ppm) in drinking water ad libitum for 3 months (Domingo et al. 1985).

Findings included:
- Dose related increases in plasma total protein, urea and uric acid concentrations were noted (statistically significant at 50 ppm only).
- Dose-related histopathological lesions of kidneys (corticomedullar microhemorrhagic foci), spleen (hypertrophy and hyperplasia), and lungs (mononuclear cell infiltration, mainly perivascular).
- Dose related accumulation of SMV was noted in kidneys and spleen (10 ppm) and in liver, kidneys, heart, spleen, and lungs (50 ppm).
Eight-week neurobehavioral rat study

A limited neurobehavioral oral study was conducted for SMV (0, 4.1, 8.2, or 16.4 mg/kg/day representing 1.71, 3.42, or 6.84 mg elemental vanadium/kg/day) in adult male Sprague-Dawley rats (n=12/group) for 8 consecutive weeks (Sanchez et al. 1998). Significant learning disability was noted in all SMV groups. Significant reductions in general activity during the first 5 min (but not at later time period) were noted in the 8.2, or 16.4 mg SMV/kg/day groups. Decreased body weight gain was observed in the 16.4 mg/kg/day group from weeks 3 and lasted at week 11 (three weeks after dosing cessation). Dose-related accumulation of SMV was observed in all tissues measured on week 11 of the study (liver, spleen, kidneys, brain, bone, and muscle).

Postnatal development day 10-21 in rats

The postnatal developmental toxicity of SMV was evaluated in a small Wistar rat study (Soazo and Garcia 2007). In this study, dams (4 dams with 4 litters/group) were intraperitoneally injected 0 or 3 mg SMV/kg/day (1.25 mg elemental vanadium/kg/day) in distilled water from the 10th to the 21st postnatal day (PND). SMV exposure caused significant delays in eye opening, decreases in forelimb support and locomotor activity when compared to the controls. Decreased myelin fiber density in corpus callosum and cerebellum were also reported in the SMV group, suggesting a delay in the CNS development.

NTP inhalation carcinogenicity studies for vanadium pentoxide in rats and mice

F344/N rats and B6C3F1 mice (n=50/sex/species/group) were exposed to VP at concentrations of 0, 0.5 (rats only), 1, 2, or 4 (mice only) mg/m³, by whole-body inhalation for 6 hours per day, 5 days per week for 104 weeks (NTP 2002). Alveolar/bronchiolar (A/B) neoplasms (adenoma or carcinoma) were significantly increased in all mice exposed to VP ($p \leq 0.01$; 22/50, 42/50, 43/50, or 43/50 in the 0, 1, 2, or 4 mg/m³ groups, respectively). Increased incidences of A/B neoplasms were also noted in male rats exposed to 0.5 and 2 mg/m³. A marginal increase in A/B neoplasms was also observed in female rats exposed to 0.5 mg/m³. Increases in chronic inflammation, interstitial fibrosis, alveolar and bronchiolar epithelial hyperplasia, and squamous metaplasia were also observed in both mice and rats. Similar survival rates were noted except significantly lower survival in male mice exposed to 4 mg/m³ (27/50 vs. 39/50 in controls). Reduced body weights were noted in male mice exposed to 4 mg/m³ and exposure concentration related body weight decrease in all female mice but not in rats and male mice with lower exposures. Abnormal breathing was observed particularly in mice exposed to 2 or 4 mg/m³. These results are considered positive in mice and male rats (increased the incidence of lung neoplasms) but equivocal in female rats.
Appendix 2. Proximal renal tubular function changes, potentially from vanadium impurity in albumin

Heinemann et al. (2000) examined the possibility that vanadium, found as an impurity in albumin solutions, could cause renal damage in patients with existing reduced renal function. The case of a 60-year-old male who underwent an elective coronary bypass revascularization was reported.

In the intensive care unit, on the first postoperative day, the patient received 70 mL of a 20% albumin solution that was administered over 20 minutes via a central venous catheter. The albumin solution contained a 677 μg/L of vanadium with a free fraction of 299 μg/L; constituting a total dose of 47.4 μg intravenous vanadium. During his hospital stay, his renal function was assessed as clinically stable as laboratory evaluations of serum creatinine remained between 0.8 and 1.3 mg/dL. Through the measurement of peak and periodic vanadium blood levels, the authors estimated the vanadium half-life to be approximately 125 hours in this patient. Urinary vanadium excretion rates were also measured. The authors also recorded urinary excretion rates of α₁ microglobulin (α₁-M), N-acetyl-β-D-glucosaminidase (β-NAG), α-glutathione S-transferase (α-GST), and π-glutathione S-transferase (π-GST) as markers of proximal and distal renal tubular damage in this patient.

These markers were also observed in six patients undergoing “similar surgical procedures,” but without administration of albumin solution; used as a control group. The urinary excretion of the microproteins α₁-M and β-NAG, as expected (Dehne et al. 1995), were elevated in all seven patients for several days. However, in the albumin treated individual, α-GST was excreted at a rate over that of the control patients starting about 17 hours after the infusion and reaching a peak level on day three. The albumin treated patient’s mean urine levels of α-GST reached 9 times the upper limit of normal, and was statistically significantly different from that seen in the control group. In the albumin treated patient, the increased excretion rates of π-GST followed the time course of α-GST excretion; however, at peak, only marginally exceeded the upper reference limit. The α-GST excretion rates in the control group were below the detection limit in all urine samples. The authors postulate that the proximal tubular function of the patient given albumin may have been adversely affected by the vanadium secondary to the patient’s reduced renal function as consequence of his cardiac surgery. They also stipulated that α-GST was possibly a more sensitive marker than α₁-M and β-NAG of proximal tubular damage. The authors followed π-GST levels as a marker for possible distal tubular damage.
Tab 6

Artemisinin
Tab 6a

Artemisinin Nominations
Nominated by: McGuff Compounding Pharmacy Services, Inc., Alliance for Natural Health USA, American Association of Naturopathic Physicians, and Integrative Medical Consortium

<table>
<thead>
<tr>
<th>Column A—What information is requested?</th>
<th>Column B—Put data specific to the nominated substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the name of the nominated ingredient?</td>
<td>ARTEMISIA / ARTEMISININ</td>
</tr>
</tbody>
</table>
| Is the ingredient an active ingredient that meets the definition of “bulk drug substance” in § 207.3(a)(4)? | Yes. There is ample information in Pubmed. Please access this link: [http://www.ncbi.nlm.nih.gov/pubmed/19588433](http://www.ncbi.nlm.nih.gov/pubmed/19588433)  
Artemisinin-based combination therapy for treating uncomplicated malaria.  
Sinclair D1, Zani B, Donegan S, Olliaro P, Garner P.  
Author Information: International Health Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK, L3 5QA.  
Abstract  
BACKGROUND: The World Health Organization recommends uncomplicated P. falciparum malaria is treated using Artemisinin-based Combination Therapy (ACT). This review aims to assist the decision making of malaria control programmes by providing an overview of the relative benefits and harms of the available options. |
| Is the ingredient listed in any of the three sections of the Orange Book? | Not for ARTEMISIA / ARTEMISININ |
| Were any monographs for the ingredient found in the USP or NF monographs? | Not for ARTEMISIA / ARTEMISININ |
| What is the chemical name of the substance? | ARTEMISIA / ARTEMISININ |
| What is the common name of the substance? | ARTEMISIA / ARTEMISININ |
| Does the substance have a UNII Code? | 9RMU91N5K2 (artemisinin) |
| What is the chemical grade of the substance? | Not graded |
| What is the strength, quality, stability, and purity of the ingredient? | A valid Certificate of Analysis accompanies each lot of raw material received.  
Artemisinin is supplied as a white, crystal powder extract. |
| How is the ingredient supplied? | TSCA Chemical Inventory  
(EPA): This product is NOT on the EPA Toxic Substances Control Act (TSCA) inventory.  
WHMIS Classification (Canada): Not controlled under WHMIS (Canada). |
<p>| Is the substance recognized in foreign pharmacopeias or registered in other countries? | Information not known |
| Has information been submitted about the substance to the USP for consideration of monograph development? | Information not known |
| What dosage form(s) will be compounded using the bulk drug substance? | Oral, injection |
| What strength(s) will be compounded from the nominated substance? | Capsule strength can range from 50 mg to 500 mg per capsule. Injectable have been compounded at 60 mg, 120mg, 180mg, 240mg |
| What are the anticipated route(s) of administration of the compounded drug product(s)? | Oral, injection |</p>
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has the bulk drug substance been used previously to compound drug product(s)?</td>
<td>Yes</td>
</tr>
<tr>
<td>What is the proposed use for the drug product(s) to be compounded with the nominated substance?</td>
<td>Malaria, Helminthes including many worms and protozoa, particularly toxoplasmosis, gram positive infections both gastrointestinal and chronic elsewhere, Streptococcus spp. and Enterococcus spp causing GERD, stomach ulcers. (Topic for cancer tumor research and liver cancer.)</td>
</tr>
<tr>
<td>What is the reason for use of a compounded drug product rather than an FDA-approved product?</td>
<td>Malaria has become drug resistant in many areas so more tools are needed, Artemesia increases efficacy of the available and can be used independently. Generics such as Mebendazole has been discontinued in the US. Artemesia is more successful in eradicating toxoplasmosis than FDA drugs available. Ant biotics often causes overgrowth and reoccurrence of infections while properly dosed. Artemisia can kill gram positive gastrointestinal infections, showing clean stool panels on re-test.</td>
</tr>
</tbody>
</table>
Estimate patient population: 10-20% of ulcers, heartburn patients would not find resolution of their symptoms with FDA approved drug products.

Kangwan N, Park JM, Kim EH, Hahm KB.

At least 30% of patients who have had undiagnosed toxoplasmosis have already been treated with several rounds of antibiotics. About 10% would not tolerate the excipients or preservatives from making the pyrimethamine or sulfadiazine.

Isolated study on Toxoplasmosis treatment with Artemisia:
Toxoplasma gondii: effects of Artemisia annua L. on susceptibility to infection in experimental models in vitro and in vivo.
de Oliveira TC1, Silva DA, Rostkowska C, Bêla SR, Ferro EA, Magalhães PM, Mineo JR.

Review demonstrates the anti-inflammatory effects and inhibition of viruses (e.g. Human cytomegalovirus), protozoa (e.g. Toxoplasma gondii), helminths (e.g. Schistosoma species and Fasciola hepatica) and fungi (e.g. Cryptococcus neoformans).
Artemisinins: pharmacological actions beyond anti-malarial.
Ho WE1, Peh HY2, Chan TK3, Wong WS4.
Tab 6b

FDA Review of Artemisinin
I. INTRODUCTION

Artemisinin has been nominated for inclusion on the list of bulk drug substances for use in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for use in the treatment of malaria, helminthic infections, protozoal (particularly toxoplasmosis) infections, gram positive infections, *Streptococcus* spp. and *Enterococcus* spp. causing gastroesophageal reflux disease, stomach ulcers, and cancer. The nominated routes of administration are oral administration and injection. This review focuses on only its use in treating malaria, helminthic infections, protozoal (particularly toxoplasmosis) infections, stomach ulcers, and cancer because adequate support was not provided for the other nominated uses.

We have reviewed available data on the physicochemical characteristics, safety, effectiveness, and historical use in compounding of this substance. For the reasons discussed below, we believe the evaluation criteria weigh against placing artemisinin on the list of bulk drug substances that can be used to compound drug products in accordance with section 503A of the FD&C Act (503A Bulks List).

II. EVALUATION CRITERIA

A. Is the substance well characterized, physically and chemically, such that it is appropriate for use in compounding?

*Chemical structure*

The chemical structure of artemisinin and its derivatives are shown below.
Artemisinin, the parent compound and its semi-synthetic derivatives are a family of sesquiterpene trioxane lactone agents that have been investigated largely for their anti-malarial activity against *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) in malaria treatment programs worldwide. In addition, artemisinin and its derivatives have been investigated for inhibitory effects against *Toxoplasma gondii*, schistosomal infections and as a treatment option for gastric ulcers and various cancers. The nomination identified oral capsules ranging in strength from 50 mg to 500 mg per capsule and injections at 60 mg, 120 mg, 180 mg, and 240 mg as the anticipated compounded dosage forms. This memo focuses on artemisinin. As will be discussed throughout the document, information for some of the derivatives is also provided because the mode of action is thought to be similar among all the substances.

Databases searched for information on artemisinin in regard to this section of the review include PubMed, SciFinder, Analytical Profiles of Drug Substances, the European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, and the United States Pharmacopeia (USP)/NF.

1. **Stability of the API and likely dosage forms**

Although artemisinin contains a reactive peroxide group, it is surprisingly stable under neutral conditions. It is stable up to 200°C for 2.5 min or 150°C in neutral solvents (Lin et al. 1985; Zeng et al. 1983). However, artemisinin is very labile in acidic or basic environments due to its lactone nature (Ho et al. 2004). The estimated shelf life of artemisinin reported in the literature is about 3 years once kept under nitrogen atmosphere (Xiao et al. 2013). There are no further literature reports discussing the effect of oxygen and humidity on the stability of artemisinin. It is likely that artemisinin will be stable under ordinary storage conditions.

2. **Probable routes of API synthesis**
Although a total synthesis has been established for artemisinin, it is not yet feasible for industrial application due to its complexity and low yield (Avery et al. 1987). Currently, the majority of artemisinin supply still comes from the extraction of the plant *Artemisia annua*, commonly called Chinese wormwood or sweet wormwood.¹ Artemisinin concentration is highest in the leaves and inflorescences of *Artemisia annua*, and it is usually extracted with hexanes, petroleum ether, or other organic solvents. Over the years, many extraction methods have been developed with different solvent systems and different manufacturing procedures (Hale et al. 2007; Christen et al. 2001; Elsohly et al. 1990; Kumar et al. 2004).

3. **Likely impurities**

Possible impurities may include:

- Residual solvents from the extraction process and purification procedures, such as hexanes and petroleum ether; and
- Trace amount of degradation product of artemisinin (ring-opening reaction of the lactone functional group).

4. **Toxicity of those likely impurities**

Residual solvents such as toluene may be highly toxic, depending on specific manufacturing processes. The degradation products are unlikely to be highly toxic. Further characterization of the toxicity of these substances is not warranted, pending the availability of additional information about the likelihood of their presence and amounts in the bulk drug substance.

5. **Physicochemical characteristics pertinent to product performance, such as particle size and polymorphism**

Artemisinin is a colorless crystal that is practically insoluble in water. It has been reported that artemisinin is insoluble in olive oil, which is used as a solvent to make intramuscular injections.² No further information on the influence of particle size and polymorphism on bioavailability was found in the literature.

6. **Any other information about the substance that may be relevant, such as whether the API is poorly characterized or difficult to characterize**

¹ *Artemisia annua* is commonly known as *Chinese wormwood* or *sweet wormwood* and is also commonly referred to as *sweet annie*. All of these common names might be used by growers, gardeners, in literature, or by herbalists to refer to *Artemisia annua*. It is important not to confuse the above common names with wormwood or absinthe (*Artemisia absinthium*) and mugwort (*Artemisia vulgaris*), as these two species have different uses compared to *Artemisia annua*, despite all three being in the same genus, *Artemisia* (Natural Medicines, 2017).

² Throughout the literature, the oil solubility of artemisinin is characterized as poor without specific reference to a source (Woodrow et al. 2005).
Artemisinin has been characterized with proton nuclear magnetic resonance ($^1$H NMR) spectroscopy, Carbon-13 nuclear magnetic resonance ($^{13}$C NMR) spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and mass spectrometry (MS).

**Conclusions:** Artemisinin is a natural product. The compound is likely to be stable under ordinary storage conditions. The nominated compound is easily characterized with various analytical techniques, and the production of this compound has been well established.

**B. Are there concerns about the safety of the substance for use in compounding?**

The safety and efficacy sections of this review provide a summary of information on artemisinin and its derivatives. The derivatives were developed because of limited solubility, especially for parenteral formulations, low bioavailability, and a short half-life of artemisinin by various routes of administration. The artemisinin structure contains an endoperoxide bridge, which is thought to be the part of the structure responsible for the activity against malaria. All of the derivatives maintain this endoperoxide bridge structure. As such, any efficacy or safety associated with the derivatives may be relevant to the consideration of efficacy and safety for artemisinin. There is a greater wealth of clinical data available for the derivatives than there is for artemisinin.³

1. **Nonclinical assessment**

The nonclinical data discussed below are derived from public information collected from the following databases: PubMed, TOXLINE, and DART. In addition, the following World Health Organization (WHO) online publications were used as references⁴:

- Assessment of the safety of artemisinin compounds in pregnancy (Nov. 2007),
- WHO Prequalification Programme Guideline, and

   a. Pharmacology of the drug substance and its likely impurities

**Artemisinin and Derivatives**

Artemisinin, also known by its Chinese name *qinghaosu*, is a botanical compound isolated from the leaves of the plant *Artemisia annua* that has been used in traditional Chinese medicine for the treatment of fever. In the 1970s, artemisinin was isolated in a Chinese herbal drug discovery program seeking to find chemicals with activity against *Plasmodium* species (Ho et al. 2014). Though artemisinin is potent, its short half-life, poor bioavailability, and reliance on plant cultivation spurred a search for semi-synthetic and completely synthetic alternatives (Ho et al.

³ More details on the studies conducted with artemisinin derivatives can be found in the appendices of this review. This review, however, evaluates only artemisinin and does not address the eligibility of artemisinin derivatives for use in compounded drug products.

⁴ The WHO publications can be found on these links: http://apps.who.int/iris/bitstream/10665/43797/1/9789241596114_eng.pdf, and http://apps.who.int/prequal/info_applicants/Guidelines/Nonclinical_Overview_Artemisinin-Derivatives.pdf
Commonly available artemisinin derivatives include artemether, artesunate, artemether, and dihydroartemisinin (also known as artenimol). Dihydroartemisinin is also an active metabolite of some of the derivatives.

Artemisinin and its semi-synthetic derivatives have been investigated largely for their anti-malarial activity against *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) in malaria treatment programs worldwide. In addition, artemisinin and its derivatives have been investigated for inhibitory effects against *Toxoplasma gondii* and schistosomal infections, as well as a treatment option for gastric ulcers and various cancers.

### Mechanism of Action

#### Effects on *Plasmodium* (antimalarial)

Although not completely understood, the antimalarial activity of the artemisinin class is believed to be a result of their highly reactive peroxide bridge, which has been shown to generate reactive oxygen species (Fenton reaction) and carbon-centered radical molecules that bind and modify proteins and DNA of *Plasmodium* parasites, causing death to the microorganism (Efferth and Kaina 2010; O’Neill et al. 2010). The sarcoplasmic endoplasmic reticulum ATPase gene of *Plasmodium* has been suggested as a possible parasite-specific target, which is targeted by the endoperoxide bridge of artemisinin and its derivatives. The heme iron found within the parasite catalyzes the cleavage of the endoperoxide bridge. Then, carbon-centered radicals form that alkylate and damage the macromolecules in the parasite by damaging the enzyme sarcoplasmic-endoplasmic reticulum calcium ATPase (SERCA), which is apparently critical for parasite survival (Medhi et al. 2009). In addition, artemisinin and its derivatives may exert their antimalarial effects as well as effects on other non-plasmodial cells by inhibiting a calcium ATPase (PfATP6) gene encoded by *P. falciparum*, which is similar to SERCA; mutations in PfATP6 may mediate resistance to artemisinin (Krishna et al. 2010). A similar mechanism of action is proposed for the therapeutic effects of artemisinin and its derivatives in treating *Toxoplasma gondii* and *Schistosoma* infections.

Artemisinin is effective against the asexual erythrocytic forms of *P. falciparum* and *P. vivax*, but it is inactive against extra-erythrocytic forms (sporozoites, liver schizontes or merozoites). In vitro activity of artemisinin against *P. falciparum* typically showed EC$_{50}$ (half maximal effective concentration) values at or below 10 nM, demonstrating parasite sensitivity to artemisinin (Gupta et al. 2001; Ramharter et al. 2002; Sponer et al. 2002). In vivo efficacy was demonstrated in mice, rats, and monkeys infected with drug-sensitive *P. berghei* and *P. knowlesi* malaria strains. Nonclinical models infected with the human malarial strain, *P. falciparum*, are difficult and costly to develop, making extrapolation from nonclinical models to human infections challenging (WHO 2003).

When used as a monotherapy to treat malaria, artemisinin results in incomplete parasite clearance unless high dosages are used over several days. This approach may reduce compliance and increase the risk of toxicity. The use of artemisinin derivatives results in rapid clearance of the malaria parasites from the blood because these are fast-acting substances; however, their short biological half-life precludes a long-lasting activity. For this reason, artemisinin and its
derivatives are preferably used in combination with longer acting drugs that have a slower onset of activity. Combination therapy with other antimalarial agents also helps in preventing drug resistance and offers complete and rapid eradication of the parasite, thus reducing survival of resistant strains (Medhi et al. 2009).

The WHO Roll Back Malaria Programme recommends a combination therapy strategy, where artemisinin containing therapies (ACT) are used wherever emerging, high-resistant infections to the most commonly used antimalarial agents are encountered (WHO 2003).

Effects on helminthic infections (anti-schistosomiasis)

Helminths, specifically trematode worms of the genus *Schistosoma*, cause schistosomiasis in tropical areas. Artemisinin and its derivatives, such as artemether, artesunate, and dihydroartemisinin, have shown some inhibitory effect against the schistosomal infection, including *S. mansoni*, *S. haematobium*, and *S. japonicum* in animals when used at high doses (Ho et al. 2014; Saeed et al. 2016). Specifically, in vivo studies revealed that artemether orally administered to mice and hamsters at a dose of 300 mg/kg caused a dose- and time-dependent inhibition of *S. mansoni*, *S. haematobium* and *S. japonicum* infections (Shuhua et al. 2000a; Shuhua et al. 2000b; Xiao et al. 2000). In one study, a single oral dose of artemether reduced total *S. mansoni* numbers with the highest worm reduction rates seen when artemether was given on day 14 (75%) or day 21 (82%) after infection in mice (Xiao et al. 2000). In another experiment, it was shown that the highest worm reduction rates (≥ 94%) were achieved when artemether was given in 3 to 5 doses weekly, with a 100% worm reduction rate after 5 weekly doses of artemether (on days 7, 14, 21, 28 and 35 after infection) (Xiao et al. 2000).

Other treatment options conducted with artemisinin derivatives including eight different combinations of artemether, artesunate and dihydroartemisinin (in various sequences administered on three separate days or as a mixture of all three compounds on three consecutive days) reduced the total *S. japonicum* burden by 79.5–86% when given on days 6-8 post-infection, or by 73.8-75.7% when given on 34–36 post-infection in mice at 300 mg/kg/day (Li et al. 2011).

Artemether and artesunate administered to female mice at 300 mg/kg/day appear to have inhibitory effects against praziquantel-resistant *S. japonicum*, where they reduced the total worm burden in mice infected with praziquantel-sensitive *S. japonicum* or mice infected with the resistant isolate (approximately 74 % for both when artesunate was given on days 7–8 post infection) (Wang et al. 2014).

In summary, the mechanism of action of artemisinin and its derivatives in the treatment of schistosomiasis appears to be related to the cleavage of its endoperoxide moiety in the presence of ferrous iron leading to metabolic and oxidative stress in worms, morphological damage, reduced egg and worm rates, as well as reduced granuloma rates in the host (Saeed et al. 2016; Utzinger et al. 2007).

Effects on helminthic infections other than schistosomiasis
An in vitro study was conducted to assess trematocidal effects of *Artemisia annua* and other plant derivatives against *S. mansoni*, *Fasciola hepatica*, and *Echinostoma caproni*. Although this study was able to show fasciocidal activity of a pure form of artemisinin at a dose of 2 mg/ml, the authors caution that it would be difficult to correlate in vitro with in vivo activity given the trematodes colonize the liver, bile duct, etc. and whether or not there would be accumulation of plant secondary metabolites in such circumstances is unknown (Ferreira et al. 2011).

Artemether and artesunate were found to be effective against *F. hepatica*, *C. sinensis*, and *E. caproni* in rodent infection models (Keiser and Utzinger 2007). Artemether and artesunate in sheep infected with *F. hepatica* were able to achieve worm burden reduction; 91% at a dose of 140 mg/kg im, and 92% at a dose of 40 mg/kg, respectively, although embryotoxicity and toxicity were of potential concern (Keiser et al. 2010; Keiser and Utzinger 2007).

Effets on *Toxoplasma gondii* (*T. gondii*)

*T. gondii* is an obligate, intracellular, apicomplexan protozoan which can cause a chronic asymptomatic infection (de Oliveira et al. 2009; Ho et al. 2014). Artemisinin and its derivatives affect different steps of *T. gondii*’s life cycle by inhibiting replication, growth, and attachment to and invasion of host cells (D'Angelo et al. 2009). In vitro studies showed that artemisinin and one of its derivatives, artemether, inhibited *T. gondii* replication (D'Angelo et al. 2009; Hencken et al. 2010; Ho et al. 2014; Jones-Brando et al. 2006). In two separate references, artemisinin had an IC$_{50}$ (50% inhibitory concentration) of 0.64 μMand 8.0 μM against *T. gondii* and a TC$_{50}$ (50% cytotoxic concentration) ≥320 μM or >1,130 μM, respectively (Hencken et al. 2010; Jones-Brando et al. 2006). Artemether had an IC$_{50}$ of 0.31 μM and 0.7 μM, while the TC$_{50}$ was ≥320 μM and 740 μM, respectively (Hencken et al. 2010; Jones-Brando et al. 2006).

Nonperoxidic artemether derivatives did not show an inhibitory effect against *T. gondii* (Jones-Brando et al. 2006), indicating that the endoperoxidic structure is critical to the inhibitory effect against *T. gondii*. Artemisinin-induced calcium-dependent protein secretion and perturbed calcium homeostasis in *T. gondii* seems to be mediated by an inhibition of the parasite sarcoplasmic endoplasmic reticulum Ca$^{2+}$ ATPase (SERCA) leading to Ca$^{2+}$ overload and growth inhibition. This calcium-mediated mechanism is considered to be a key action for the inhibitory activity for *T. gondii* (Ho et al. 2014; Nagamune et al. 2007).

The prophylactic and treatment effects of artemether on *T. gondii* were evaluated under in vitro conditions in two experiments. In the first experiment, Vero cells (African green monkey kidney cells) were infected with *T. gondii* tachyzoites and then treated with artemether; in the second experiment, tachyzoites were exposed to artemether, then Vero cells were infected with treated tachyzoites. Results showed that in both situations, while showing low cytotoxicity on cell lines, artemether produced a higher inhibitory effect than sulfadiazine (positive control), and apoptosis in tachyzoites rose with increasing concentrations of artemether. Although pretreatment of tachyzoites with artemether was more effective compared to sulfadiazine, when artemether was used to treat Vero cells already infected with *T. gondii*, both drugs showed similar efficacy (Mikaeiloo et al. 2016). The authors concluded that the presence of the endoperoxide bridge in artemether may help eliminate the bradyzoite stage of *T. gondii* (sulfadiazine and pyrimethamine
are ineffective against bradyzoite stages). Under the conditions of these experiments, artemether showed both a prophylactic and a treatment effect against *T. gondii*.

Synthesized novel unsaturated, carba (carbon) derivatives of artemisinin were tested in vitro for their activity against *T. gondii* tachyzoite growth inhibition and cytotoxicity, and inhibition of parasite invasion of host cells (D'Angelo et al. 2009). Some of the derivatives tested were able to inhibit *T. gondii* growth and replication and reduce attachment to the host cells and invasion of host cells effectively in the absence of cytotoxicity.

In particular, two of the new generation artemisinin derivatives, artemiside and artemisone, displayed better inhibition than artemisinin or artesunate against *T. gondii* in vitro. When compared to artesunate or dihydroartemisinin, artemisone, a second-generation artemisinin derivative obtained from dihydroartemisinin, showed less neurotoxicity in vitro and in vivo (Dunay et al. 2009).

The ability of the new artemisinin derivatives to inhibit multiple steps of *T. gondii*'s lytic cycle is associated with a mobilization of intracellular calcium stores within the parasites leading to premature secretion of the parasite’s microneme protein MIC2, which is essential for invasion of host cells.

The efficacy of the new artemisinin derivatives was tested in mouse models of acute and chronic toxoplasmosis. Although the acute model showed modest dose and vehicle dependent efficacy, the chronic model using the thiozole artemisinin derivative showed decreased mouse brain cyst burden and was apparently more effective than artemether (Schultz et al. 2014).

In summary, artemisinin is not generally used to treat *T. gondii* because newer derivatives of artemisinin have been developed that promise a better therapeutic profile against *T. gondii* in the absence of cytotoxicity.

**Effect on gastric ulcers**

The use of *Artemisia* extracts (in isopropanol or ethanol) to improve the “quality of ulcer healing (QOUH)” index is based on the potential mechanism of efficient remodeling of regenerated gastric mucosa; intervention of several growth factors; abundant gastric mucins, including trefoil proteins like trefoil peptide (pS2/TFF1); and significant suppression of inflammatory cytokines like IL-2, TNF-alpha, COX-2 and nitrosative stress. The oxidative and anti-inflammatory actions of *Artemisia* extracts are also listed as contributing properties towards QOUH action in gastric ulcer models (Kangwan et al. 2014).

The mechanism of action for the anti ulcerogenic activity of three sesquiterpene lactones artemisinin (compound 1), dihydro-epideoxyarteannuin B (compound 2) and deoxyartemisinin (compound 3) isolated from *Artemisia annua* L. was evaluated using either an ethanol or an indomethacin (a cyclooxygenase inhibitor) induced ulcer model in male Wistar rats at a dose of 100 mg/kg. Artemisinin compounds were isolated from the sesquiterpene lactone-enriched fraction obtained from the crude ethanolic extract of *Artemisia annua* L. Under the conditions of this study, artemisinin did not inhibit the ulcerative lesion index in the ethanol model where an
increase in the ulcerative lesion index was noted. Prior treatment of the rat with indomethacin, on the other hand, blocked the anti-ulcerogenic activity of compounds 2 and 3, resulting in a decrease in the ulcerative lesion index. This experiment suggested that the anti-ulcerogenic activity of compounds 2 and 3 may be related to an increase in prostaglandin synthesis (Foglio et al. 2002).

The effect of amodiaquine (30 mg/kg; positive control) and artemisinin (2.86 mg/kg) on indomethacin-induced ulcers was conducted in a rat study. Under the conditions of this study, amodiaquine led to an increase in the ulcer index, whereas artemisinin led to a decrease in the ulcer index (7.33 ± 0.92 mm as compared to the indomethacin ulcer index of 8.58 ± 1.44 mm; p < 0.05). Treatment with indomethacin was associated with a decrease in mucosal contents of prostaglandins and an increase in leukotrienes due to inhibition of cyclo-oxygenase levels, which lead to lipid peroxidation. The authors proposed that artemisinin potentially attenuates this cascade of events, leading to a decrease in gastric ulcers (Ajeigbe et al. 2008).

In a screening study of natural product-based molecules for *Helicobacter pylori* activity potential, an observation was made that in vitro, artemisinin exhibited bacteriostatic and bacteriocidal activity against *H. pylori*. When tested against *H. pylori* in a mouse study, β-artecyclopropylmether, a new artemisinin derivative, was the most potent against both resistant and sensitive strains of *H. pylori*, had strong bactericidal kinetics, retained functional efficacy at gastric pH, did not elicit drug resistance, and imparted sensitivity to resistant strains. Using a chronic infection model, β-artecyclopropylmether exhibited the potential to reduce *H. pylori* burden and was not cytotoxic (Goswami et al. 2012).

Artemisone was tested in vitro to investigate its activity against *H. pylori*. Under the conditions of the study, artemisone showed a longer half-life, increased bioavailability, and no neurotoxicity in nonclinical studies when compared with other artemisinin derivatives. Furthermore, artemisone appeared to be more potent than dihydroartemisinin and retained its activity against all *H. pylori* isolates at different pH levels that mimicked gastric pH and worked synergistically with the antibiotics tested (i.e., amoxicillin, clarithromycin and metronidazole) (Sisto et al. 2016).

In summary, artemisinin derivatives appear to increase prostaglandin levels in the gastric mucosa and prevent formation of gastric ulcers. Second generation artemisinin derivatives (e.g., artemisone) have longer half-lives, are resistant to pH changes in the gastric milieu, and have synergistic activities with other *H. pylori* therapies and thus provide a promising therapeutic effect for treating *H. pylori* infections and gastric ulcers.

Effect on cancer

Artemisinin and its derivatives have been used as potential therapeutics in the treatment of various tumor types using human cell lines (in vitro) and in animals (cell lines, in vivo models).

In vitro studies showed that artemisinin suppressed invasion and metastasis of hepatocellular carcinoma cell lines (Weifeng et al. 2011).
Dihydroartemisinin inhibited cell proliferation and induced apoptosis in the hepatocellular carcinoma (HCC) HepG2 cell lines in vitro by increasing intracellular production of reactive oxygen species and calcium (Ca^{2+}) (Gao et al. 2011). In another study, dihydroartemisinin was shown to inhibit cell growth in vitro and in vivo by inducing cell cycle arrest and apoptosis, which was associated with mitochondrial membrane depolarization, release of cytochrome c, activation of caspases, and DNA fragmentation (Zhang et al. 2012).

Dihydroartemisinin was shown to have a synergistic anti-proliferative effect when combined with cisplatin in human lung carcinoma cells, an action likely mediated by inhibition of tumor vascularization resulting from reduced expression of hypoxia-inducible factor-1 alpha and vascular endothelial growth factor (VEGF) resulting in tumor cell apoptosis (Zhang et al. 2013). Dihydroartemisinin was associated with a loss of mitochondrial transmembrane potential, release of cytochrome c and activation of caspases, which contributed towards apoptosis in HCC cells (Qin et al. 2015).

The antitumor activity of artesunate in HCC alone and in combination with sorafenib was evaluated in a mouse model. At a dose of 30 mg/kg/day, artesunate apparently showed cytotoxic effects in vitro and in vivo, reduced VEGF and phosphatidylinositol-glycan biosynthesis class F protein levels, and decreased tumor angiogenesis. The decrease in tumor neovascularization and combination therapy with sorafenib was apparently more effective than sorafenib alone (Vandewynckel et al. 2014).

The anti-HCC properties were studied in vitro and in vivo for artemisinin, dihydroartemisinin, artemether and artesunate. Structure and dose-dependent anti-tumor activities were noted including apoptosis, which increased when artemisinin derivatives were combined with gemcitabine. Dihydroartemisinin showed the most potent cytotoxic, antiproliferative, proapoptotic and cell cycle regulatory effects (Hou et al. 2008).

b. Pharmacokinetic data

Artemisinin was rapidly absorbed with a peak concentration reached at 1 hour after oral administration of 900 mg/kg [3H]-artemisinin to rats. Drug concentrations were highest in the liver, followed by the brain, plasma, and lung. Low but detectable levels were also seen in the kidneys, muscle, heart, and spleen. Artemisinin tissue levels decreased gradually with nearly 80% of total excretion of radioactivity (primarily metabolites) seen in urine by 24 hours post-treatment (China Cooperative Research Group 1982a).

Primary metabolism of artemisinin and its derivatives involve hepatic bioreduction by CYP 2B6 and to a lesser extent CYP 2A6, to the dihydro-form of artemisinin, dihydroartemisinin, as well as various glucuronide conjugates (Maggs et al. 2000; Svensson and Ashton 1999).

Information on artemisinin derivatives’ pharmacokinetics can be found in Appendix 1.

5 Sorafenib is a kinase inhibitor drug approved for the treatment of primary kidney cancer (advanced renal cell carcinoma), advanced primary liver cancer (hepatocellular carcinoma), and radioactive iodine resistant advanced thyroid carcinoma.
c. Toxicokinetics

The toxicokinetic (TK) profile of artemisinin was obtained following administration of a single oral dose (40 mg/kg) or repeated, once daily oral dosing of artemisinin (40 mg/kg) for 5 consecutive days in male rats (Table 1) (Yan et al. 2008). Artemisinin was rapidly absorbed with a short plasma half-life following a single dose or repeated dosing (both \(t_{1/2}\) and \(T_{\text{max}}\) values of approximately 1 hour). However, its systemic exposure (AUC, CL/F & \(C_{\text{max}}\)) decreased significantly with repeated dosing where its clearance was nearly 4 times faster in the repeat dose group compared to the single dose group; this observation was likely due to auto-induction caused by first pass metabolism.

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC(_\infty) (h·ng·mL(^{-1}))</th>
<th>CL/F (L·h(^{-1}))</th>
<th>(t_{1/2}) (h)</th>
<th>(C_{\text{max}}) (ng·mL(^{-1}))</th>
<th>(T_{\text{max}}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=6)</td>
<td>127 ± 37</td>
<td>378 ± 163</td>
<td>1</td>
<td>84 ± 21</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Group 2 (n=6)</td>
<td>40 ± 21</td>
<td>1366 ± 796</td>
<td>1.1</td>
<td>18 ± 10</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

*Group 1: single dose of artemisinin (40 mg/kg)*
*Group 2: repeated dose, 5 consecutive daily doses (40 mg/kg)*

Information on artemisinin derivatives’ toxicokinetics can be found in Appendix 1.

d. Acute toxicity

Limited information was found in the literature for single dose toxicity studies of artemisinin. Studies were reported in mice, rats, pigeons, and dogs (China Cooperative Research Group 1982b). The main findings of the studies point to transient neurological signs including convulsions and significant decreases in the reticulocyte count. Species differences in the susceptibility to toxic effects ranged from the pigeon as the most sensitive species to the rat as the least sensitive species.

A single dose of artemisinin resulted in a lethal dose for 50% of animals (LD\(_{50}\)) of 4228 mg/kg for oral (in water suspension) and 3840 mg/kg for intramuscular injection (in oil suspension) in mice. The LD\(_{50}\) values for a single oral or intramuscular injection to Wistar rats were 5576 mg/kg or 2571 mg/kg, respectively. Clinical signs preceding death included respiratory difficulties and respiratory arrest followed by cardiac arrest. No apparent central nervous system (CNS) symptoms were noted in mice and rats at the doses used under the conditions of this study.

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6 *Acute toxicity* refers to adverse effects observed following administration of a single dose of a substance, or multiple doses given within a short period (approximately 24 hours). Endpoints captured in acute toxicity studies usually include mortality and gross clinical observations. Acute toxicity data are usually superseded by data obtained from longer term toxicity studies.
Dose related, transient CNS effects were observed following a single dose of artemisinin given intramuscularly at 0, 400, or 800 mg/kg in dogs (n=2 dogs/group). No deaths were reported during the recovery period for either the control or high dose groups. Clinical signs included tonic and clonic convulsions, which were seen among the 800 mg/kg treated group as well as hyperactivity and vocalization in the 400 mg/kg group, which occurred at about 15 minutes after injection; the latter signs subsided within 30 minutes. A dose-related significant decrease in reticulocyte count was noted at 48 hours after dosing which returned to control values during the recovery period. No differences in histopathological findings were noted for viscera (organs examined were not specified in the publication) or bone marrow among vehicle and drug treated groups recovery groups.

In the same publication described above (China Cooperative Research Group 1982b), neurological signs were noted in several treated species (pigeons, guinea pigs, rabbits, cats) when artemisinin was administered as a single intramuscular dose. Signs included restlessness, tremors, and incoordination followed by inhibited activity, slow respiration, delayed sensation, and disappearance of righting reflex.

Information on artemisinin derivatives’ acute dose toxicity is included in Appendix 2.

e. Repeat dose toxicity

Repeat dose toxicity studies have been briefly reported for artemisinin in rats, dogs, and monkeys (China Cooperative Research Group 1982b). The majority of the studies do not meet current standards for conducting toxicology studies (e.g., small sample size, limited toxicological endpoints, study included one sex, or study did not identify the sex of animals tested, unknown number of animals used per group).

Significant dose and duration dependent toxicities including mortality, neurotoxicity, and hemotoxicity were observed in several animal studies for artemisinin and its derivatives. These adverse effects are generally considered to be a class effect (Efferth and Kaina 2010). Furthermore, these toxicities appear to be related to the routes of administration, the physical-chemical properties of the derivatives, and the treatment formulations. A summary of the key toxicological findings for artemisinin and its derivatives is presented below.

In a 5 day repeat dose dog study (n=4, sex was not specified), animals were orally administered artemisinin at 100 mg/kg/day. Animals did not show any clinical signs or any changes in respiration, cardiac rate, or cardiac rhythm (China Cooperative Research Group 1982b).

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7 The recovery groups consisted of a control and a high dose group which were assessed on day 10 after the end of the dosing period.

8 Repeated-dose toxicity studies consist of in vivo animal studies that seek to evaluate the toxicity of the test substance by observing the changes that emerge in clinical observations, clinical chemistry, gross pathology, and histology endpoints when the test substance is repetitively administered daily for a predetermined period of time.
In a 7 day repeat dose rat study (n=4/group, sex was not specified), rats were administered artemisinin intramuscularly in oil suspension at 0, 200, 400, or 600 mg/kg/day. No changes in body weight, food consumption, or serum transaminase levels were noted. Some engorgement and slight degeneration of heart, liver, spleen, lung, and kidney were reported in the 400 and 600 mg/kg/day treatment groups (China Cooperative Research Group 1982b).

In a 14 day repeat dose rat study (n=4/group for the terminal or 1 week recovery group, sex was not provided), rats were intragastrically administered artemisinin at 0, 250, 500, or 1000 mg/kg/day. No changes were seen in body weight, ECG, serum chemistry, urine, necropsy, or histopathology of major organs (heart, liver, spleen, lung, kidney, brain and stomach) at 24 hours or one week after the final dose (China Cooperative Research Group 1982b).

In a 14 day repeat dose monkey study (n=2 or 3/sex/group), Rhesus monkeys were injected intramuscularly with artemisinin at 0, 24, 48, 96, or 192 mg/kg/day. Death was reported in 3 out of 4 monkeys at 192 mg/kg/day, and 1 out of 6 monkeys at 96 mg/kg/day within 3 days after the last dose. Toxicity was characterized by reduced appetite, decreased activity, decreased heart rate, and significant hematopoietic inhibition in the bone marrow resulting in reductions in red and white blood cell numbers as well as other associated blood parameters. Microscopic examination revealed cytoplasmic coagulation and mitochondrial swelling in the cardiac muscle, slight cloudy swelling of epithelial cells in renal tubules, and slight glycogen accumulation and vacuolation in hepatic parenchymal cells in the 96 and 192 mg/kg/day groups. At 48 mg/kg/day, reductions in red blood cell numbers and associated parameters were noted. At 24 mg/kg/day, reduction in reticulocytes was noted. All findings in the 48 or 96 mg/kg/day groups were reversible within 22 days after the end of the treatment period (China Cooperative Research Group 1982b).

Information for artemisinin derivatives’ repeat dose toxicity can be found in Appendix 2.

f. Neurotoxicity

Neurotoxicity is a major toxicity observed in animals for artemisinin and its semisynthetic derivatives and is recognized as a class effect of artemisinin. Major neurotoxicological effects in mice, rats, dogs, and monkeys [summarized in a review, (Efferth and Kaina 2010)] include behavioral changes (tremor, restlessness, lethargy), abnormalities in balance and coordination (gait disturbance, jerking limb movements), changes in auditory discrimination task tests, loss of spinal reflex, pain response reflex, and loss of brainstem and eye reflexes. Histological examinations revealed extensive damage (chromatolysis, necrosis, swollen cell bodies, nuclear shrinkage, vacuolization of cytoplasm, axonal degeneration, etc.) in brainstem nuclei of the reticular formation (medullary nucleus gigantocellularis, lateral reticular, and reticulotegmental nuclei), the vestibular system (inferior and lateral nuclei), and the auditory system (trapezoid and superior olivary nuclei). However, forebrain regions (cerebral cortex, basal ganglia, thalamus, and hypothalamus) were either unaffected or rarely affected.

Neurotoxicity of artemisinin and its derivatives is not only related to dose levels and dosing duration, but also related to the administration route, the physical-chemical properties of the derivatives, formulations, species, and age of animals (Erickson et al. 2011; Li and Hickman
In general, the oil-soluble arteether and artemether were more neurotoxic in animals than the water-soluble artesunate. Intramuscular administration was more neurotoxic than oral administration.

For further details on neurotoxicity findings of artemisinin and its derivatives, refer to Appendix 3.

g. Hepatotoxicity

Data discussing preliminary hepatotoxicity potential for artesunate can be found in Appendix 3.

h. Genotoxicity

Artemisinin did not show genotoxic activity at concentrations up to 300 μg/plate in the in vitro bacterial reverse mutation assay (Ames test). Similarly, artemisinin was negative at concentrations up to 846 mg/kg in the in vivo micronucleus test conducted in mice (China Cooperative Research Group 1982b).

Information on artemisinin derivative genotoxicity can be found in Appendix 4.

i. Developmental and reproductive toxicity

The literature includes an extensive evaluation of the embryofetal stages of development upon exposure to artemisinin and its derivatives’ toxicities, but a limited number of studies evaluating other stages of development have been conducted (fertility, early embryonic development, prenatal and postnatal development).

*Embryofetal effects*

A large number of studies have been conducted for artemisinin and its derivatives where several animal species were exposed during various stages of gestation. A more detailed review of the mechanism of action for developmental and reproductive toxicity-related toxicities for artemisinin and its derivatives can be found in Appendix 5.

Artemisinin is associated with an increase in the incidence of embryolethality, late resorptions, and malformations (external, visceral, and skeletal), all of which have been observed in rodents,

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9 The genotoxicity assessment battery usually consists of a gene mutagenicity assay (for single dose trials) and a variety of clastogenicity/genotoxicity assays. To support multiple dose administration in humans, additional genotoxicity testing assessment is usually conducted to detect chromosomal damage in mammalian systems.

10 Developmental and reproductive toxicity studies are usually designed to assess the potential adverse effects in humans of both sexes and include females from various age groups that will be exposed to the proposed substance. Developmental toxicity or teratogenicity refers to adverse effects (can include embryo-fetal mortality, structural abnormalities, functional impairment, or alterations to growth) and can occur in pups either as a result of the exposure of their parents to the substance, before the pups’ birth, or by direct exposure of the pups to the substance after birth.
rabbits, and monkeys and are considered an artemisinin class effect with a steep dose response curve. Although the most sensitive period for the embryolethal effect appears to be around gestation day (GD) 10 to 14 with the peak day being GD 11 in rats, the most sensitive day for the malformations appears to be around GD 10 in rats (White and Clark 2008).

Pregnant Wistar rats were orally administered artemisinin at 14, 28, or 223 mg/kg/day during GD 1-6, GD 7-12, or GD 13-19. Artemisinin administration caused embryonic loss in a dose dependent manner: at 14 mg/kg/day, the lowest dose tested, half of the embryos died (50% embryonic deaths) when rats were dosed on GD 7-19. This was compared to an embryonic loss (100%) when artesiminin was administered on GD 7-19 at 28 or 223 mg/kg. Artemisinin also resulted in total embryonic death (100%) when administered on GD 9-11 or GD 12-14 at 223 mg/kg. Umbilical hernia was detected in 6.1% of the fetuses at 223 mg/kg/day when administered on GD 6-8. Similar results were reported in mice (China Cooperative Research Group 1982b).

In another rat study, Wistar female rats (7-12/group) were orally gavaged at 0, 7, 35, or 70 mg/kg/day artemisinin (extracted from *Artemisia annua* L., suspended in 1% Tween 80) from GD 7 to 13 or 14 to 20. Artemisinin caused significant post-implantation losses in a dose-related manner. When artemisinin was given on GD 7-13, 100% post-implantation losses occurred in the 35 (n=8) and 70 (n=8) mg/kg groups, but no significant loss was seen in the 7 mg/kg group (n=9). Similar findings were noted when artemisinin was administered from GD 14 to 20 (30±11%, 28±14%, 87±9%, or 100% in the 0, 7, 35, or 70 mg/kg/day groups, respectively, mean±standard error). No significant maternal toxicity was reported except for reduced weight gain (Boareto et al. 2008).

Information on artemisinin derivative reproductive toxicity can be found in Appendix 5.

**Pre- and postnatal development**

Pregnant rats were administered artemisinin from GD 14-20. Pups were monitored for growth and sexual development from their delivery until weaning. In the high-dose group (35 mg/kg group), three dams (3/12) delivered viable pups, but with significantly smaller litter size (1±1 vs 8±1 in the control group). However, none of the pups survived to postnatal day (PND) 4. There were no significant differences in pregnancy outcomes and offspring body weight and sexual development between the control and 7 mg/kg/day groups except significantly reduced numbers of sperms in the cauda epididymis in the 7 mg/kg/day group when compared to controls. There was no report regarding neurobehavioral observation in offsprings (Boareto et al. 2008).

In a pre- and postnatal developmental toxicity study in rats where artemisone was administered (0, 1, 2 and 4mg/kg bw/day), postnatal survival, pup body weight gain, and motor activity were adversely affected at the highest tested dose (4mg/kg/day) (Schmuck et al. 2009).

In summary, artemisinin and its derivatives have been studied for their impact on fertility and various stages of embryonic development and pre- and postnatal stages in several animal species. Adverse findings were both dose and time of exposure dependent (sensitive window of exposure during embryonic development). Toxicities range from total embryonic loss to multiple
malformations (cardiac, visceral, skeletal) among surviving pups and seem to be most sensitive during the mid to late stage phases of development. Adverse effects were also noted for spermatogenesis (reduced epididymal sperm counts, abnormal shape of sperm).

Information on artemisinin derivatives’ reproductive effects can be found in Appendix 5.

j. Carcinogenicity

No carcinogenicity studies were located for artemisinin or its derivatives.

**Nonclinical Summary**

Significant toxicities including mortality, neurotoxicity, hematotoxicity, embryolethality, and teratogenicity with a steep dose–response relationship were demonstrated as a class effect for artemisinin and its derivatives. In general, the toxicities are dose, treatment frequency, and duration related. In addition, toxicities were related to administration routes, the physical-chemical properties of the derivatives, and the formulation.

Orally administered artemisinin is rapidly absorbed and cleared from the plasma, distributed primarily to the liver, and to a lesser extent to the brain and lungs. Artemisinin metabolites are mainly excreted via the urinary route and little remains unchanged as the parent drug. Toxicity studies showed that the cardiovascular and neurological systems are target organs for toxicity in monkeys and dogs, but the significance of these findings to humans are not well understood. Artemisinin is not mutagenic but artesunate appears to be positive in genetic toxicology tests, which may be linked to the endoperoxide bridge activity. No carcinogenicity studies have been reported in animals for either artemisinin or its derivatives. Artemisinin and its derivatives cause lethality and developmental toxicities in pregnant animals at doses relevant to human therapeutic doses, particularly in mid- and late stages of development in various species (rat, rabbit, monkey), suggesting a considerable risk for teratogenicity and embryo-fetal lethality in humans from orally administered artemisinin.

2. **Human safety**

The following database(s) were consulted in the preparation of the clinical portion of the review: PubMed, Embase, and Web of Science.

A search of the databases for articles related to artemisinin was difficult to conduct because using the search term *artemisinin* also yielded many articles that included artemisinin derivatives. In the literature, these groups of chemicals are collectively characterized as “artemisinins.” As was noted under section B of this review, because of the poor bioavailability and short half-life of artemisinin, efforts were undertaken to develop derivatives of artemisinin to

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11 Studies that assess cancer risk in animals are used as predictive tools to evaluate the potential for drugs to result in tumors when used by humans on a chronic basis. Carcinogenicity studies are conducted if the clinical use is expected to be continuous for a minimum of 6 months of life, or if intermittent clinical use is expected to total 6 months or more of life.
improve on the clinical pharmacology of the drug and maintain the endoperoxide structure that has been shown to be necessary for artemisinin’s effect.

a. Clinical pharmacokinetics

Artemisinin has been extensively evaluated in human single and multiple dose pharmacokinetic studies via oral, rectal and intramuscular administration. Artemisinin has low solubility in water and oil and is not an ideal substance for intravenous administration because of difficulties in formulating a product. As noted earlier in the review, derivatives of artemisinin that maintain the structure of an endoperoxide bridge were developed to improve the solubility and bioavailability of the molecule.

There is contradictory information in the literature and in textbooks suggesting that artemisinin and its derivatives are all metabolized to dihydroartemisinin. Studies available in the literature have evaluated the pharmacokinetics of artemisinin where dihydroartemisinin was not detected within the limits of detection of the assay. Dihydroartemisinin is an active molecule against malaria because it contains the endoperoxide structure that is necessary for its activity against malaria. For some of the derivatives that are quickly metabolized in humans, such as artesunate, the anti-malarial effect is probably through dihydroartemisinin (Benakis et al. 1997). If artemisinin is metabolized to dihydroartemisinin, it represents a very small percent of the metabolism.

The clinical pharmacology for artemisinin follows.

Single Dose Pharmacokinetics

In a single dose study of artemisinin (500 mg) in eleven Vietnamese patients with malaria, the elimination half-life of artemisinin was $2.72 \pm 1.76$ h. The mean $T_{\text{max}}$ was $2.88 \pm 1.71$ h. The mean maximum plasma concentration ranged from 69 to 932 ng/ml. By 24 hours, the concentration ranged from 1 to 8 ng/ml. The results from this study did not differ substantially from healthy subjects. No dihydroartemisinin was detected in any of the samples (De Vries et al. 1997).

Pharmacodynamics (PD)

Artemisinin is fast acting in the killing of malaria, but has a short half-life and does not appear to have active metabolites. In a study of 38 patients in Tanzania with symptomatic malaria infection, the pharmacokinetics of artemisinin alone or in combination with mefloquine was studied. Seventeen patients received two 500 mg doses of artemisinin on day 1 followed by 250 mg twice daily for four days. Twenty-one patients received a single 500 mg dose of artemisinin followed by 750 mg co-administered with 250 mg mefloquine three times daily on day 1 followed by 250 mg artemisinin three times daily on day 2. All patients cleared parasitaemia almost within one day. Reinfection or recrudescence occurred in 41% of the artemisinin monotherapy patients by 28 days, whereas there were none in the combination group at 28 days. The artemisinin AUC values were higher and the clearance was lower in the combination therapy group, suggesting a possible metabolic interaction (Alin et al. 1996).

Bioavailability
The absolute bioavailability of artemisinin is not known because there are no adequate intravenous formulations for comparison. A four-way crossover study in 10 healthy males evaluated an aqueous formulation administered by oral, rectal, and intramuscular routes of administration and an oil suspension (olive oil) by intramuscular injection. The bioavailability of the oil suspension was much greater than the aqueous formulation administered by intramuscular injection. The oral bioavailability of the aqueous formulation was 32% compared to the intramuscular oil suspension injection. The artemisinin aqueous suspension was poorly absorbed after the rectal and intramuscular administration. There was relatively large variability in peak concentration and duration of the intramuscular oil suspension among treated subjects. The elimination half-life of the intramuscular oil suspension is approximately 3.5 times the elimination from the oral aqueous suspension, suggesting that the absorption of the intramuscular dose into the circulation influences the elimination half-life (Titulaer et al. 1990).

**Metabolism**

The following diagram shows the metabolism of artemisinin and some of its derivatives. Artemisinin is mostly metabolized by cytochrome CYP2B6, CYP3A4 and to a minor extent by CYP2A6. None of the metabolites of artemisinin has the endoperoxide bridge, and they are inactive against malaria. Artesunate, artemether and arteether are metabolized by various cytochromes to dihydroartemisinin (active metabolite) (https://www.pharmgkb.org/pathway/PA165378192#PGG) (PharmGKB 2010; Whirl-Carrillo et al. 2012).


Drug Interactions

Artemisinin induces cytochromes CYP3A, CYP2B6 and CYP2C19, and reduced activity of cytochromes CYP1A2 and CYP2D6 (Asimus et al. 2007; Svensson et al. 1998; Simonsson 2003). Drug-drug interactions may occur with drugs that are metabolized through these pathways. (https://www.pharmgkb.org/pathway/PA165378192#PGG)

Multiple Dose Pharmacokinetics and PD

In a study by Ashton et al. (1998), 30 male Vietnamese patients with uncomplicated malaria were treated with 500 mg of artemisinin daily for 5 days by the oral or rectal route (n=15/group). Parasite clearance and artemisinin pharmacokinetics were measured. The relative bioavailability of the rectal formulation was 30% compared to the oral formulation. Mean parasite clearance times were 25 and 29 hours for oral and rectal administration, respectively. The table that follows shows the pharmacokinetic parameters on day 1 and 5 for oral and rectal administration.
Table 2: Pharmacokinetic parameters on day 1 and 5 for oral and rectal administration

<table>
<thead>
<tr>
<th></th>
<th>Oral (n = 15)</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Rectal (n = 15)</th>
<th>Day 1</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC(0-t) (hr · ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2434 ± 1372</td>
<td>468 ± 724*</td>
<td>698 ± 446†</td>
<td>181 ± 257*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(1674, 3194)</td>
<td>(66, 869)</td>
<td>(451, 945)</td>
<td>(38, 323)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AUC(0-∞) (hr · ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2780 ± 1717§</td>
<td>686 ± 854‡</td>
<td>865 ± 518¶</td>
<td>395 ± 305§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(1742, 3817)</td>
<td>(112, 1260)</td>
<td>(467, 1263)</td>
<td>(~90, 880)</td>
<td></td>
<td></td>
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<tr>
<td><strong>CL_{oral} (L/hr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>299 ± 326§</td>
<td>1618 ± 1188‖</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(102, 495)</td>
<td>(820, 2416)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>t_{1/2} (hr)</strong></td>
<td>2.0 ± 0.5§</td>
<td>1.9 ± 0.9‖</td>
<td>2.0 ± 1.4¶</td>
<td>3.5 ± 2.6§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(1.6, 2.3)</td>
<td>(1.3, 2.6)</td>
<td>(0.9, 3.0)</td>
<td>(~0.6, 7.7)</td>
<td></td>
<td></td>
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<tr>
<td><strong>C_{max} (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>706 ± 414</td>
<td>134 ± 136*</td>
<td>185 ± 92†</td>
<td>41 ± 45‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(477, 935)</td>
<td>(59, 210)</td>
<td>(134, 236)</td>
<td>(16, 66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>t_{max} (hr)</strong></td>
<td>Median 2.5</td>
<td>2</td>
<td>4</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.5-6</td>
<td>1-6</td>
<td>2-10</td>
<td>1-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>t_{lag} (hr)</strong></td>
<td>Median 1</td>
<td>1</td>
<td>1</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.5-1.5</td>
<td>0-4</td>
<td>0.5-2.5</td>
<td>0.5-2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MRT (hr)</strong></td>
<td>Mean ± SD</td>
<td>4.5 ± 1.3§</td>
<td>4.8 ± 1.4‖</td>
<td>4.8 ± 2.2¶</td>
<td>7.3 ± 4.3#</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3.8, 5.3)</td>
<td>(3.9, 5.8)</td>
<td>(3.1, 6.6)</td>
<td>(0.4, 14.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Both AUC and C_{max} values were approximately 5-fold lower on day 5 vs. day 1 values. The elimination half-life with oral dosing was not different on day 1 vs. day 5. The mean drug clearance with oral dosing was 299 and 1618 L/h on day 1 vs. day 5, respectively. These results suggest that artemisinin induces the enzymes responsible for its metabolism (Ashton et al. 1998).

Clinical Pharmacokinetics Summary

Artemisinin and its derivatives are rapidly absorbed with a short elimination half-life following a single dose or repeated dosing. Systemic exposure to artemisinin (AUC & C_{max}) decreases significantly with repeated dosing likely due to auto-induction of drug metabolism. Repeat dose toxicity studies for artemisinin should be interpreted with caution as exposure to the drug may not be constant over time.

The oral bioavailability of the aqueous formulation was 32% compared to the intramuscular oil suspension injection. The bioavailability of intramuscular injectable formulations varies depending on the formulation (aqueous vs. oil), making the intramuscular route of administration unreliable for dosing purposes.

Artemisinin and its derivatives are extensively metabolized by the liver (primarily mediated by CYP2B6 with a secondary contribution from CYP3A4) to inactive metabolites. Because of its
effects on multiple cytochromes, artemisinin is likely to have significant drug-drug interactions with drugs metabolized through the cytochrome P450 pathways. Artemisinin was shown to induce CYP3A, CYP2B6 and CYP2C19, and inhibit CYP1A2 and CYP2D6. It is possible that artemisinin acts as a perpetrator to change the exposure of other concomitantly administered drugs that are substrates of these cytochrome P450 isoforms in patients. These interactions have not been fully explored.

b. Reported adverse reactions

*FDA Adverse Event Reporting System (FAERS)*

The Office of Surveillance and Epidemiology conducted a search of the FAERS database for reports of adverse events for *Artemisia*/artemisinin through August 31, 2016, and retrieved 11 cases. Two of the cases reported the use of artemisinin and the remaining cases used an artemisinin derivative or an herbal product from an *Artemisia* species.

The following two cases describe adverse events associated with use of artemisinin.

**Case 1:** A 60-year-old male patient took artemisinin for malaria prophylaxis while traveling to India; concomitant medications included a type of echinacea drug and his usual multivitamin. Five days after the last dose of artemisinin, he was hospitalized with jaundice and acute cholecystitis. Liver biopsy revealed drug-induced hepatitis. The patient’s wife and two friends also took artemisinin but did not experience similar events.

There appears to be a temporal relationship between ingestion of artemisinin and echinacea and the liver biopsy confirmed drug-induced hepatitis. Causality is unclear because the role of artemisinin is confounded by ingestion of echinacea, which can rarely cause hepatitis. The patient was from (b) and used artemisinin for malaria prophylaxis. Artemisinin and its derivatives are not on the CDC list of recommended drugs for prophylaxis of malaria when traveling to India (CDC 2016c).

**Case 2:** A 69-year-old patient being treated with anastrozole and artemisinin for breast cancer as well as radiation developed elevated liver enzymes (alanine aminotransferase = 700 U/L, aspartate aminotransferase = 400 U/L). Treatment with anastrozole and artemisinin were discontinued and liver enzyme tests gradually returned to normal. Anastrozole was restarted and liver function tests remained normal 8 weeks later at the time of the report. Concomitant medications included olmesartan and levothyroxine.

Anastrozole and olmesartan may be associated with liver dysfunction. In addition, radiation therapy may also be associated with radiation hepatitis (Khozouz et al. 2008). There was a positive dechallenge associated with discontinuation of anastrozole and artemisinin. Rechallenge with anastrozole did not result in elevations of liver function tests. The report did not state whether artemisinin was restarted. The case was reported from the USA.

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12 See CDC at [https://www.cdc.gov/malaria/travelers/country_table/i.html](https://www.cdc.gov/malaria/travelers/country_table/i.html).
The remaining cases involved ingestion of artemisinin derivatives or *Artemisia* herbal ingredients. Synopses for the cases can be found in Appendix 9. These cases reported prolonged QT interval, stroke, fracture, warfarin drug interaction, skin ulcerations, seizure, and back pain.

**Conclusions Regarding FAERS Reports**

The FAERS reports have limited utility because reporting of adverse events is voluntary and the reports are missing data and include confounding events. The reports involved patients who were treated for malaria as well as other indications, including cancers. The adverse events described above may be associated with an underlying illness or a concomitant medication.

There is a potential for drug-drug interactions between artemisinin and artemisinin derivatives with concomitant medications, as described in the FDA-approved labeling for the fixed-dose, combination drug Coartem, which contains the artemisinin derivative artemether and lumefantrine. In case 2 of appendix 9, the rise in INR\(^{13}\) may have been related to an interaction between *Artemisia absinthium* and warfarin. There are some reports in the medical literature describing specific drug interactions with artemisinin, but they have not been extensively studied. One example describes the drug interactions between artemisinin derivatives and HIV-anti-virals (Kiang et al. 2014).

Although the rise in hepatic enzymes associated with ingestion of artemisinin derivatives is suggestive of a causal relationship and is of concern, the cases were confounded by concomitant medications also known to cause hepatic dysfunction. For further discussion regarding the potential for artemisinin-associated hepatotoxicity, see Appendix 9.

**Center for Food Safety and Nutrition Adverse Event Reporting System (CAERS)**

The Center for Food Safety and Nutrition (CFSAN) collects reports of adverse events involving food, cosmetics, and dietary supplements in the CAERS. A search of CAERS was conducted for adverse events associated with *Artemisia/artemisinin* on July 7, 2016, and retrieved 14 cases. Eight cases were reported with artemisinin exposure and are described below. Six cases were reported with exposure to artemisinin from various *Artemisia* species other than *Artemisia annua*. *Artemisia annua* has the highest concentration of artemisinin of the *Artemisia* species, but all of the *Artemisia* species listed contain some artemisinin.\(^{14}\) The synopses of the cases involving exposure to artemisinin from *Artemisia* species other than *Artemisia annua* are located in Appendix 10 and include cases of vertigo, liver toxicity, seizure, angioedema and syncope.

A discussion of the eight CAERS cases reported with artemisinin exposure follows.

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\(^{13}\) The prothrombin time (PT)—along with its derived measures of prothrombin ratio (PR) and international normalized ratio (INR)—are assays evaluating the extrinsic pathway of coagulation. This test is also called "ProTime INR" and "PT/INR."

\(^{14}\) The leaves and flowers of *Artemisia* species contain greater amounts of artemisinin compared to the roots and stems. *Artemisia annua* contains the greatest percent of artemisinin by weight (approximately .4 - .45 %). Other species contain lessor amounts of artemisinin in the leaves (0.05 - .25%) and flowers (0.05 - .35%) (Mannan et al. 2010).
**Case 1:** Report # 71288 (8/12/2004). A patient with breast cancer was on multiple alternative therapies, including artemisinin (purchased from University of Washington) and Laetrile (synthetic form of amygdalin, plant substance found in nuts, fruit pips, etc.) for an unknown period. The patient had gone to a clinic in Mexico to receive chemotherapy when she was receiving alternative therapies. The patient eventually died.

This patient with breast cancer apparently did not receive traditional oncology therapy; the cause of her death was likely related to the metastasis of her cancer. Her other medical history, not described in the report, may also have contributed. Additionally, as mentioned above, she was on multiple alternative therapies, including artemisinin. It is difficult to ascribe causality of her death to artemisinin, given her illness and confounding multiple alternative therapies.

**Case 2:** Report #88248 (9/9/2005). A 67 year old Vietnam veteran with prostate cancer (diagnosed in June 2005), with a past history of hypothyroidism (diagnosed in summer 2005) and morphine addiction (severe leg wound in Vietnam war), and who participated in a complementary and alternative medicine (CAM) treatment program that involved exercise, a vegan diet, fever treatments, and as described in the report, CAM artemisinin for about 20 days at a lifestyle center in was reported to have drug-induced liver injury (DILI).

The patient was at the center from to , had an elevated prostate-specific antigen (PSA) and normal baseline labs, including liver function tests (LFTs) and thyroid hormones (TSH and T4). The patient started CAM products on , was discharged home after 20 days of treatment, and continued CAM treatment at home. His PSA apparently declined from 7 to 1.6; however, he developed mild jaundice and on 9/9/05, his liver function tests were abnormal (aspartate aminotransferase (AST) 347, alanine aminotransferase (ALT) 172, bilirubin 3.4, and alkaline phosphatase (AP) 172). On 9/15/05, the patient was very fatigued, had scleral icterus, and had the following liver function test results: AST 158, ALT 247, and bilirubin 12.61. CAM products were discontinued.

Five days later on 9/20/05, the AST was 215, ALT 292, AP 262, and bilirubin 25.17. The patient had severe jaundice, scleral icterus, itching, dark urine, and light-colored stools. Icterus and pruritus progressed, and he was admitted to a local hospital from . The patient was prescribed multiple medications for his itching and ciprofloxacin for presumed urinary tract infection (UTI). A gastrointestinal (GI) consult on suggested painless jaundice possibly related to herbal product-induced cholestasis. Medications for itching (Benadryl, parenteral Narcan, Atarax, Ativan, temazepam, cholestyramine and Actigall) continued. On , the patient was discharged and referred to , where he was seen by the hepatologist on 10/11/05 with disabling pruritus and weakness. His bilirubin was 11.9, AST 106, ALT 105, and AP 163. Hepatitis serology was negative; he was also considered to possibly have paraneoplastic syndrome (reason unclear). A liver biopsy was not considered to be helpful; his liver function tests continued to improve and about a week later by 10/14/05, the AST was 91, ALT 93, AP 112 and bilirubin 9.2, which declined on 10/21/05 to AST 71, ALT 76, AP 125 and bilirubin 6.2. A bulleted summary of the lab test results for AST, ALT, AP, and bilirubin from 9/9/05 to 10/21/05 are below.
The patient’s symptoms and labs continued to improve, and by 12/8/05, he had no itching and felt well.

The report mentions the patient received fever treatments, consumed a vegan diet, and exercised, and that CAM artemisinin was prescribed to the patient. Although it is not entirely clear if CAM therapy included other herbal drug products, it appears from the case description that artemesinin was the main herbal drug therapy.

Based on the temporal association of severe rise in ALT, AST, and bilirubin, coupled with the modest rise in AP over a course of two to three weeks (and negative hepatitis serology), it appears this patient’s liver dysfunction may have been related to ingestion of artemisinin-containing herbal drug products. The report did not describe doses of CAM artemisinin that were prescribed to the patient; however, the total duration of use appears to be about 5 weeks.

Although jaundice was present at the monthly follow-up visit, it likely developed before the visit, indicating onset of liver dysfunction about two to three weeks after starting treatment. The light-colored stools and severe rise in bilirubin appear to point to an obstructive effect (direct bilirubin levels are not available). Additionally, the positive dechallenge (i.e., fall in ALT, AST and bilirubin levels associated with discontinuation of CAM products) appears to be suggestive of a causal relationship. Per the report, the patient’s highest ALT level of 297 would have been approximately 5 x the upper limit of the normal (ULN) range (7 – 55 U/L); AST level of 347 would have been approximately 8 x ULN (5 – 40 U/L); bilirubin of 25.17 would have been approximately 25 x ULN (0.3 – 1.2 mg/dL); and AP level of 262 would have been approximately 1.5 x ULN (20 -140 IU/L). These values are suggestive of Hy’s law, based on Hy Zimmerman’s observations that a patient is at high risk of a fatal DILI if given a medication that causes hepatocellular injury severe enough to cause hyperbilirubinemia (FDA 2009). Although the report did not indicate lab value units, it is presumed that since this patient was in the United States, lab values were standardized values. The R-value was about 3, suggestive of a mixed hepatocellular/cholestatic injury.

\[ R = \frac{\text{Initial ALT/ULN}}{\text{AP/ULN}}; \text{patients presenting with an R value >5 have hepatocellular liver injury, R = 2–5 denotes mixed liver injury, and R <2 is indicative of cholestatic liver injury. The R value helps the clinician to identify other etiologies of acute liver injury to consider and exclude (i.e. differential diagnosis). Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal. Data from Fontana et al. (2010). [obtained from http://www.nature.com/nrgastro/journal/v8/n4/box/nrgastro.2011.22 BX1.html ; Fontana reference is Nature Reviews Gastroenterology and Hepatology 8, 202-211 (April 2011) }\]
Although levothyroxine is associated with elevations in liver function tests, given that it was continued throughout the episode, it is unlikely that it contributed to the pattern of DILI observed in this patient.

**Case 3:** Report #89846 (12/4/2006). A patient with previous history of Lyme disease took Allergy Research Group brand artemisinin – 200 mg on 10/12/06, 300 mg on 10/13/06, 200 mg on 10/14/06 – after which she apparently developed vertigo, dyspnea, dysarthria, paraesthesia, and blood pressure fluctuations, and then stopped treatment. Her MRI and auditory evoked potentials were normal and she recovered 5 to 6 weeks after the incident.

Data on this case were limited. Concomitant medication history is unknown and causality is unclear. There does appear to be a temporal relationship between ingestion of artemisinin capsules and patient’s symptoms.

**Case 4:** Report #111604 (10/31/2008). A patient on travel to Africa took 100 mg artemisinin for malaria prophylaxis for about 14 days. The patient started artemisinin on approximately 10/7/08 and continued through 10/21/08, after which he developed progressive increase in anorexia, vomiting, and biluria at 6.5 on 10/25/08, and 14.9 on 11/7/08. A liver biopsy showed “eosinophil hepatic withdraw drug effect” and was complicated by a subcapsular hemorrhage. Patient was started on steroids with apparent improvement in liver enzymes. The report includes: HBSAG (-), AMA (-), HEPCAB (-), EBV(-), HEPA IGM (-), MRI ‘Cholangial’ (-).

The data on this case were limited, and LFTs were not fully reported. Viral etiologies, though not fully reported, appeared to have been assessed, and an MRI appeared to have been conducted to assess obstructive etiologies. Based on the temporal relationship and clinical presentation, it appears that ingestion of artemisinin for 14 days may have been associated with liver dysfunction. Per the report, the patient consumed 100 mg of artemisinin daily for 14 days. Although the patient’s weight is unknown, assuming an average 50 kg patient, the dose of artemisinin would have been 2 mg/kg/day. The liver biopsy, which showed possible eosinophilia, could be suggestive of drug-induced liver injury (Kleiner et al. 2014). It is unclear if the patient was started on steroids as a cautionary measure. DILI cases are usually treated with supportive care; corticosteroids are not typically used to treat DILI.

**Case 5:** Report #121516 (12/23/2009). A patient taking an artemisinin dietary weight loss supplement for Babesia infection for about 90 days apparently experienced liver dysfunction with lethargy, jaundice, and dark urine. Hospitalization was required.

Past medical history, concomitant medications, and current medical history, including lab data, were not provided. Babesiosis itself may be associated with hepatosplenomegaly and jaundice. It is unclear if the patient had ongoing liver dysfunction related to Babesiosis that may have been exacerbated by artemisinin, or ingestion of artemisinin for 90 days produced the liver dysfunction.

**Case 6:** Report #130779 (6/16/2010). A patient with a previous history of Lyme disease started taking the dietary supplement Tricycline (contains artemisinin and berberine) on (b)(6) (5 capsules daily, increased to 15 capsules daily). After a month, around (b)(6) he had vomiting,
jaundice, pruritus, weight loss, confusion, “high liver enzymes” and bilirubin. He was hospitalized and saw a hepatologist. About 2 months after starting Tricycline, on the ALT was 133, total bilirubin 15, and AP 196. On 8/10, the bilirubin was 10.9, and “liver enzymes” were decreasing.

Tricycline, marketed by the Allergy Research Group, contains berberine sulfate, an active constituent of goldenseal, in addition to artemisinin and other ingredients (citrus seed extract from grapefruit and black walnut hulls). There appears to be a temporal relationship between ingestion of Tricyline and development of liver dysfunction. However, it is unclear if this was related to artemisinin only, or if other ingredients such as berberine may also have contributed. Per Medline Plus, berberine has been reported to worsen jaundice and kernicterus in newborns, but cases of drug induced liver injury in adults associated with berberine were not readily found in our review.

**Case 7**: Report #130780 (10/8/2010). A patient taking Allergy Research Group’s dietary supplement Tricycline for parasite cleansing (around 5/10) took one bottle (90 capsules) over two weeks, beginning with 1 capsule per day and increasing to 6 capsules per day. The patient began experiencing jaundice, dark urine, pruritus, asthenia, vomiting, diarrhea, and required hospitalization. Her liver enzymes were elevated and she had pancreatic cysts. Labs were as follows:

- 6/12/10, AST 272, SLT 368
- 6/16/10, AST 597, AST 395, ALP 450, BR 4.8, direct BR 4.8 (AST reported twice in the report; likely that ‘597’ refers to ALT, given that on 6/17/2010, ALT was 506)
- 6/17/10, AST 293, ALT 506, ALP 363, BR 3.6, Direct BR 2.2
- 6/21/10, AST 269, ALT 424, ALP 340, BR 3.3, direct BIR 1.1
- 6/28/10, AST 250, ALP 299, BR 2.2, Direct BIR 1.1
- 7/20/10 – results normal

The comments related to berberine in case 6 apply to this case as well. In addition, this patient’s liver enzyme elevations are suggestive of drug-induced liver injury with marked elevations of ALT, AST and bilirubin. The ALP levels were also markedly elevated, suggestive of obstructive process.

**Case 8**: Report #160494 (11/23/2012). A patient traveling in Africa took artemisinin 100 mg capsules, 2 caps bid (twice a day) between 10/31-11/17/12. He experienced the following symptoms after about 2 weeks of treatment: nausea, decreased appetite, and dark urine. Coming back to the United States, he saw his doctor on 11/20/12, and then went to the emergency room on His “liver enzymes” were “extremely” elevated (lab results unavailable) and an MRI showed possible gallstones. He had a cholecystectomy and a liver biopsy on and was on antibiotics from Apparently the gall bladder showed sludge but there were no gall stones and the liver biopsy showed “drug-induced hepatitis.”

LFTs apparently indicated high “liver enzymes”; viral etiologies were not reported and post cholecystectomy, no gall stones were found, although the MRI had been suggestive. Despite limited data, given the temporal association, the “high liver enzymes,” and the liver biopsy
findings, it appears this patient may have had drug-induced liver dysfunction that may be related to artemisinin. The patient consumed 200 mg of artemisinin bid for 2 weeks. Although the weight is unknown, assuming an average 50 kg patient, dose would have been 8 mg/kg/day.

Keeping in mind the limitations associated with voluntary adverse event reporting, the CAERS data for artemisinin (8 case reports above and the 6 case reports described in Appendix 10) had several gaps (e.g., missing data, confounding events, and simultaneous use of multiple herbal and dietary supplements) making causality assessments challenging.

Notwithstanding these limitations, data were suggestive of artemisinin and artemesia supplements being associated with liver dysfunction, neurological adverse events and allergic reactions. The neurological and allergic reactions are generally in keeping with the adverse event profile of artemisinin. Although mild elevations of liver enzymes have been described for artemisinin derivatives when used in the malaria setting, the CAERS data, albeit limited, and, in particular, the three reports in which only artemisinin appears to have been ingested, suggest a potential for hepatotoxicity to be associated with these supplements when used for off-label indications such as cancer and malaria prophylaxis.

As discussed below, the temporal relationship, positive dechallenge, elevations of liver enzymes and bilirubin, and liver biopsy findings in some cases were suggestive of artemisinin-based herbal supplements being associated with liver dysfunction and potentially with drug-associated liver injury (satisfying Hy’s Law\(^\text{16}\)).

In case 2, although the dose of artemisinin was unknown, the patient took artemisinin for a total of about 5 weeks and likely had clinical manifestations of liver dysfunction by about 3 weeks. The lab results revealed significantly elevated AST, ALT and bilirubin and modest elevations of AP about 4 weeks after onset of treatment. The levels continued to rise, with peak bilirubin levels being 23.17 about 6 weeks after onset of treatment. The patient had severe jaundice including scleral icterus and intense pruritus and needed hospitalization. Eventually there was clinical and lab improvement over several weeks after discontinuing treatment.

In case 4, the patient took 100 mg of artemisinin for 14 days after which he developed signs of liver dysfunction. The liver biopsy may have been suggestive of drug-induced injury. The dose may be assumed to be approximately 2 mg/kg.

\(^{16}\) Briefly, Hy’s Law cases have the following three components:
- 3-fold or greater elevations above the upper limit of normal of alanine aminotransferase (ALT) or aspartate aminotransferase (AST);
- Elevation of serum total bilirubin to >2xULN, without initial findings of cholestasis (elevated serum ALP);
- No other reason can be found to explain the combination of increased aminotransferase and total serum bilirubin, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury.
In case 8, the patient took 200 mg artemisinin bid for 14 days, after which he developed signs of liver dysfunction. His liver biopsy showed “drug-induced hepatitis” and liver enzymes “extremely elevated.” Dose may be assumed to be about 8 mg/kg.

Although data on purity/potency and lack of contaminants or other active ingredients in the artemisinin supplements were not available, the liver dysfunction appears to be related to increased duration of treatment and was noted at least 2 weeks after starting treatment and appeared to resolve a couple of weeks after stopping treatment, indicating a positive dechallenge and possible causality. Co-relation with dose was unclear but suggestive that higher doses may be causative; overall dose and duration of treatment for artemisinin in the CAERS cases appeared to be higher than typical malaria treatment doses (approximately 4 mg/kg/day for 3 days). Viral and obstructive etiologies, although not fully reported, appeared to not have been contributory. Elevations in liver enzymes and bilirubin in case 2 approached Hy’s Law. Cases 4 and 8 reported liver biopsies that appeared to indicate DILI.

In patients taking Tricycline, cases # 6 and 7, who had drug-induced liver dysfunction, it is unclear if causality could be ascribed to artemisinin, given that Tricycline contains other ingredients such as berberine which may also have contributed. However, the liver enzymes were markedly elevated in case 10.

b. Clinical reports

Adverse events with artemisinin and its derivatives include gastrointestinal disturbance (nausea, vomiting, diarrhea), dizziness, tinnitus, and bradycardia. Transient reticulocytopenia, neutropenia, and elevated liver enzymes have been reported. Electrocardiographic abnormalities including QTc prolongation have been reported in studies with artemisinin-based combination therapy, but most studies have not found significant electrocardiographic changes. Severe type 1 hypersensitivity allergic reactions have been reported in clinical trials and have been estimated to occur in approximately 1 in 3000 patients (Nosten and White 2007).

Appendix 11 provides summaries of case reports and clinical studies from the literature for adverse events reported with artemisinin derivatives. The adverse events include cases of neutropenia, hearing loss, severe allergic reactions, hepatotoxicity, and bone marrow toxicity.

Neurotoxicity

Genovese and Newman (2008) suggests that there needs to be more research to better understand the neurotoxicity induced by artemisinin and its derivatives and to better correlate animal to human findings. The authors hypothesize that the toxicity may be related to sustained, rather than peak, levels of circulating drug. Drug-drug interactions between artemisinin and its derivatives and other drugs used in combination, such as lumefantrine, piperaquine, and sulfadoxine, as they relate to brainstem neurotoxicity, is also not known, although clinical studies conducted for Coartem were not generally suggestive of severe neurotoxicity. Widespread clinical use of WHO-approved artemisinin-based combination therapy for treatment of malaria has not generally been associated with severe neurotoxicity.
Additional references related to artemisinin derivatives are provided in Appendix 12.

**Embryotoxicity**

According to Efferth and Kaina (2010), a meta-analysis of 14 studies in 945 women exposed to artemisinin during pregnancy, of which 123 were in the first and 822 in the second or third trimester, showed no adverse pregnancy outcomes. These studies were not large enough to detect small differences in adverse event rates.

Four artemisinin-based treatments were studied in a total of 3428 pregnant women who had falciparum malaria. All patients were in the second or third trimester of pregnancy. The treatment groups included combination therapy with artemether–lumefantrine, amodiaquine–artesunate, mefloquine–artesunate, or dihydroartemisinin–piperaquine. A total of 44 congenital malformations were reported without specific details other than rates being provided: artemether–lumefantrine group (17/832 newborns = 2.0%), amodiaquine–artesunate group (8/776 newborns = 1%), dihydroartemisinin–piperaquine group (6/767 = 0.8%), and in the mefloquine–artesunate group (13/780 newborns = 1.7%). There were 78 still births with no significant difference between groups for the number of live births (The PREGACT Study Group 2016).

**Hematological toxicity**

There is limited information for the effect of artemisinin on hematologic cells. There are literature reports that this class of drug decreases reticulocyte counts with short duration of use, but resolves with discontinuation of the drug (Efferth et al. 2010).

Hematologic toxicity reported with artemisinin derivatives are available in Appendix 13 and include cases of reduced reticulocyte counts and hemolysis.

**Hepatotoxicity**

The following two cases illustrate additional scenarios where artemisinin may be related to adverse health effects.

**Case 1:** Kumar (2015) describes a patient with no risk factors for liver disease, no concomitant medications, and with normal liver function test 6 months before presentation who took 125 mg of artemisinin (obtained from a health food store with no other active ingredients) orally two to three times a day for general health maintenance. After about 6 weeks the patient presented with a 1-week history of jaundice and arthralgia. Her total bilirubin was 15.4 mg/dL, ALT was 675 U/L (normal < 40), AST was 175 U/L (normal < 40), AP was 208 U/L (normal < 126) and international normalized ratio (INR) was 1.1. Hepatitis A, B, C, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) serology was negative, as were autoantibodies (antinuclear antibody (ANA), anti-smooth muscle antibody, anti-mitochondrial antibody). Ultrasound, MRI, and magnetic choloangiopancreatography were normal. A liver biopsy one week after presentation showed cholestatic hepatitis with marked cholestasis and mild portal and lobular inflammation.
Based on the temporal relationship, a diagnosis of drug-induced liver injury was made, artemisinin was discontinued, and there was gradual clinical and biochemical improvement. Liver function tests (LFTs) remained normal 1 year later. The Roussel Uclaf Causality Assessment Method score was 7, suggesting a probable association. The “R” value was > 10 at presentation suggesting a hepatocellular pattern of liver injury, but subsequently evolved into a prolonged cholestatic phase with eventual recovery.

The authors point out that typical weight-based dosing for artemisinin and its derivatives in combination with other drugs as part of anti-malarial therapy has not generally resulted in severe liver injuries. This report of severe liver injury secondary to artemisinin use suggests a need to characterize the risk of hepatotoxicity given artemisinin’s widespread use.

The temporal relationship of development of hepatitis after about 6 weeks of initiating artemisinin with no concomitant medical history or other medications, along with positive dechallenge, are suggestive of a causal relationship, making it likely that the patient had artemisinin-induced liver toxicity. The patient consumed 125 mg artemisinin tablets 2 to 3 times a day (i.e., 250 – 375 mg/day). Although weight is unknown, assuming an average 50 kg patient, the dose would have been 5 to 7.5 mg/kg/day for 6 weeks. Marked elevations in ALT and bilirubin, the high AST levels, and moderate elevations of alkaline phosphatase are suggestive of Hy’s law. The R-value at > 10 was suggestive of hepatocellular injury.

**Case 2**: The CDC (2009) (MMWR report dated 8/14/2009) was notified on 8/27/08 that a 52-year-old patient in (b) (6), taking two 100 mg artemisinin capsules orally three times a day (total 600 mg/day; dose of 7.5 mg/kg/day of artemisinin) for a week developed hepatitis temporally associated with an herbal supplement containing artemisinin.

His past medical history included lactose intolerance and irritable bowel syndrome, but no history of hepatic dysfunction or alcohol abuse. Two weeks earlier, he had visited a naturopathic provider for abdominal discomfort and was diagnosed as having an “unidentifiable protozoan” and was prescribed a 6-week course of an herbal supplement (manufactured and sold in the US) containing 100 mg artemisinin, two capsules orally three times a day, resulting in a dose of 7.5 mg/kg/day of artemisinin. About a week into therapy, the patient developed abdominal pain and dark urine; three days later he stopped the supplement and three days after that, he visited his doctor. On 8/21/08 (about two weeks after starting artemisinin), his ALT was 898 U/L (normal 10-55 IU/L), AST was 280 U/L (normal 10-40 IU/L), bilirubin was 3.1 mg/dL (normal 0.2-1.2 mg/dL) and alkaline phosphatase (AP) was 258 IU/L (normal 40-150 IU/L). Five months earlier, all labs had been normal. Hepatitis serology was negative. He was hospitalized on artemisinin discontinued, and gradually over 2 weeks, all labs improved and were normal by 9/4/08 (about two weeks after discontinuing artemisinin).

On 9/8/08, samples from the patient’s supply were sent to the CDC for analysis with high-performance liquid chromatography; samples were also sent to (b) (6) to identify clinically relevant organic contaminants by mass spectrometry. The CDC analysis showed 94 mg and 97 mg of artemisinin in two samples of the supplement, and no contaminants or additional organic active pharmaceutical ingredients were found in the other samples.
The report discusses that the patient’s hepatitis may have resulted from the 10-day ingestion of artemisinin containing supplement, that there were no other etiologies, that the hepatitis resolved upon discontinuation of artemisinin, and that further studies are required to assess causality. The report also mentions that because the chemical structure of the artemisinin and its derivatives are similar and they are metabolized to the same active compound (i.e., dihydroartemisinin), the therapeutic window for these compounds is similar and the 7.5 mg/kg/day dose was higher than the 4 mg/kg/day for 3 days of artesunate (when used in combination therapy for malaria).

The temporal association of the development of hepatitis within about 1 week of starting therapy with no concomitant medical history or other medications, and positive dechallenge within about 2 weeks of discontinuation suggests a causal relationship, making it likely that the patient had artemisinin-induced liver injury. The marked elevations, especially of ALT values, the high AST and bilirubin levels, and the moderate elevation of AP are suggestive of Hy’s Law. The R-value at about 9 is suggestive of hepatocellular injury.

Clinical and research information on DILI from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Library of Medicine (NLM) suggests that the mechanism of action by which artemisinin derivatives cause liver injury is unknown, but it may be related to an idiosyncratic immunological reaction to a hepatic metabolite (NIDDK and NLM 2016).

**Overall Human Safety Summary**

Safety information on artemisinin and artemisinin derivatives is largely obtained from their use as anti-malarial therapy (typically used in conjunction with other drugs), where they are generally considered safe and well-tolerated when administered for several days. However, use has been associated with neurotoxicity, ototoxicity, hematologic toxicity (neutropenia, decreased reticulocyte counts, delayed hemolysis), and allergic reactions.

Use in the malaria setting is typically at doses of approximately 4 -6 mg/kg/day for 3 days. When artemisinin and artemisinin derivatives are used at higher doses and for longer durations, they appear to have the potential of causing drug-induced liver injury.

The CAERS data have cases with elevations of liver enzymes, including bilirubin, suggestive of liver injury associated with the use of artemisinin when used for longer durations than the treatment of malaria.

The case reports (CDC 2009; Kumar 2015) that were associated with massive elevations in liver enzymes and bilirubin (suggestive of Hy’s law) were also suggestive of the potential for artemisinin to cause hepatotoxicity and perhaps drug-induced liver injury, which may be dose-related. Although these data are suggestive of a dose-related toxicity, it is unclear if the potential for hepatotoxicity is indeed dose-related or idiosyncratic. The hepatotoxicity appears to be reversible on discontinuation of treatment. Further studies are required.

The safety of artemisinin and artemisinin derivatives for long-term/chronic use is unknown. Genotoxicity, cytotoxicity, acute and chronic toxicities (hepatotoxicity, renal toxicity,
neurotoxicity and hematologic toxicity), cumulative and synergistic toxicity, as well as drug-drug interactions (e.g., interaction with warfarin) need to be evaluated before use in a chronic setting, including as an add-on treatment in the oncology setting.

Additionally, if artemisinin and its derivatives are able to induce auto-induction, it is feasible that this would lead to requirement of higher doses with longer durations of treatment to achieve therapeutic blood levels. It is unclear if this could lead to toxicities, lack of compliance, and resistance, among other things.

The concern regarding use in pediatric patients and women of child-bearing potential, including pregnant women (to corroborate the findings of embryo/fetal toxicity in animals), also needs further evaluation.

Severe toxicities observed in animal studies, such as neurotoxicity, embryo/toxicity, and hematologic toxicity, do not appear to be observed in human studies. These toxicities may be related to intramuscular use of depot formulations in animal studies which may lead to sustained drug release profiles versus largely oral use in humans which are not associated with sustained exposures. In addition, toxicities appear to be related to long-term rather than short-term use and may be related to higher cumulative drug exposures. Physical-chemical properties of artemisinin derivatives and their individual PK properties may also contribute to their unique safety profiles.

It is difficult to assess the overall safety of artemisinin derivatives because use largely occurs in countries with limited infrastructure and inadequate pharmacovigilance programs, and the clinical studies reviewed were not designed or adequately powered to appropriately detect safety-related issues and rare adverse events. It is likely adverse events associated with use of artemisinin in dietary supplements have been underreported as well. As a result, potential interactions, between artemisinin and drugs, including drugs with a narrow therapeutic index such as warfarin or digoxin may go unrecognized.

Conclusions

For short duration of dosing for malaria, artemisinin appears to be relatively safe. However, there is evidence of serious adverse events with repeat dosing, the most concerning being drug induced hepatitis. Numerous reports can be found in the literature of elevations of transaminases and bilirubin in patients taking repetitive doses of artemisinin, Artemisia and artemisinin derivatives leading to hospitalization. In most cases, stopping the artemisinin resulted in resolution of the liver function abnormalities. In cases of liver toxicity, patients were ingesting daily doses for the treatment of cancer, unspecified protozoan infection, and as prophylaxis for malaria when traveling in endemic areas. Because of artemisinin induction or inhibition on multiple cytochromes P450 enzymes, there is a high likelihood of significant interactions with drugs that have not been fully characterized.

c. The availability of alternative approved therapies that may be as safe or safer

Based on the information discussed above, artemisinin is relatively safe with short-term use (less than one week) for the treatment of malaria. For longer duration of use, there are reports of
serious adverse events, as noted above. There are a number of drug products that have been FDA-approved to treat each of the conditions listed in the nomination. A list of alternative therapies can be found in section II.C.3.

C. Are there concerns about whether a substance is effective for a particular use?

1. Reports of trials, clinical evidence, and anecdotal reports of effectiveness, or lack of effectiveness, of the bulk drug substance

The efficacy data for artemisinin and its derivatives in the treatment of malarial, helminthic and protozoal infections, as well as gastric ulcers (including *H. pylori*) and various cancers, are discussed below.

*Malaria Infections*

Malaria is caused by a parasite of the genus *Plasmodium* with several different species including *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*. Transmission to humans occurs via a sting from an infected mosquito. Illness can be mild to severe and include fever, chills, body aches, sweats, and gastrointestinal symptoms. Severe disease can involve neurologic complications, renal failure, metabolic acidosis, hypoglycemia, severe anemia, hypotension, and abnormalities of coagulation.

Malaria was eliminated from the United States in the 1950s. Since then, approximately 1500 to 2000 cases are reported in the United States annually almost exclusively in recent travelers or immigrants from areas of the world where malaria is still endemic. In rare cases, there are occasional outbreaks of locally transmitted disease that occur in the United States because local mosquitoes bite individuals such as recent travelers carrying the malaria parasite (CDC 2016a). For travelers where malaria is endemic, malaria prophylaxis is necessary. The CDC publishes lists of the appropriate drugs for prophylaxis in each country based on the malaria drug resistant strains present in each country. Artemisinin is not a drug recommended by the CDC for prophylaxis of malaria.

Artemisinin and its derivatives have a well-established efficacy profile for the treatment of malaria and are one of the mainstays of therapy for malaria in the world. Semi-synthetic derivatives of artemisinin are recommended by the World Health Organization as standard of care for treatment of malaria in combination with the other antimalarial drugs, known as artemisinin-based combination therapy. One of the derivatives, artemether, is part of the fixed-dose, combination drug, Coartem (artemether/lumefantrine) tablets. Coartem is indicated for the treatment of uncomplicated malaria due to *Plasmodium falciparum* and is the only artemisinin-derivative-containing antimalarial drug currently approved by FDA. Intravenous artemunate, followed by an oral antimalarial drug such as doxycycline or clindamycin, is recommended for the treatment of severe malaria. Intravenous artemunate is not marketed in the United States; however it is available from the CDC.\(^\text{17}\)

\(^\text{17}\) See CDC at [https://www.cdc.gov/malaria/diagnosis_treatment/artesunate.html](https://www.cdc.gov/malaria/diagnosis_treatment/artesunate.html).
However, neither artemisinin nor its derivatives are recommended for use as monotherapy for the treatment of malaria because of incomplete parasite clearance unless used at high doses, which can reduce compliance and increase the risk of toxicity. Furthermore, artemisinin derivatives are fast-acting substances and are quickly cleared from the blood precluding a long-lasting activity. For this reason, artemisinin and its derivatives are preferably used in combination with longer-acting drugs that have a slower onset of activity. Combination therapy with other antimalarial agents also helps in preventing drug resistance and offers complete and rapid eradication of the parasite, thus reducing survival of resistant strains (Medhi et al. 2009).

Helminthic Infections: Schistosomiasis

Helminthic infections such as those caused by trematodes (e.g., schistosomiasis, clonorchiasis, opisthorchiasis) and nematodes (e.g., *Ascaris lumbricoides*, roundworms) are a considerable public health burden. Among helminthic infections, schistosomiasis is the most extensively studied and as discussed below, artemisinin derivatives have been used to investigate various forms of schistosomiasis worldwide in nonclinical and clinical studies.

Schistosomiasis is a waterborne helminthic infection, affecting about 200 million people worldwide (Saeed et al. 2016). The three common types of schistosomiasis infections include those caused by *S. mansoni*, *S. haematobium*, and *S. japonicum*. According to the CDC, 85% of the cases are in Africa, largely related to *S. mansoni* and *S. haematobium*; in addition, *S. haematobium* is found in the Middle East, and *S. japonicum* is found in Indonesia, China and Southeast Asia (CDC 2015). Schistosomiasis is uncommon in the United States; however, travelers may acquire the disease in endemic areas.

Artemisinin derivatives alone and in combination with other drugs, primarily praziquantel (PZQ), have been evaluated in various nonclinical and clinical studies against the three commonly occurring schistosomal infections, *S. mansoni*, *S. haematobium* and *S. japonicum*. The rationale for combination therapy with PZQ is based on the complementary modes of action of artemisinin derivatives (on eggs and juvenile worms) and PZQ (on adult worms), thereby affecting the entire life cycle of the helminth. Animal models, including mice, rabbits, hamsters, and dogs, have been used to investigate the antischistosomal effects of artemether; artesunate, arteether and dihydroartemisinin on *S. mansoni*, *S. haematobium* and *S. japonicum* (Saeed et al. 2016). Study findings suggest that artemether and arteether may be effective against PZQ resistant strains of *S. japonicum*. Studies that assessed combinations of these derivatives with PZQ showed higher worm and egg reduction rates compared to monotherapy with any of the artemisinin derivatives.

Clinical studies from the literature describe the use of artemisinin derivatives for the treatment or prophylaxis of schistosomiasis. A brief synopsis of each study and a summary table are located in Appendix 6.

18 PZQ (Biltricide) is a broad-spectrum anthelmintic used for the treatment of a wide variety of parasitic fluke and tapeworm infections. It is administered orally.
In summary, although clinical trials lacked standardization and showed mixed results, artemisinin derivatives (at doses ranging from 4 mg/kg for artesunate to 6 mg/kg for artemether) generally in combination with PZQ appear to have a prophylactic and treatment effect against the three commonest types of schistosomiasis (S. mansoni, S. haematobium and S. japonicum). Monotherapy appears ineffective for prophylaxis and treatment; this may be due to the differential action of artemisinin derivatives on eggs and juvenile worms as opposed to their action on adult worms. If the treatment is given during the larval stage of the schistosomiasis, the eggs may have been prevented from becoming adult worms.

In some studies where artemether treatment was provided after PZQ, the rationale to provide repeated doses of artemether during the transmission season was to prevent any eggs or juvenile worms from developing into a full-blown infection. Repeat doses may help kill eggs/juvenile worms not killed in the previous cycle.

Complimentary modes of action of PZQ (adult worms) and artemisinin derivatives (eggs, juvenile worms) may be beneficial for prophylaxis and treatment. PZQ, the current treatment of choice, appears to be more efficacious at prevention and treatment of schistosomiasis than the artemisinin derivatives. Further rigorous and well-conducted clinical trials would be needed to determine optimal dosing regimens and overall safety and efficacy of artemisinin and its derivatives when used in combination with PZQ or other drugs.

Assessments are largely based on single drug regimens. In endemic areas, multiple regimens may be required and may include higher doses and longer durations of treatment; as noted above, safety assessments under these conditions, including toxicities and drug-drug interactions, are unknown. There is worldwide concern about the use of artemisinin derivatives in endemic areas where both malaria and schistosomiasis co-exist because it may lead to development of resistance of malaria parasites towards artemisinin derivatives.

**Helminthic Infections other than Schistosomiasis**

The following section presents a discussion of artemisinin derivatives that have been explored in the treatment of helminthic infections other than schistosomiasis (e.g., fascioliasis, clonorchiasis).

Clinical trials investigating the treatment of helminthic infections other than schistosomiasis included one study evaluating artemisinin and five studies evaluating artemisinin derivatives.

A pilot study was conducted comparing PZQ 25 mg/kg o.d. for three days to oral artemisinin 500 mg BID for 5 days on 21 patients with clonorchiasis in Vietnam. Results showed that 5 weeks after treatment, in the artemisinin treated group, reduction of egg count was insignificant (from 1103 to 542 eggs per gram feces (epg), p > 0.05, while in the PZQ group, it was from 1632 epg to 37 epg, p < 0.001. Proportion of patients (95% C.I.) with C. sinensis eggs in their stool on week 5 was 90% in the artemisinin group and 71% in PZQ group (p >0.05). The eradication rate (95% confidence limit) at week 5 was 29% (11 – 52%) in the PZQ group versus 10% (1 – 30%)
in the artemisinin group. No serious AEs noted (Tinga et al. 1999). For clonorchiasis, Artemisinin at 500 mg bid for 5 days was not efficacious compared to PZQ.

For the artemisinin derivative studies, a brief synopsis of each study and a summary table is located in Appendix 7.

In summary, based on the exploratory studies above and in Appendix 7, there is no evidence that artemisinin and its derivatives used at doses ranging from 4 to 10 mg/kg/day (malaria and higher than malaria doses) are efficacious in treating helminthic infections other than schistosomiasis. In endemic areas, it is feasible that higher doses, longer durations, and multiple regimens may be required over a relatively short period of time; additionally, combination treatment with other drugs may also be required based on the mode of action of artemisinin and its derivatives (i.e. on eggs and immature worms). Given the propensity for helminthes/flukes to accumulate in the liver, gall bladder, etc., the effect of possible drug accumulation in these organs is also unclear. Further clinical studies would be needed to elucidate the usefulness of artemisinin derivatives alone and in combination with other drugs in the treatment of helminthic infections other than schistosomiasis.

Protozoal Infections: Toxoplasmosis

Toxoplasmosis is a protozoal infection caused by an obligate intracellular parasite, Toxoplasma gondii. Humans can be infected by T. gondii through ingestion of undercooked meat from infected animals, by contact with feces from infected cats, or congenitally via placental transfer from mother to fetus. T. gondii can cause systemic infection and widespread organ damage in neonates and immunocompromised patients. Acute toxoplasmosis infection in the immunocompetent patient is usually self-limiting, resulting in fever, malaise, and lymphadenopathy. Ocular infection resulting in chorioretinitis is largely related to congenital infection or infection occurring in immunocompromised patients.

According to the CDC (last updated 3/26/2015), it is estimated that 22.5% of the population in the United States 12 years and older have been infected with toxoplasma. It is also considered to be the leading cause of death attributed to food illness in the US.

Pyrimethamine and sulfadiazine (+ folic acid) are the drugs of choice (FDA-approved) for the treatment of toxoplasmosis. The dose is 50-75 mg qd + 1-4 gm of sulfonamide for 1 to 3 weeks depending on response, after which the dose is reduced to about one half of each drug for an additional 4-5 weeks.

We were unable to locate clinical studies reporting the use of artemisinin in treating T. gondii. Non-clinical information for potential effects in vitro or animal models are discussed in the non-clinical section of this review.

Based on the nonclinical studies, it appears that artemisinin derivatives may have a prophylactic and treatment effect against T. gondii. Further nonclinical and clinical efficacy and safety studies are needed to assess these effects, especially studies of the newer artemisinin derivatives and newer delivery systems that may have the potential to be efficacious against T. gondii.
Unlike its use in malaria, the shorter half-life of artemisinin and its second-generation commonly used derivatives may preclude chronic use, which may be expected in toxoplasmosis. In addition, there might be safety concerns such as neurotoxicity and hepatotoxicity related to higher overall exposures to artemisinin and its derivatives (higher doses, longer durations and multiple treatment regimens).

**Gastric Ulcers**

It has been proposed that artemisinin derivatives may be efficacious against gastric ulcers. Discussion below includes the anti-gastric ulcer effects per se, as well as the anti-*Helicobacter pylori* (*H. pylori*) effects of artemisinin and its derivatives.

Depending upon the etiology, there are multiple classes of drugs to treat gastric ulcers. It has been proposed that artemisinin and its derivatives may prevent development of gastric ulcers by increasing gastric mucosal prostaglandin levels. Prostaglandins protect the gastric mucosa by decreasing gastric acid secretion and increasing mucosal blood flow, bicarbonate and mucus production. Another proposed mechanism is the action against *H. pylori*, which has been known to be associated with peptic ulcer disease.

A search of the databases was unable to find clinical studies conducted to assess safety and efficacy of artemisinin derivatives for the treatment of gastric ulcers. Section II.B.1.a of this review describes relevant nonclinical in vitro and in vivo studies.

**Anti-tumor activity**

Artemisinin derivatives have been proposed to have anti-tumor activity based on multiple actions. Clinical studies discussing the use of artemisinin derivatives for their anti-tumor activity are discussed below. Multiple treatment classes are available to treat various kinds of cancers. They have been proposed to inhibit tumor growth and metastases by various actions, including decreasing cell proliferation, triggering apoptosis, and reducing angiogenesis (Efferth et al. 2003; Ho et al. 2014; Krishna et al. 2008; Sertel et al. 2010). These actions may be attributed to the following proposed mechanisms based on in vivo and in vitro studies:

- **Endoperoxide component** aids the formation of reactive oxygen species such as superoxide anion and hydroxyl radicals capable of causing cellular damage by oxidative stress.

- **Artemisinin** reacts with iron to form cytotoxic carbon-centered radicals that are potent alkylating agents capable of inducing oxidative damage and inducing apoptosis in cancer cells (Singh and Panwar 2006).

- **Given that cancer cells contain more free iron than normal cells, artemisinin and its derivatives may be selectively more toxic to cancer cells compared to normal cells** (Efferth et al 2007; Efferth and Kaina 2010; Efferth et al. 2003; Firestone and Sundar 2009; O'Neill et al. 2010). Tagging artemisinin to transferrin enhances selectivity and toxicity of artemisinin towards cancer cells (Lai et al. 2009; Singh and Panwar 2006).
Administration of iron-enhancing compounds with artemisinin and its derivatives may help increase iron content inside cancer cells and increase efficacy of artemisinin drugs (Singh and Panwar 2006).

- Down-regulation of VEGF may help inhibit angiogenesis (Chen et al. 2014)
- Inhibition of NF-κB by dihydroartemisinin may help block metastasis (Wang et al. 2011; Wu et al. 2011).

We were unable to locate any clinical studies that evaluated the effect of artemisinin for the treatment of cancer. There are some case reports and clinical trials with artemisinin derivatives. These are summarized in Appendix 8.

As discussed above, based on in vitro studies and pilot or exploratory clinical studies and the case reports and clinical trials summarized in Appendix 8, it appears that artemisinin and its derivatives may have anti-tumor activities. However, there is insufficient information to support the usefulness of artemisinin and its derivatives in the treatment of cancer. The propensity for synergistic toxicity and drug-drug interactions that may be expected to occur in this setting should be assessed appropriately.

**Overall Summary of Efficacy**

Artemisinin has been useful in the treatment of malaria. It has poor bioavailability and a short half-life. Artemisinin derivatives were developed to improve the bioavailability and to allow for the development of more dependable parenteral formulations. Because of their short half-life and potential for drug resistance, these drugs should not be used as monotherapy.

Artemisinin derivatives have been studied in patients infected with schistosomiasis. There is some evidence that artemisinin derivatives in combination with other therapies may improve clinical response. For other helminthic infections, there are no supporting data.

Artemisinin has not been studied in humans for the treatment of *T. gondii*.

There is no evidence to support the use of artemisinin as an anti-ulcer therapy.

There have been limited reports of the use of artemisinin derivatives in the treatment of some cancers, but there is insufficient information in those reports.

2. *Whether the product compounded with this bulk drug substance is intended to be used in a serious or life-threatening disease*

The diseases proposed in the nomination are serious or life-threatening for some patients.

3. *Whether there are any alternative approved therapies that may be as effective or more effective.*
Malaria Infections

FDA has approved many prescription drug products for the treatment and/or prophylaxis of malaria. The guidelines for malaria treatment depend on multiple factors, including the severity of disease and the presence of drug resistance where the infection was acquired (CDC 2013a). In general, FDA-approved drug products indicated for the treatment of malaria include atovaquone-proguanil, Coartem (artemether-lumefantrine), chloroquine phosphate, halofantrine, hydroxychloroquine sulfate, mefloquine hydrochloride, primaquine, pyrimethamine-sulfadoxine, quinidine sulfate, quinidine gluconate, quinine sulfate, sulfadiazine and doxycycline. Drug products indicated for the chemoprophylaxis of malaria include atovaquone-proguanil, doxycycline, mefloquine hydrochloride, pyrimethamine, and pyrimethamine-sulfadoxine. Intravenous formulations of quinine are not available in United States.

Quinidine gluconate injection, an antiarrhythmic drug, is the only FDA-approved drug for the treatment of severe malaria due to *Plasmodium falciparum*.

Treatment guidelines on the clinical use of these antimalarials for uncomplicated and severe malaria are published by the Centers for Disease Control (CDC 2013). Many of these antimalarial products are also approved for malaria prophylaxis for travelers to malarious areas of the world (CDC 2016b).19

Tetracycline or clindamycin in combination with other antimalarials are used off-label for the treatment of malaria and are included in the Centers for Disease Control malaria treatment guidelines.20 Trimethoprim/sulfamethoxazole (Bactrim) has shown benefit for the prevention of malaria in studies of HIV-infected and HIV-exposed children in sub-Saharan Africa (Mbeye et al. 2014).

Helminthic Infections (schistosomiasis, fascioliasis, clonorchiasis, opisthorchiasis, loiasis)

PZQ is indicated for the treatment of infections from all species of Schistosoma for all forms of schistosomiasis, as well as for infections from *Clonorchis sinensis/Opisthorchis viverrini*. According to its label, PZQ may not prevent progression from asymptomatic infection to acute schistosomiasis or from acute to chronic phase schistosomiasis. Because loiasis is uncommon in the United States, diethylcarbamazine citrate is no longer an FDA-approved product. However, physicians can acquire the drug from the CDC after positive laboratory confirmation (CDC 2013b). There are no FDA-approved drug products indicated for the treatment of fascioliasis. The treatment of choice is triclabendazole, which can be obtained from the CDC. Other drugs used off-label for fascioliasis include albendazole, biothionol, metronidazole, and nitazoxanide.

Toxoplasmosis

19 See the CDC at http://www.cdc.gov/malaria/travelers/country_table/k.html.
Pyrimethamine and sulfadiazine are FDA-approved drug products indicated for the treatment of toxoplasmosis. Sulfadiazine is prescribed as adjunctive therapy to pyrimethamine. Other antimicrobials used off-label for toxoplasmosis include atovaquone, azithromycin, clindamycin, and sulfamethoxazole.

**Gastric ulcers**

Depending on the etiology, there are multiple classes of drugs for the treatment of gastric ulcers. For ulcers caused by *H. pylori* infection, the following treatment regimens are recommended for the eradication of *H. pylori* (Table 3), which combine antibiotics with acid suppression.

Examples of FDA-approved proton pump inhibitors (PPI) to reduce gastric acid secretion include dexlansoprazole, esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole. PPIs are also recommended for nonsteroidal anti-inflammatory drug (NSAID)-associated and idiopathic ulcers (i.e., not related to *H. pylori* and NSAIDs) (McColl 2009).

| Table 3: Treatment regimens recommended for eradication of *H. pylori* infection |
|-------------------------------|----------------|------------------|
| **Recommendation** | **Regimen** | **Definition** |
| First line | | |
| Recommended option | Bismuth quadruple | PPI + bismuth + metronidazole\(^a\) + tetracycline |
| Recommended option | Concomitant nonbismuth quadruple | PPI + amoxicillin + metronidazole\(^a\) + clarithromycin |
| Prior treatment failure | | |
| Recommended option | Bismuth quadruple | PPI + bismuth + metronidazole + tetracycline |
| Recommended option | Levofloxacin-containing therapy | PPI + amoxicillin + levofloxacin |

\(^a\)Metronidazole may be substituted with tinidazole. PPI = proton pump inhibitor

*Adapted from Fallone et al. (2016)*

**Cancer**

There are a number of FDA-approved drug products that have been established to be safe and effective for the treatment of various types of cancer.

**D. Has the substance been used historically as a drug in compounding?**

The following databases were consulted in preparation of this section: PubMed, Natural Medicines, European Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, USP/NF, and Google.

1. **Length of time the substance has been used in pharmacy compounding**

*Artemisia annua* has been used in Chinese traditional medicine as a treatment for fever and malaria for over 2000 years (Klayman 1985; Li and Wu 1998). Artemisinin was first isolated.
from the leafy portions of *Artemisia annua* by Chinese scientists in 1971 (Klayman 1985). Artemisinin was discovered in part by Youyou Tu, a Chinese scientist, who was awarded the 2015 shared Nobel Prize in Physiology or Medicine (Callaway and Cyranoski 2015). There is insufficient information available to determine how long artemisinin has been used in pharmacy compounding.

2. *The medical condition(s) it has been used to treat*

Artemisinin and its derivatives have been used to treat malaria for years. Results from a Google search using the terms *artemisinin compounding pharmacy* yielded multiple dietary supplements. To our knowledge, no compounded artemisinin product for clinical use is currently available in the United States.

3. *How widespread its use has been*

Insufficient data are available from which to draw conclusions about the extent of use of artemisinin in compounded drug products.

4. *Recognition of the substance in other countries or foreign pharmacopeias*

Artemisinin is available in combination with piperaquine (Artepharm Co. Ltd., China) or naphthoquine (KPC Pharmaceuticals, Inc., China) for the treatment of malaria through Chinese manufacturers; their regulatory status in countries other than the United States is unknown. Neither product is prequalified by the World Health Organization (WHO) (WHO List of Prequalified Medicinal Products, 2016) and according to the WHO’s guidelines for the treatment of malaria, the two combination products are not currently recommended for general use due to insufficient data from clinical trials (Guidelines for the Treatment of Malaria, 3rd Edition).

A search of the British Pharmacopoeia (BP 2016), the European Pharmacopoeia (8th Edition, 2016, 8.8), and the Japanese Pharmacopoeia (16th Edition) did not show any listings for artemisinin. Artemisinin is listed in the Chinese Pharmacopoeia (2010 Edition) and is categorized as an antimalarial. In addition, artemisinin is listed as a pharmaceutical substance, or active pharmaceutical ingredient (API), in the WHO’s International Pharmacopoeia (5th Edition, 2015). According to the International Pharmacopoeia, artemisinin is mainly used as a starting material for artemisinin-derived APIs and not as an API.

**Conclusions:** There is insufficient information to determine the historical use of artemisinin in pharmacy compounding. Based on internet searches, artemisinin does not appear to be available as a compounded product in the United States. Artemisinin is listed in the Chinese Pharmacopoeia and is available in combination with piperaquine or naphthoquine through Chinese manufacturers.
III. RECOMMENDATION

We have reviewed the criteria and the information currently available as described in section II above to evaluate artemisinin for the 503A Bulks List. A balancing of the criteria weighs against artemisinin being placed on that list based on the following:

1. Artemisinin is likely to be stable under ordinary storage conditions. It is easily characterized with various analytical techniques and the production of this compound has been well established.

2. Artemisinin has clinically significant effects on different cytochrome P450 enzymes. It has been shown to induce its own metabolism within several days of treatment. It has been shown to induce CYP3A, CYP2B6 and CYP2C19. It reduces activity of CYP1A2 and CYP2D6. This is likely to lead to significant drug interactions when used for repetitive daily therapy. The constellation of drug interactions has not been fully explored. Treatment for malaria was generally limited to 1 to 2 days of treatment and for some regimens of up to 5 days. If artemisinin is used for other conditions, such as anti-ulcer therapy and anti-cancer therapy where repetitive doses would be required, it may interfere with other concomitant therapies used to treat the diseases.

For 1 or 2 day dosing for malaria, artemisinin appears to be relatively safe. However, with repeat dosing there is evidence of serious adverse events, the most concerning being drug induced hepatitis. There are numerous reports in the literature of elevations of transaminases and bilirubin in patients taking repetitive doses of artemisinin leading to hospitalization. In most cases, stopping the artemisinin resulted in resolution of the liver function abnormalities. In cases of liver toxicity, patients were ingesting daily doses for the treatment of cancer, unspecified protozoan infection and as prophylaxis for malaria when traveling in endemic areas.

3. For the treatment of malaria, artemisinin is likely an effective therapy. However, malaria is a relatively rare disease in the United States, and almost all cases are reported to occur in recent travelers who have returned from endemic areas. There are numerous FDA-approved medications available in the United States for the treatment of malaria, including the artemisinin derivative artether. Artemisinin must be given in combination with another therapy for the treatment of malaria because of its short half-life. It has poor oral bioavailability due to excess first pass metabolism. It cannot be given intravenously because of poor water and oil solubility. Intramuscular absorption is erratic and dependent on whether it is in oil or aqueous solution. It affects numerous cytochrome P450 enzymes and is likely to have significant interactions with drugs metabolized by those cytochromes.

For helminthic and other infections, there is insufficient information on the effectiveness of artemisinin in infected patients. Other therapies are available for the treatment of these diseases.

For use as an anti-ulcer therapy and anti-cancer therapy, there is insufficient information on the effectiveness of artemisinin.
4. There is insufficient information on which to determine the extent of use of artemisinin in pharmacy compounding. It does not appear to be currently available from compounding pharmacies in the United States.

Based on this information the Agency has considered, a balancing of the four evaluation criteria weighs against artemisinin being added to the 503A Bulks List.
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WHO 2003. Assessment of the safety of artemisinin compounds in pregnancy. pp. 1-14


## ACRONYM LIST

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin-Based Combination Therapy</td>
</tr>
<tr>
<td>API</td>
<td>Active Pharmaceutical Ingredient</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>CAERS</td>
<td>CFSAN’s Adverse Event Reporting System</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>DART</td>
<td>Developmental and Reproductive Toxicology</td>
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<tr>
<td>FAERS</td>
<td>FDA Adverse Event Reporting System</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FD&amp;C</td>
<td>Federal Food, Drug, and Cosmetic Act</td>
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<td>FT-IR</td>
<td>Fourier Transformed Infrared Spectroscopy</td>
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<td>GD</td>
<td>Gestation Day</td>
</tr>
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<td>GERD</td>
<td>Gastroesophageal Reflux Disease</td>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>IND</td>
<td>Investigational New Drug</td>
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<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median Lethal Dose</td>
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<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NF</td>
<td>National Formulary</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No Observed Adverse Effect Level</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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APPENDIX 1. PHARMACOKINETICS FOR ARTEMISININ DERIVATIVES

Pharmacokinetics
In a single dose, intravenous study where 5 mg/mL/kg radiolabeled $[^{14}C]$ artesunate (20 μCi/kg) (n=5) was administered to rats, approximately 68% of $[^{14}C]$ artesunate radioactivity in all the measured tissues was found in the small intestine and its contents, followed by spleen, liver, adrenals, kidneys, eyes, and bone marrow at 1 hour post-dosing. After 24 hours, the radioactivity rapidly declined in all tissues except in the spleen, which had higher radioactivity up to 96 hours post-dosing. Approximately 1% of the total radioactivity dose was detected in brain. The radioactivity in whole blood was consistently higher (by 2-4 fold) than that in plasma throughout the experiment (i.e., 0-192 hours), suggesting that artesunate binds to red blood cells. Urinary excretion was the major route of elimination, where approximately 56% of the dose was eliminated in urine followed by 39% in feces over a period of 192 hours. In an in vitro protein binding experiment, about 75% of radiolabeled artesunate was bound to human plasma proteins and 82% was bound to rat plasma proteins (Li et al. 2006).

Toxicokinetics
The TK profile of artesunate and dihydroartemisinin (the primary metabolite) was evaluated following the administration of single intravenous (0.75 or 1.5 mg/kg) or oral (10 or 17 mg/kg) doses of artesunate to female rats (4/group/time point) on gestational day (GD) 11 (Clark et al. 2008b). Estimations of AUC$_{0-t}$ were limited by the number of sampling times for which quantifiable concentration data were obtained (1 hour following intravenous administration and 3 hours post-oral administration). Following oral administration, exposure to dihydroartemisinin was greater than that to artesunate. When compared on a molar basis, the AUC$_{0-t}$ for dihydroartemisinin was approximately 5- or 11-fold greater than the corresponding value for artesunate following doses of 10 or 17 mg/kg, respectively. However, following intravenous administration, the AUC$_{0-t}$ for dihydroartemisinin was only 1-and 0.6–fold of the corresponding value for artesunate at 0.75 or 1.5 mg/kg, respectively.

Artesunate
Artesunate has a very short plasma half-life (T$_{max}$ and t$_{1/2}$) with similar oral half- life to that seen for artemisinin (approximately 1 hour) but higher than that seen following intravenous (3 hours) dosing (Table A1). A dose-related increase in systemic exposure (AUC$_{0-t}$ and C$_{max}$) to artesunate was seen following either the oral or intravenous routes of administration. Systemic exposure to artesunate, in terms of AUC$_{0-t}$, was approximately an order of magnitude greater following administration of the intravenous doses used in this study (0.75 and 1.5 mg/kg) than following oral administration (10 and 17 mg/kg). The oral bioavailability of artesunate was estimated to be less than 1% following administration of either 10 or 17 mg/kg artesunate (Clark et al. 2008b).

Table A1. Toxicokinetics of artesunate in female rats following a single oral or intravenous dose of artesunate on GD 11 (adapted from Table 5 of article) (Clark et al. 2008b).
Dihydroartemisinin

In the same study discussed above (Clark et al. 2008b), systemic exposure to dihydroartemisinin was calculated; both AUC\(_{0-t}\) and C\(_{\text{max}}\) increased with an increasing dose of artesunate following either an intravenous or an oral route of administration (Table A2).

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>10 (oral)</th>
<th>17 (oral)</th>
<th>0.75 (i.v.)</th>
<th>1.5 (i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{0-t}) (ng·h/mL)</td>
<td>8.27</td>
<td>19.4</td>
<td>160</td>
<td>466</td>
</tr>
<tr>
<td>t (h)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng/mL)</td>
<td>24.6</td>
<td>58.9</td>
<td>727</td>
<td>2300</td>
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<tr>
<td>T(_{\text{max}}) (h)</td>
<td>0.30</td>
<td>0.27</td>
<td>0.09</td>
<td>0.12</td>
</tr>
<tr>
<td>Fpo (%)</td>
<td>0.4</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

N=4 animals per time point
AUC\(_{0-t}\)=area under the plasma concentration-time curve from the start of dosing to the last quantifiable time-point
t=half-life
C\(_{\text{max}}\)=maximum observed plasma concentration
T\(_{\text{max}}\)=time at which C\(_{\text{max}}\) was observed
Fpo=oral bioavailability

Table A2. Toxicokinetics of dihydroartemisinin in female rats following a single oral or intravenous dose of artesunate on GD 11 (adapted from Table 6 of article) (Clark et al. 2008b).

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>10 (oral)</th>
<th>17 (oral)</th>
<th>0.75 (i.v.)</th>
<th>1.5 (i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{0-t}) (ng·h/mL)</td>
<td>30.6</td>
<td>147</td>
<td>119</td>
<td>219</td>
</tr>
<tr>
<td>t (h)</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng/mL)</td>
<td>49.0</td>
<td>179</td>
<td>218</td>
<td>498</td>
</tr>
<tr>
<td>T(_{\text{max}}) (h)</td>
<td>0.52</td>
<td>0.54</td>
<td>0.09</td>
<td>0.12</td>
</tr>
</tbody>
</table>

N=4 animals per time point
AUC\(_{0-t}\)=area under the plasma concentration-time curve from the start of dosing to the last quantifiable time-point
T=half life
C\(_{\text{max}}\)=maximum observed plasma concentration
T\(_{\text{max}}\)=time at which C\(_{\text{max}}\) was observed

In another TK study conducted in female Cynomolgus monkeys, monkeys (n=3/group) were dosed with a single dose of artesunate (10 mg /kg, oral or intravenous injection in 1% w/v methylcellulose for oral, in 0.9% w/v aqueous sodium chloride solution for intravenous injection containing a final concentration of 2% v/v DMSO and 0.2% 2- hydroxypropyl-b-cyclodextrin for IV) (Clark et al. 2008a).

Following a 10 minute intravenous infusion, concentrations of artesunate declined rapidly and were unquantifiable beyond 1.5 hour after the end of the infusion period. Estimation for the terminal half-life and mean residence time were 0.3 hour and 0.08 hour, respectively.

Dihydroartemisinin was quantifiable until at least 4 hours after the end of the infusion, and
exposure to dihydroartemisinin, in terms of mean AUC_{0-t}, was approximately twice that of artesunate (when calculated on a molar basis) (Table A3).

Following oral administration of artesunate at 10 mg/kg, mean AUC_{0-t} and C_{max} values for artesunate were 32.4 ng.h/mL (range=15.9 to 49.6 ng.h/mL) and 29.4 ng/mL (range=1.6 to 42.4 ng/mL), respectively. Comparison to the AUC_{0-t} data obtained in the same animals following intravenous administration indicated that the oral bioavailability of artesunate was low in the female Cynomolgus monkey, with a mean value of only 1.9 (±1.3)% Systemic exposure to dihydroartemisinin was approximately 3-fold that of artesunate (when calculated on a molar basis) with mean AUC_{0-t} and C_{max} values of 73.5 (range 13.6 to 113) and 62.4 ng.h/mL (range 9.13 to 98.7) ng/mL, respectively (Table A3).

Table A3. Toxicokinetic of artesunate and dihydroartemisinin in female monkeys following a single oral or intravenous doses of 10 mg/kg artesunate (adapted from Table 10 of article) (Clark et al. 2008a).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>AUC_{0-t} (ng.h/mL)</th>
<th>C_{max} (ng/mL)</th>
<th>T_{max} (h)</th>
<th>Cl_p (mL/min/kg)</th>
<th>V_{ss} (L/kg)</th>
<th>t_{1/2} (h)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Artesunate</strong></td>
<td>Mean&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1840</td>
<td>10800</td>
<td>0.17</td>
<td>94</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>SD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>431</td>
<td>1780</td>
<td>0.08-0.17</td>
<td>20</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td><strong>DHA</strong></td>
<td>Mean&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2270</td>
<td>2910</td>
<td>0.25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>SD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>453</td>
<td>290</td>
<td>0.25-0.42</td>
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<tr>
<td><strong>Artesunate</strong></td>
<td>Mean&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.4</td>
<td>29.4</td>
<td>0.50</td>
<td>1.9</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>SD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.9</td>
<td>15.9</td>
<td>0.50-1.50</td>
<td>1.3</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td><strong>DHA</strong></td>
<td>Mean&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.5</td>
<td>62.4</td>
<td>1.50</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>SD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.7</td>
<td>47.1</td>
<td>1.00-3.00</td>
<td>ND</td>
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</tbody>
</table>

<sup>a</sup>N=3 except, for the V_{ss}, t_{1/2} and MRT of artesunate, n=2 (insufficient data for 3rd animal). Cl_p, total plasma clearance; V_{ss}, volume of distribution at steady-state; t_{1/2} terminal half-life; MRT, mean residence time; SD, standard deviation (n=3); ND, not determined.

<sup>b</sup>Median for T_{max}

<sup>c</sup>Range for T_{max}
APPENDIX 2. GENERAL TOXICOLOGY STUDIES FOR ARTEMISININ DERIVATIVES

Single Dose Toxicity
Artesunate was administered to mice, rats, and dogs at very high doses using several routes (intravenous, intraperitoneal, intramuscular injection). Clinical signs included CNS adverse effects (sedation, analgesia, increased barbiturate sleeping time) in mice treated with 200 mg/kg, lowered body temperature in rats treated with 450 mg/kg, and cardiovascular changes (decreased heart rate and blood pressure, as well as electrocardiogram, ECG, changes) in anesthetized dogs treated with 320 mg/kg artesunate (Zhao 1985).

Repeat Dose Toxicity
Repeat dose toxicity studies for artemisinin derivatives including artesunate, artemether, dihydroartemisinin, arteether, and artelinate (artemisinin derivative) were conducted in mice, rats, dogs, and monkeys. Neurological, hematological, cardiac, hepatic, and renal toxicities were reported. Some key publications are reviewed below.

Three day repeat dose toxicity study in rats for artelinate and artesunate
In this study, both *P. berghei*-infected and uninfected Sprague-Dawley rats (with >5% parasitaemia as severe malaria; 4-6/group, sex not specified) were used. Infected rats were intravenously administered artelinate at doses ranging from 40 mg/kg/day to 320 mg/kg/day or artesunate at doses ranging from 120 mg/kg/day to 720 mg/kg/day (in 0.45% NaCl/0.1% L-lysine) for 3 consecutive days and observed for 2 weeks after the final dose. Uninfected rats were administered a single intravenous dose of artelinate at doses ranging from 40 mg/kg to 320 mg/kg/day or artesunate at doses ranging from 60 mg/kg/day to 720 mg/kg/day and observed for 2 weeks after the final dose (Li et al. 2007).

Dose related mortality were observed for artesunate (0/6, 2/5, or 5/5 in the 240, 480, or 720 mg/kg infected groups, respectively; 0/4, 1/4, 2/4, or 4/4 in the 120, 240, 480, or 720 mg/kg uninfected groups, respectively; all died on Day 1 except for 1 on Day 2 in the 720 mg/kg/day infected group). LD$_{50}$ was 488 mg/kg/day or 351 mg/kg in infected or uninfected rats, respectively. Artesunate was more toxic than artelinate with an LD$_{50}$ of 177 mg/kg or 116 mg/kg in infected or uninfected rats, respectively. Similarly, all rats died on Day 1 except for 1 on Day 2 in the 160 mg/kg/day infected and uninfected groups.

Dose related tail necrosis was observed after 3 artesunate intravenous injections (2/6, 5/6, or 6/6 in the 30, 60 or 120 mg/kg groups, respectively). However, the necrosis did not occur at higher doses if the injection site was switched to the femoral vein. The tail lesions in the 30 mg/kg group recovered after cessation of dosing but severe tail necrosis in higher dose groups was irreversible and the animals were euthanized.

At 240 mg/kg/day dose of artesunate, hemoglobinuria (Day 1-3 but not on Day 4 or later), significant alterations in urinary volume (increase on Day 1 then decrease with peak decrease on Day 3), and a marked increase in blood urea nitrogen concentration on Day 4 were noted in infected and in uninfected rats. All parameters returned to normal on Day 11.
Minimal to moderate renal tubular necrosis and regeneration were observed for artemunate in both infected (0/5, 0/3, or 2/3 in the 0, 120 or 240 mg/kg groups, respectively) and uninfected rats (0/4, 1/5, 3/5, 0/4, or 4/4 in the 0, 40, 80, 120 or 240 mg/kg groups, respectively, on Day 1 post dosing). In the uninfected rats, incidence and severity was reduced in the recovery group (incidence: 0/4, 3/3, 3/3, 1/4, or 4/4 on Day 1 post dosing vs. 0/4, 1/5, 3/5, 0/4, or 4/4 on Day 8 post dosing in the 0, 40, 80, 120 or 240 mg/kg groups, respectively; severity: 1.52 ± 0.21 on Day 1 post dosing vs. 0.31 ± 0.18 on Day 8 after dosing in the 240 mg/kg group).

Four, 8, or 13 week repeat dose toxicity study in dogs for artemether and artesunate

In a dog study, beagle dogs (n=2/sex/group/time point) were administered artesunate (orally in 0.9% physiological saline) or artemether (intramuscularly in peanut oil) at 6 mg/kg/day for 4, 8, or 13 weeks. A 4-week recovery group after 13 weeks of dosing was also included. Treatment related adverse cardiac, neuropathological, and erythropoiesis effects were observed (Yin et al. 2014).

Cardiac findings included statistically significantly prolonged QT intervals (adjusted for heart rate as QT<sub>500</sub>) in male and female dogs in the artemether group from week 3-13 compared to the vehicle control group. Furthermore, there were statistically significant decreases in heart rate among dogs treated with artesunate. Inflammation, fatty infiltrations, and cell degeneration in cardiac conduction system were present in the artesunate group only (1/4 or 2/4 at 8 or 13 week necropsies, respectively, but none was seen at 4 or 17 week necropsies).

Adverse neuropathological alterations were observed in the artemether group starting from week 8 necropsies and afterwards. Observed abnormalities consisted of neuronal chromatolysis, swollen cell bodies, eccentric nuclei, neuronal degeneration, and neuronal necrosis. The incidence was related to the treatment duration. For example, in the artemether group, neuronal necrosis was present in 2/4 dogs at 8 weeks and 4/4 at 13 weeks. Neuronal lesions were noted in 1/4 dogs even after the 4 week recovery period. In addition, hepatocyte necrosis was present in the artemether group only (3/4 or 2/4 at 4 or 8 week necropsies, respectively, but none at 13 or 17 week necropsies).

Mild inhibition of erythropoiesis in sternal bone marrow was noted in both artesunate and artemether treated groups with more severe findings in the artemether groups (at 4 weeks, 2/4 or 4/4 in the artesunate or artemether group, respectively; 2/4 at 8 or 13 weeks in the artemether group). The inhibition of erythropoiesis was reversible as evidenced by the absence of inhibition in the recovery groups.

Five month repeat dose toxicity study in rats for artemether

Sprague-Dawley rats (n=2 or 3/sex/group/time point) were orally administered 0, 80 or 400 mg/kg artemether in 1% tragacanth once every 2 weeks for 5 months (10 doses in total). Dose-related, reversible reductions of reticulocyte counts were noted. Transient focal vesicle degeneration of the liver tissue (1/6) or congestion of the duodenum, edema, and infiltration of inflammatory cells in the lamina propria of the intestinal villi (1/6) were observed in the 400
mg/kg group but not in the 80 mg/kg group. No CNS lesion was observed in either treatment group (Xiao et al. 2002).
APPENDIX 3. ADDITIONAL NONCLINICAL NEUROTOXICOLOGY STUDIES FOR ARTEMISININ DERIVATIVES

Twenty eight day repeat dose neurotoxicity study in mice for artemether, artesunate, and dihydroartemisinin

In a 28 day neurotoxicity study using the Swiss albino female mice model, **artemether** was administered intramuscularly in an oil preparation and **artesunate** was administered intramuscularly in an aqueous preparation at 30, 50, or 100 mg/kg/day. The animals were killed on Day 120 (Nontprasert et al. 1998). Dose-related decreased body weight gain, neurotoxicity and mortality were observed for both drugs but with higher incidence and severity in the artemether groups. Neurotoxicity signs including abnormal balance and gait were observed in 0/10, 6/12, 24/28 mice in the 30, 50, or 100 mg/kg/day artemether groups, respectively, and 0/10, 2/12, 4/34 in the 30, 50, or 100 mg/kg/day artesunate groups, respectively. Deaths were observed in 8/36 or 2/36 in the 100 mg/kg/day artemether or artesunate groups, respectively. Abnormalities of balance and gait were irreversible in 16/24 animals in the 100 mg/kg/day artemether group and in 1/6 animals in the 50 mg/kg/day artemether group. In contrast to the artemether group, neuronal abnormalities were reversible in all artesunate groups.

To further characterize the factors affecting neurotoxicities, additional neurotoxicity studies were conducted in Swiss albino female mice (Nontprasert et al. 2000; 2002a; 2002b). **Artemether** or **artesunate** in either aqueous or oil preparation was intramuscularly or orally administered daily for 28 consecutive days. The period of observation was either 120 days for mice developing clinical signs of neurotoxicity or 180 days for mice that did not develop signs after the final day of drug administration.

All animals were dead in the 150 and 200 mg/kg/day **artemether** intramuscular groups (n=20). In contrast, there was one (1/20) or three (3/20) deaths only in the 150 or 200 mg/kg/day artesunate intramuscular groups, respectively. Neurological signs such as abnormalities of gait and balance preceded death in the majority of animals administered intramuscular artemether; this observation was only noted for one animal in the artesunate intramuscular groups. These results show that artemether, a lipid-soluble derivative, was significantly more neurotoxic than artesunate, a water-soluble derivative when either drug was administered via the intramuscular route.

In addition, **artemether** administered orally in an oil based formulation was more toxic than when given in an aqueous solution. At 200 mg/kg/day, there were 27% (12/45) deaths when artemether was dissolved in oil while none when artemether was given in an aqueous suspension. Furthermore, intramuscular administration was more toxic than oral administration. All animals (20/20, 100%) were dead when artemether was administered intramuscularly at 200 mg/kg/day while only 12 of 45 animals were dead (27%) when the same dose was administered orally.

Neuropathological changes were observed in mice administered **artemether** intramuscularly at doses ≥50 mg/kg/day but not at 25 mg/kg/day. Neuropathological changes consisted of neuronal chromatolysis, and eccentric and swollen nuclei and neuronal degeneration. Degenerative neurons were often surrounded by phagocytic microglial cells. The incidences of
neuropathological changes were dose related; 0% (0/5), 40% (2/5), or 82% (14/17) in the 25, 50, or 100 mg/kg/day, respectively. Furthermore, the severity of the damage to the trapezoid nucleus expressed both as a severity score and as the percentage of affected neurons, was dose-related. All mice with neuropathological damage also showed clinical abnormalities. No neuropathological abnormalities were found in control (0/20), oral artemether (0/14 at 200 mg/kg/day), or intramuscular artesunate (0/17 at 150-250 mg/kg/day, aqueous formulation) groups.

In similarly designed neurotoxicity studies in Swiss albino female mice (20/group), dihydroartemisinin was orally gavaged once daily for 28 consecutive days at 50, 100, 150, 200, 250 or 300 mg/kg in an aqueous suspension. In addition, dihydroartemisinin, artesunate, or artemether was orally gavaged twice daily for 28 consecutive days at 150 mg/kg/dose (300 mg/kg/day) in aqueous suspensions. Dose-related abnormal balance and gait, and deaths were noted in both dihydroartemisinin groups. While there was no deaths in the 50, 100, or 150 mg/kg/day groups, there was one (1/20), three (3/20), or six (6/20) deaths in the 200, 250, or 300 mg/kg/day groups, respectively. Interestingly, there were fewer deaths (2/20) when dihydroartemisinin was given twice daily at 150 mg/kg/dose (vs. 6/20 once daily at 300 mg/kg). There was 0/20 or 1/20 death when artesunate, or artemether orally gavaged twice daily for 28 days at 150 mg/kg/dose (300 mg/kg/day). Abnormal balance and gait were observed in a few animals in the high dose dihydroartemisinin groups (≤3 animals in the 250 or 300 mg/kg/day dihydroartemisinin groups) and in the artesunate, or artemether groups (1 or 2 animals in the artesunate, or artemether 150 mg/kg/dose twice daily groups) but not in animals in the lower doses of dihydroartemisinin (≤ 200 mg/kg) groups. Microscopic examination of the trapezoid nucleus in animals with abnormal balance and gait revealed no discernible neuronal cell-body damage.

Repeat dose neurotoxicity studies in neonatal/juvenile rats for arteether

Neurotoxicity studies regimens were conducted in neonatal/juvenile Sprague–Dawley rats with two different doses of arteether (Erickson et al. 2011). First, rat pups were administered arteether intramuscularly at 0, 5, 10, 30, 60, or 90 mg/kg/day in sesame oil beginning on postnatal day (PND) 7 for 7 consecutive days. Dose-related mortality and morbidity were observed. By Day 8, almost all animals in the 60 and 90 mg/kg groups (7/8 or 8/8 rats in the 60 and 90 mg/kg groups, respectively) were either found dead or were euthanized due to moribund conditions. Mortality (2/8) was also seen in the 30 mg/kg group. Lack of body-weight gain, lethargy, and weakness were observed in treated rats. In addition, difficulty in paddling with forelimbs and treading with hind limbs was noted in rats in the 90 mg/kg group. Brain histopathology examination did not reveal any brain lesions in any of the treated rats.

A longer duration repeat dose study was performed in rat pups (6/group/time point) which were administered arteether at 0, 1, 5, or 10 mg/kg for 5 or 8 treatment cycles beginning on PND 7 (one cycle = seven daily treatment followed by 7 days of no treatment). Dose and treatment duration related neurotoxicities were observed. Neuromotor toxicity signs such as progressive tremor, jerky limb movements and hopping around were first noted in 2 rats in the 10 mg/kg group at the end of Cycle 7; one pup was found dead and another was euthanized within two days. By the end of Cycle 8, all remaining rats in the 10 mg/kg group (n=4) were euthanized.
after displaying similar signs of neuromotor toxicity. In addition, uncoordinated landing in the 
righting reflex test was observed in 3/6 rats in both the 1 and 5 mg/kg treated groups on PND 
111.

Characteristic brainstem lesions of artemisinin and its derivatives such as satellitosis, gliosis, 
progressive necrosis, and vacuolation were observed in the motor neurons of the trapezoid, 
superior olivary nuclei, and vestibular nuclei. The lesions were observed in animals in the 5 and 
10 mg/kg groups at interim necropsies (approximately one week after the last dose on Day 69, 
Cycle 5) but not in the 1 mg/kg group.

Repeat dose hepatotoxicity studies for artesunate

Two articles have been published which report on the potential effects on the liver as a result 
of artesunate exposure.

In the first paper, rats were exposed to increasing doses of artesunate [control (group 1), 1 
mg/kg (group 2), 2 mg/kg (group 3) and 4 mg/kg (group 4)] over a period of 5 days to mimic the 
clinical regimen for treating malaria. In the highest dose group, significant increases were noted 
for AST, ALT, Alk phos & total serum albumin when compared to control values, suggesting 
potential liver toxicity (see Table A4 below). No data were collected for organ weight or 
histopathology evaluation of the liver to establish the extent of hepatotoxicity after 5 days of 
daily treatment with artesunate (Omotuyi et al. 2008).

Table A4. The effect of artesunate administration on the total serum albumin (g/dL), serum 
enzyme activity (U/L) and malondialdehyde level of the liver (nmol/L).

<table>
<thead>
<tr>
<th>Plasma enzymes</th>
<th>Alkaline phosphatase (U/L)</th>
<th>Aspartate transaminase (U/L)</th>
<th>Alanine transaminase (U/L)</th>
<th>Total serum albumin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>4.83 ± 1.09</td>
<td>1.30 ± 0.02</td>
<td>1.40 ± 0.98</td>
<td>1.72 ± 1.90</td>
</tr>
<tr>
<td>Group B</td>
<td>6.28 ± 1.76</td>
<td>2.68 ± 0.94</td>
<td>1.73 ± 0.43</td>
<td>1.89 ± 0.78</td>
</tr>
<tr>
<td>Group C</td>
<td>15.87 ± 3.54a</td>
<td>3.20 ± 1.02</td>
<td>2.83 ± 0.02</td>
<td>0.88 ± 0.54a</td>
</tr>
<tr>
<td>Group D</td>
<td>26.22 ± 3.59a</td>
<td>4.80 ± 1.47a</td>
<td>4.63 ± 1.13a</td>
<td>0.29 ± 0.21a</td>
</tr>
</tbody>
</table>

Values are reported as mean ± standard deviation from 10 rats. aValues differ significantly when 
compared with the control (p < 0.05).

In the second paper, male Wistar rats were treated with artesunate in the presence or absence 
of folic acid for 5 days (Table 1). The study results show that artesuante use was associated with a 
dose dependent increase in AST, ALT and Alk Phos levels, indicating potential liver toxicity 
whereas supplementation with folic acid attenuated the increase of these enzymes (Udobre et al. 
2009). Similar to the Omotuyyi paper above, this study did not report on organ weight or 
histopathology evaluation of the liver to establish the extent of hepatotoxicity after 5 days of 
daily treatment with artesunate.
Table 1: The effect of different doses of artemisinin (artesunate) alone and artemisinin with folic acid on serum aspartate amino transferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg kg(^{-1}) b.wt.)</th>
<th>AST activity (U L(^{-1}))</th>
<th>ALT activity (IU L(^{-1}))</th>
<th>ALP activity (IU L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.750 mg ART</td>
<td>46.56±3.52*</td>
<td>24.670±8.39</td>
<td>170.23±22.15*</td>
</tr>
<tr>
<td>B</td>
<td>1.50 mg ART +0.750 mg FA</td>
<td>34.50±4.84*</td>
<td>27.090±0.71</td>
<td>158.49±26.79*</td>
</tr>
<tr>
<td>C</td>
<td>1.500 mg ART</td>
<td>51.26±2.79*</td>
<td>22.690±4.93*</td>
<td>181.01±13.59*</td>
</tr>
<tr>
<td>D</td>
<td>1.500 mg ART +1.500 mg FA</td>
<td>49.50±6.36*</td>
<td>25.000±8.49*</td>
<td>171.68±10.19*</td>
</tr>
<tr>
<td>E</td>
<td>3.000 mg ART</td>
<td>75.36±5.75*</td>
<td>320.330±7.09*</td>
<td>189.76±6.27*</td>
</tr>
<tr>
<td>F</td>
<td>3.000 mg ART +1.500 mg FA</td>
<td>54.00±4.24*</td>
<td>24.500±0.71*</td>
<td>183.83±16.61*</td>
</tr>
<tr>
<td>G</td>
<td>6.000 mg ART</td>
<td>86.00±131*</td>
<td>18.400±2.73*</td>
<td>200.40±16.77*</td>
</tr>
<tr>
<td>H</td>
<td>6.000 mg ART +1.500 mg FA</td>
<td>80.61±5.42*</td>
<td>21.000±4.24*</td>
<td>191.23±13.40*</td>
</tr>
<tr>
<td>I</td>
<td>Normal saline (control)</td>
<td>20.75±4.10</td>
<td>26.650±3.93</td>
<td>121.33±20.91</td>
</tr>
</tbody>
</table>

ART: Artemisinin, FA: Folic acid, Control: Physiological saline. The results are expressed as Mean±SD. n = 8, *Statistical significance (p<0.05)
APPENDIX 4. GENOTOXICITY STUDIES FOR ARTEMISININ DERIVATIVES

Artesunate

A number of studies have reported on the genotoxic effects for artesunate which seem to act by inducing DNA damage via oxidative stress. This mechanism of action is hypothesized to be related to the cleavage of the endoperoxide bridge of artesunate which leads to the formation of reactive oxygen species and carbon-centered radicals (Olliaro et al. 2001; O'Neill et al. 2010; Wang et al. 2010).

Artesunate induced apoptosis in vitro in a concentration (at ≥ 30 μg/mL not at 10 μg/mL, 96 hours treatment) and duration-related manner (146 hours > 122 hours > 96 hours treatment) (Li et al. 2008). Artesunate also induced DNA single-strand breakage in a concentration-related manner (at ≥ 30 μg/mL but not 10 μg/mL, 24 hours treatment) in a COMET assay using the Chinese hamster ovary cell line, CHO-9. Artesunate similarly induced DNA double-strand breakage as indicated by increased gamma-H2AX phosphorylation (at ≥ 5 μg/mL not at 1 μg/mL, 24 to 48h treatment). Cells with defective DNA repair (base excision repair, homologous recombination or nonhomologous end joining) were more sensitive than corresponding wild-type cells to artesunate. In addition, artesunate induced the mutagenic oxidative DNA damage by 8-oxo-guanine (Efferth and Kaina 2010).

In vitro COMET and micronucleus assays were conducted for artesunate at 0.5, 1, or 2 μg/mL using human lymphocytes (Mota et al. 2011). Significant concentration-related increase (p < 0.05 at all levels) in DNA damage index and micronuclei frequency were observed. Artesunate induced apoptosis and necrosis as well.

The genotoxic and cytotoxic effects of artesunate were studied in an in vitro study using mammalian DNA obtained from three healthy non-smokers. Human lymphocytes were exposed to different concentrations of artesunate for 3 hours. A statistically significant (p < 0.05), concentration-dependent increase in DNA damage was observed in all evaluated concentrations compared to the negative control (Mota et al. 2011).

An in vivo COMET and micronucleus assays were conducted for artesunate in male Swiss albino mice. A single dose of artesunate was administered by oral gavage at doses of 0, 5, 50, or 100 mg/kg in water. The results demonstrated that artesunate induced significant DNA damage in liver cells at all doses and caused dose-related and statistically significant increase (at 50 and 100 mg/kg) in micronucleated polychromatic erythrocytes (Aquino et al. 2011).

In another in vivo study, Swiss albino male mice were used to test the effect of artesunate on sperm DNA integrity. Artesunate was administered IP at 40 mg/kg (in physiological saline) as a single dose or for 3 days at 13.4 mg/kg/day, evaluation was conducted at 24 hours and on day 35 (n=5/time point) following exposures in vivo. A positive control (2 mg/kg b.w. of mitomycin C) was used. The COMET assay revealed a significant increase in DNA strand breaks in spermatozoa as evidenced by approximately 3-fold increase in % tail DNA (p < 0.001) after 35 days of artesunate (single dose or 3 day treatment) compared with controls. The significant reduction in glutathione and super oxide dismutase and the increase in lipid peroxidation...
(malonaldehyde level) in hepatic tissues suggest oxidative stress in artesunate treated mice (Singh et al. 2015).

**Artemether**

The cytotoxic and genotoxic effects of artemether were evaluated to compare gastric cancer cell lines to human lymphocytes exposed to the same conditions. Results showed that artemether induces cytotoxic and genotoxic alterations in gastric cancer cell lines and cytotoxic effects in human lymphocytes likely related to reactive oxygen species, which at high concentrations can cause DNA breakage via oxidative damage (Alcantara et al. 2013).

In conclusion, while artemisinin did not have genotoxic activity, the literature has extensive data showing the genotoxic effect of artesunate. The mechanism of action seems to be related to the breakage of the endoperoxide bridge, which generates reactive oxygen species and causes cell death and mutations.
APPENDIX 5. DEVELOPMENTAL AND REPRODUCTIVE STUDIES FOR ARTEMISININ DERIVATIVES

Fertility Effects
Artesunate treatment in combination with other antimalarial agents\textsuperscript{21} appears to have an adverse effect on sperm parameters where a significant ($p < 0.05$) decrease in total sperm count and sperm motility, increase in abnormal sperm cells (morphology), debris and premature sperm cells were seen. The effects were maximal at the subclinical doses and synergistic in the combination therapies, compared to monotherapy of any of the individual agents. Artesunate, amodiaquine and artesunate/amodiaquine caused 54%, 28% and 84% decreases in total sperm count, with 13%, 31% and 64% decreases in sperm motility, respectively. Similar synergistic responses were obtained with artesunate/sulfadoxine/pyrimethamine. These results were explained in terms of the oxidative effects of these agents, through generation of free radicals, resulting in pathological damage to testicular structure/function (Obianime and Aprioku 2009).

Exposure to artesunate (2.9mg/kg/day) for 5 days did not alter the fertility endpoints for treated male rats except for a decrease in serum testosterone. A longer exposure to artesunate (6 weeks of daily exposure to 2.9 mg/kg) significantly reduced sperm characteristics (motility and viability, but not the number of sperm), increased levels of serum follicle stimulating hormone and luteinizing hormone, a decrease in serum testosterone levels. Histopathological evaluation of the 5 days treatment group showed mild effects on the testicular and epididymal structures, compared to the 6 week treatment group which showed visible reduction in epididymal sperm content, disorganization of the seminiferous tubule architecture, visible lesions in the testicular and epididymal tissues and arrested spermatogenesis; all of which showed some improvement during the recovery period (Olumide and Raji 2011).

Fertility and early embryonic development were evaluated for artemisone in rats (0, 5, 20 and 80 mg/kg bw/day). At high doses, a reduction in the number of females in estrus (reduced reproductive cycles), a prolonged time to insemination, decreased numbers of corpora lutea, implantation sites, and viable fetuses were observed (Schmuck et al. 2009).

The effect of artesunate on spermatogenesis was tested. A single dose of artesunate (40 mg/kg) or 3 doses of artesunate (13.4 mg/kg/day) significantly increased the frequency of sperms with abnormal head morphology at 24 h or on day 35 after dosing (3.3±1.0, 9.1±1.3, 7.2±0.1, or 6.3±0.3% in the negative control, positive control, 40 mg/kg single dose, or 13.4 mg/kg/day for 3 days groups, respectively, at 24h). Furthermore, the single dose group resulted in significantly reduced epididymal sperm count at 24 h or on day 35 after dosing (30±1.2/epididymis x10\textsuperscript{6}, 16.6±0.7, or 21.6±1.8 in the negative control, positive control, 40 mg/kg single dose groups, respectively, at 24h) (Singh et al. 2015).

Embryofetal effects
Standard embryofetal development studies were conducted in rats and rabbits for artesunate (Clark et al. 2004). Sodium artesunate (suspended in 1% aqueous methylcellulose) was orally

\textsuperscript{21}Artesunate; artesunate/sulfadoxine/pyrimethamine; artesunate/amodiaquine and their combinants (sulfadoxine/pyrimethamine and amodiaquine).
administered to pregnant Sprague Dawley rats (0, 6, 10, or 16.7 mg/kg; GD 6-17) and New Zealand White rabbits (0, 5, 7, or 12 mg/kg; GD 7-19). Significant dose-related embryonic losses (abortions in rabbits and resorptions in both rats and rabbits) were observed at all dose levels. Artesunate caused total litter loss in 21 of 24 pregnant rats at 16.7 mg/kg/day. In addition, growth retardation, cardiovascular malformations (ventricular septal and vessel defects), skeletal defects (shortened and/or bent long bones and scapulae, misshapen ribs, cleft sternebrae, and incompletely ossified pelvic bones) were reported. Adverse developmental effects were observed largely in the absence of any apparent maternal toxicity. The rat was found to be more sensitive than the rabbit in detecting embryolethal and growth retardation effects of artesunate.

Dose-related post-implantation embryonic losses and malformations were observed for dihydroartemisinin in rats. In this study, pregnant Crl:CD(SD)BR rats were orally administered 0, 7.5 or 15 mg/kg/day dihydroartemisinin (suspended in 0.5% Methocel + 1% Tween 80) on GD 9.5 and 10.5. Cesarean section delivery was conducted on GD 11.5, 12.5, 13.5 or 15 (n=2/group/time point, except on GD 15: n=2, 4, or 0 in the 0, 7.5 or 15 mg/kg/day groups, respectively) and GD20 (n=6). Dose-related increases in post-implantation losses was noted starting on GD11.5 and pup death on GD 13.5 and afterwards. Post-implantation losses were 4%, 25%, or 97% in the 0, 7.5, or 15 mg/kg groups, respectively, on GD 13.5; 0% or 60% in the 0 or 7.5 mg/kg groups, respectively, on GD 15; or 2%, 68%, or 99% in the 0, 7.5, or 15 mg/kg groups, respectively, on GD 20. At 7.5 mg/kg/day, malformations included cardiovascular and skeletal defects, similar to that identified for other artemisinins, and were observed on GD 15 or 20. Overall, malformations were noted in 44% fetuses (10/33) from three litters with embryolethality on GD 20. Interestingly, no malformations were noted in one litter without drug-related embryolethality on GD20 (Longo et al. 2006).

Further rat studies were conducted to determine the most sensitive period to artesunate. Pregnant Crl:CD(SD)IGSBR rats were orally administered artesunate (0, 10, 17, or 30 mg/kg/day, suspended in 1% methylcellulose) on single or multiple days of gestation. The results demonstrated that embryolethality, cardiovascular malformations, and skeletal defects were observed after single doses on GD 10-14, but no developmental effects were observed before GD9 or after GD16 or 17. The most sensitive day for embryolethality was GD 11. The most sensitive day for the induction of malformations was GD 10 (White and Clark 2008).

A comparative developmental toxicity study was conducted in Crl:CD(SD)IGSBR rats (23 or 25 pregnant females/group) for artesunate (15.0 mg/kg, 39 μmol/kg in 1% methylcellulose), dihydroartemisinin (11.1 mg/kg, 39 μmol/kg), artemether (19.4 mg/kg, 65 μmol/kg), and arteether (20.3 mg/kg, 65 μmol/kg) using oral administration on GD 10. Based on the previous studies, a dose that produced approximately 50% mean postimplantation loss would be optimal for producing malformations without excessive total litter loss. Accordingly, a dose estimated to provide approximately 50% postimplantation loss was used for each artemisinin. All artemisinin treatments resulted in a marked increase in embryolethality as reflected by increased numbers of females with total litter resorption, increased postimplantation loss in live litters, and an overall increased resorption rate. Mean percent postimplantation loss was 5.7, 69.7, 55.4, 68.5, or 73.6 in the control, artesunate, dihydroartemisinin, artemether, or arteether groups, respectively. Mean male and female fetal weights was statistically reduced in all artesinin groups. At least 77% of live litters in each artemisinin group showed drug–related malformations. Similar
external (mainly short tail), visceral (mainly cardiovascular, membranous ventricular septal defect, retroesophageal right subclavian vessels), and skeletal (mainly curved scapula and misshapen humerus) malformations were observed (Clark et al. 2008b).

A dose-response embryolethality study was also conducted in rats after a single intravenous administration of artesunate at 0, 0.75, 1.5, or 3 mg/kg in phosphate buffered saline on GD11. Complete (100%) postimplantation loss occurred at 1.5 and 3 mg/kg, but no loss at 0.75 mg/kg (Clark et al. 2008b).

**Artesunate** administered orally at ≥15 mg/kg or by intravenous route at ≥ 1.5 mg/kg on GD11 caused 100% postimplantation loss. In a TK study, only the AUC0-t for dihydroartemisinin was similar between the embryolethal oral and intravenous doses (147 and 219 ng.h/mL, respectively). The similar response (total embryonic loss) between oral and intravenous exposure seen for dihydroartemisinin suggested to the authors that dihydroartemisinin may be the proximate developmental toxicant (Clark et al. 2008b).

A more recent study in Sprague Dawley rats showed that when **artesunate** (in 0.5% methylcellulose solution) was orally administered to pregnant rats (0, 2, 4, or 8 mg/kg; GD 6-15), it caused dose-related embryofetal losses at 4 and 8 mg/kg (% post-implantation loss: 3.4 ± 5.18, 4.7 ± 5.61, 19.9 ± 31.13, or 37.3 ± 40.45 at 0, 2, 4, or 8 mg/kg, respectively, with 2 or 5 litters totally resorbed at 4 or 8 mg/kg, respectively). Dose-related increases in the incidence of visceral and skeletal variations (including absent ribs or thoracic vertebrae) were observed. The number of fetuses with visceral variations were 11 (8.3%), 23 (15.6%), 29 (24.4%) and 24 (25.3%) in 0, 2, 4 or 8 mg/kg groups, respectively. The number of fetuses with skeletal variations were 3(2.1%), 31(19.5%), 27(20.6%) or 23(22.3%) in 0, 2, 4 and 8 mg/kg groups, respectively (Chung et al. 2013).

Cynomolgus monkeys were dosed with **artesunate** by the oral route at 0, 4, 12, or 30 mg/kg/day on GD 20–50 (period of organogenesis, 9-15/group) or at 0 or 12 mg/kg/day on GD 29–31 or 27-33 (considered likely to be the peak of embryonic sensitivity, in 1% w/v methylcellulose) (Clark et al. 2008a). Dose and treatment duration related embryonic deaths were noted (1/15, 1/15, 7/11, or 9/9 in the 0, 4, 12, or 30 mg/kg/day GD20-50 groups, respectively, 0/15, 0/15 or 6/11 in the 12 mg/kg/day groups treated for 3, 7, or 30 days, respectively). No drug related embryonic deaths were noted in the 4 mg/kg GD 20-50 group or in the 12 mg/kg GD 29-31 or GD 27-33 groups. Artesunate was embryolethal at ≥12 mg/kg/day when dosed for at least for 12 days at the beginning of organogenesis, but not when dosed for 3 or 7 days. Histologic examination of 3 live embryos in 30 mg/kg/day GD 20-50 group (removed by cesarean section on GD26, 32 or 36) revealed a marked reduction in embryonic erythroblasts and cardiovascular abnormalities (thinning of heart walls, and distended cardiac chambers). In 4 of 9 dams in the 30 mg/kg/day group, reticulocyte values markedly decreased from 53 to 118 x 10³/mm³ before treatment to 0 to 9 x 10³/mm³ between GD 26 and 41. A fifth animal had a lesser decrease in reticulocyte count from 74x10³/mm³ before treatment to 17 to19 x 10³/mm³ on GD 31 and 41. No malformations or other abnormalities were observed in surviving fetuses examined on GD100 at 4 or 12 mg/kg/day (0/14, 0/14, or 0/4 in the 0, 4, or 12 mg/kg/day GD 20-50 groups, respectively, 0/15, 0/13, or 0/14 in the 0, 12 mg/kg/day GD 29-31, or 12 mg/kg/day GD 27-33 groups, respectively) except for long bone lengths which were slightly decreased [2.2 to 4.6% decreases in long bone
lengths relative to crown-rump length in the 12 mg/kg/day GD 20-50 group (N=4) compared to control (N=14). In this study, a dose of 4 mg/kg/day was considered the developmental no-adverse-effect-level, at which the maternal plasma AUC was 3.68 ng.h/mL for artesunate and 6.93 ng.h/mL for dihydroartemisinin. At 12 mg/kg/day for 3 or 7 days on GD29–31 or GD27–33 (n=15) when no drug related developmental toxicities, maternal plasma AUCs were 9.84 ng.h/mL for artesunate and 16.4 ng.h/mL for dihydroartemisinin.

Mechanism of action

Further studies revealed that embryonic erythroblasts were the primary target of artesunate and dihydroartemisinin toxicity in the rat embryos after in vivo treatment (Longo et al. 2006; White et al. 2006). Depletions of primitive embryonic erythroblasts preceded embryo lethality and malformations. Depletions of primitive embryonic erythroblasts were likely responsible for anemia and hypoxia, and excess cell deaths in rats. These two studies are further summarized below.

In a rat study for artesunate, pregnant Sprague-Dawley rats were administered a single oral dose of 17 mg/kg artesunate (in 1% methylcellulose) on GD10, 10.5, or 11 and fetuses were evaluated through GD14. Paling of visceral yolk sacs was observed as early as 3 hours post artesunate dosing. Within 24 hours, marked paling and embryonic erythroblast depletion were observed, which preceded malformations and embryo death, and persisted through GD 14. Histologically, embryonic erythroblasts were reduced and cells showed signs of necrosis within 24 hours and maximal erythroblast depletion was reached by 48 hours (White et al. 2006).

The embryonic erythroblasts appear to be more sensitive than maternal reticulocytes. Artesunate, when orally administered to pregnant rats (n=12) at 17 mg/kg on GD11, caused 100% embryo lethality, but only caused 15% decrease in maternal reticulocyte counts at 24 h post dosing without any other hematological changes (White and Clark 2008). The most affected organ was the heart, where abnormalities (predominantly swollen and collapsed atrium and swollen and kinked outflow tract) occurred within 24 hours post artesunate dosing and increased in incidence and severity over time (in 100% of embryos within 48 hours post artesunate dosing). Microscopic examinations revealed cardiac myopathy as evidenced by thinned and underdeveloped heart walls and enlarged chambers. Delayed limb and tail development was noted when artesunate was administered on GD 10.5, 63% (7/9) or 90% (11/12) embryos with delayed limb development by GD 13 or 14, respectively; 56% (4/7), or 100% (13/13) embryos with shorter tails by GD 13 or 14, respectively. In addition, pale liver and necrotic cells in liver were noted. Embryos were viable through GD 13 (when dosing was done on GD 10.5), but approximately 77% of embryos were dead or resorbed by GD 14, presumably due to hypoxia and/or cardiac abnormalities.

An in vitro study using embryos on GD9.5 culture showed that dihydroartemisinin also induced primitive RBC loss. Dihydroartemisinin at 0.05 μg/mL induced irregular nuclei in primitive RBCs on GD10 and cellular and nuclear alterations (irregular cell outline and indented nuclei) and cell debris on GD10.5 although no change after 1.5 hour treatment. The embryos treated with 0.05 μg/mL dihydroartemisinin had fewer primitive RBCs (365 vs. 1242 in controls) but with higher percentage irregular shape (13.4% vs. 0%) and nuclei (20.5% vs. 0.2%) than the
control embryos. Complete structural disorganization, membrane disruption, and dissolution of the nucleus were observed in severely affected cells. In addition, a concentration-dependent decrease in glutathione/protein ratio was seen in embryonic RBCs exposed to 0.05 and 0.1 μg/mL (Longo et al. 2006).

The developmental toxicity of artesunate and dihydroartemisinin seems to be mediated through the depletion of primitive embryonic erythroblasts, which was accompanied by a prolonged, severe anemia (Longo et al. 2006; White et al. 2006; Clark et al. 2008a).

In summary, extensive embryofetal developmental toxicity studies were conducted using artemisinin derivatives (artemether, artesunate, dihydroartemisinin) in rats, rabbits, and monkeys (Chung et al. 2013; Clark, 2009; Clark et al. 2004; 2008a; 2008b; White and Clark 2008). Similar to artemisinin, significant embryonic death with a steep dose–response curve was observed for all artemisinin derivatives tested, largely in the absence of any apparent maternal toxicity. Therefore, embryonic loss is considered an artemisinin class effect. In addition, malformations with external (mainly short tail), visceral (mainly cardiovascular) and skeletal malformations was observed at lower doses. The severity of the developmental toxicities associated with administration of artemisinin derivatives was species, dose, and duration related. A single oral dose of any of the artemisinin derivatives (including artesunate, dihydroartemisinin, artemether, or arteether) during a sensitive period of organogenesis was sufficient to cause embryolethality and to increase the incidence of malformations in rats (Clark et al. 2008b).
APPENDIX 6. CLINICAL TRIALS AND REPORTS FOR TREATMENT OF SCHISTOSOMIASIS

A synopsis follows the table of Clinical Trials and Reports of Artemisinin and Artemisinin Derivatives in the Treatment of Schistosomiasis.
### Table of Clinical Trials and Reports of Artemisinin and Artemisinin Derivatives in the Treatment of Schistosomiasis

<table>
<thead>
<tr>
<th>#</th>
<th>Name/date</th>
<th>Title</th>
<th>Description</th>
<th>Findings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xiao/1996/China</td>
<td>Oral <em>artemether</em> (artemether) for prevention against <em>S. japonicum</em></td>
<td>Field studies, (n = 741). Pre-treatment with PZQ, then, artemether 6 mg/kg or placebo every 2 weeks for 3 doses</td>
<td>1 month later, 20/365 (6%) infection rate artemether, versus 51/376 (14%) placebo.</td>
<td>artemether + PZQ may have a prophylactic effect against <em>S. japonicum</em></td>
</tr>
<tr>
<td>2</td>
<td>Utzinger/2000/</td>
<td>Oral <em>artemether</em> (artemether) for prevention of <em>S. mansoni</em></td>
<td>R/DB/P-controlled, (n=289). Pre-treatment with PZQ, followed by artemether 6 mg/kg (n = 138), placebo (n = 151); every 3 weeks for 6 doses.</td>
<td>3 weeks after sixth dose, 31/128 in artemether versus 68/140 in placebo re-infected. Relative risk 0.50 (95% CI – 0.35-0.71), p = 0.00006, mean egg output lower in artemether versus placebo (19 versus 32 eggs/stool), p = 0.017</td>
<td>Artemether + PZQ may have a prophylactic effect against <em>S. mansoni</em></td>
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<td></td>
<td>Cote d'Ivoire</td>
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<td></td>
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</tr>
<tr>
<td>3</td>
<td>Borrmann/2001/Gabon</td>
<td>Oral <em>artesunate</em> (ASN) &amp; Praziquantel (PZQ) for treatment of <em>S. haematobium</em></td>
<td>R/DB/P-controlled (n=300) – assess efficacy &amp; tolerability of ASN + placebo &amp; combination of ASN + PZQ, ASN 4 mg/kg for 3 days, PZQ, placebo.</td>
<td>Cure rates – PZQ + placebo 73%, ASN + placebo 27%, PZQ + ASN 81%, placebo 20%.</td>
<td>Artemesunate not efficacious alone or in combination with PZQ to treat <em>S. haematobium</em>.</td>
</tr>
<tr>
<td>4</td>
<td>De Clercq/2002/Senegal</td>
<td>Comparison of oral <em>artesunate</em> to <em>PZQ</em> for <em>S. haematobium</em></td>
<td>Efficacy and safety of ASN and PZQ compared in two villages, n=180, n=108. Half of the patients in each village treated with ASN and half with PZQ.</td>
<td>Cure rates assessed at 5, 12 and 24 weeks after completion of treatment showed ASN to be effective against <em>S. haematobium</em>, but results with PZQ were consistently better.</td>
<td>PZQ more efficacious than ASN to treat <em>S. haematobium</em>.</td>
</tr>
<tr>
<td>5</td>
<td>N’Goran/2003/Cote d’Ivoire</td>
<td>Oral <em>artemether</em> for prevention &amp; treatment of <em>S. haematobium</em></td>
<td>R/DB/P-controlled (n=161). Pre-treatment with PZQ, followed by artemether 6 mg/kg (n = 161), placebo (n = 161) – every 4 weeks for 6 doses</td>
<td>Reinfection in artemether 49% versus 65% in placebo; protective efficacy – 0.25 (95% CI – 0.08-0.38), p – 0.007.</td>
<td>Artemether + PZQ may have a prophylactic effect against <em>S. haematobium</em>.</td>
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<tr>
<td>6</td>
<td>Inyang-Etoh/</td>
<td>Efficacy of oral <em>artesunate</em> (ASN) in urinary schistosomiasis/<em>S. haematobium</em></td>
<td>Study (n=145) study to evaluate efficacy &amp; tolerability of two doses of ASN, 6 mg/kg 2 weeks apart.</td>
<td>87 subjects took two doses. 4 weeks after second dose, 61 (70%) were egg-negative and considered cured. Study non-randomized, small sample size and no comparisons were made to PZQ.</td>
<td>Artesunate may be efficacious against <em>S. haematobium</em>, but required two doses; may affect compliance.</td>
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<td>2004/Nigeria</td>
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<td>7</td>
<td>Li/2005/China</td>
<td>Efficacy of repeat doses (9-11 doses) of oral <em>artemether</em> on <em>S. japonicum</em></td>
<td>R, DB, P-controlled study to evaluate repeat doses of artemether (n= 783) after pre-treatment with PZQ, artemether at 6 mg/kg once every two weeks for 9-11 doses.</td>
<td>1 month after treatment, eggs in 3/373 (0.8%) of artemether patients and 56/361 (15%) of placebo patients (p &lt; 0.001). In artemether group, no acute cases seen; in placebo group, 3 acute <em>S. japonicum</em> cases. No serious AEs.</td>
<td>PZQ + repeat doses of artemether may be efficacious in preventing <em>S. japonicum</em>, but compliance with 9-11 doses may be an issue.</td>
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<td>8</td>
<td>Mohamed/2009/Sudan</td>
<td>Efficacy and safety of oral artesunate (ASN) + sulfadoxine/pyrimethamine (SP) versus PZQ for S. mansoni</td>
<td>Open-label trial (n=102) to evaluate E &amp; S of oral ASN 4 mg/kg for 3 days + SP on day 0 versus PZQ.</td>
<td>Cure rate at 28 days was 59% in AS+SP group and 100% in PZQ group (p &lt; 0.001). ASN + SP had poor efficacy compared to PZQ.</td>
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<td>9</td>
<td>Hou/2008/China</td>
<td>Artemether+ PZQ in treatment of S. japonicum</td>
<td>R/DB/P-controlled trial to assess two different doses of PZQ, 60 and 120 mg in combination with artemether 6 mg/kg (n=205).</td>
<td>Efficacy rates were as follows: PZQ 60 mg + artemether=98%  PZQ 60 mg + placebo=96% PZQ 120 mg + artemether=98% PZQ 120 mg + placebo = 96%</td>
<td>PZQ at 60 mg/kg was as effective as PZQ at 120 mg/kg. Combination of PZQ + ATM did not improve efficacy compared to PZQ alone.</td>
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<td>10</td>
<td>Inyang-Etoh/2009/Nigeria</td>
<td>Oral artesunate (ASN) + PZQ for urinary schistosomiasis</td>
<td>R/P-controlled trial (n = 312) to assess combination of PZQ and ASN 4 mg/kg for 3 days.</td>
<td>Cure rates: 73% PZQ + placebo, 71% in ASN + placebo, 89% in ASN + PZQ.</td>
<td>Combination of ASN + PZQ may be efficacious.</td>
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<tr>
<td>11</td>
<td>Keiser/2010/Cote d’Ivoire</td>
<td>E &amp; S of mfefloquine, Artesunate (ASN), mfefloquine+ASN and PZQ for S. haematobium</td>
<td>Randomized, open-label, exploratory trial to assess E &amp; S of ASN (3 doses at 4 mg/kg), PZQ, and mfefloquine+ASN (n = 83).</td>
<td>Cure rates at 26 days after treatment – mfefloquine – 21%, ASN – 25%, mfefloquine+ASN – 61%, PZQ-88%.</td>
<td>ASN+mefloquine may be efficacious in treatment if S. haematobium</td>
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<td>12</td>
<td>Elmorshedy/2016/Egypt</td>
<td>Oral artemether in prophylaxis of S. mansoni</td>
<td>R/DB/P-controlled trial to evaluate prophylactic effect of artemether 6 mg/kg every 3 weeks for 5 cycles after pre-treatment with PZQ. (n=913)</td>
<td>Prevalence of infection in PZQ+artemisinin group was 6.7% versus 11.6% in the PZQ + placebo group and incidence of new infections for PZQ + artemisinin was 2.7% versus 6.5% for PZQ + placebo.</td>
<td>PZQ + artemisinin may have prophylactic effects against S. mansoni.</td>
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</table>
1) A field trial conducted in 741 subjects evaluated prophylactic effect of oral artemether against acquiring *S. japonicum* infections in China. Residents in endemic areas were pre-treated with PZQ, after which, they were randomized to oral artemether (6 mg/kg) or control (placebo) group; this was repeated 3 times, 2 weeks apart (Xiao et al. 1996).

- Approximately 1 month after last medication, 20/365 (6%) residents in the artemether group were stool positive compared to 51 out of 376 (14%) in the control group.
- 2 cases of acute schistosomiasis were seen in the control group, none in the artemether group.
- Eggs per gram of feces were 122 +/- 79 (range 12-192) in the artemether group compared to 681 +/- 909 (range 12-2,760) in the control group.
- No serious AEs were noted.

*This study shows that artemether in combination with PZQ may have a prophylactic effect against *S. japonicum*.*

2) A randomized, double-blind, placebo-controlled clinical trial conducted in 289 pediatric patients evaluated the prophylactic effect of oral artemether in preventing *S. mansoni* infection in an endemic area of western Cote d’Ivoire. Four weeks after pre-treatment with two doses of PZQ four weeks apart, patients with similar baseline characteristics who were *S. mansoni* negative were treated with placebo (n = 151) or 6 mg/kg oral artemether (n = 138) every three weeks for six doses. Adverse events (AEs) were assessed 24 hours after treatment (Utzinger et al. 2000).

- 3 weeks after the sixth dose of artemether or placebo, the incidence of reinfection in artemether group was reduced (31/128) compared to the placebo group, (68/140); the relative risk was 0.50 (95% CI 0.35-0.71), p = 0.00006.
- Mean egg output was lower in the artemether group versus the placebo group (19 versus 32 eggs/stool, p = 0.017).
- No serious adverse events were reported in the study.

*Although artemether and PZQ may have a prophylactic effect in acquiring *S. mansoni* infection, results from this study could have been improved by administering artemether earlier after last dose of PZQ and by reducing treatment intervals between artemether doses. Further research is needed to evaluate combination therapy with drugs with complementary modes of action which could also help prevent resistance. Literature cautions against wider use of prophylactic artemether given that artemisinin derivatives are one of the most rapidly acting anti-malarials and are effective against severe and multi-drug resistant malaria. Potentially, prophylactic artemether could be used in countries where either: the incidence of malarial infections are low or are found for limited periods in countries close to eradicating *S. mansoni* infections, or in cases where exposure cannot be prevented, such as travelers and emergency flood-relief workers.*
3) A randomized, double-blind, placebo-controlled trial (n=300) was conducted in pediatric patients in Gabon to investigate the efficacy and safety of oral artemesunate and PZQ in S. haematobium infections (Borrmann et al. 2001). Patients were randomized to receive:

- Artesunate (4 mg/kg once daily for 3 days) plus placebo (n = 90)
- PZQ (40 mg/kg single dose) plus placebo (n = 90)
- Artesunate (4 mg/kg once daily for 3 days) plus PZQ (40 mg/kg single dose) (n = 90)
- Placebo (n = 30)

The cure rates as assessed by egg counts were as follows: hematuria and proteinuria: 73% in PZQ plus placebo group; 27% in artesunate plus placebo group; 81% in artesunate plus PZQ group; 20% in placebo group. No serious AEs were noted.

Artesunate did not appear to be efficacious alone or in combination with PZQ.

4) A clinical trial comparing efficacy and safety of oral artemesunate was conducted in pediatric patients in two villages (n = 180, n = 108) in Senegal to evaluate the effectiveness of PZQ in the treatment of S. haematobium infections. In each village, half of the patients were treated with artemesunate (malaria doses - 8 tabs of 50 mg over 5 days, i.e., 3, 2, 1, 1, 1) and half with PZQ. Cure rates assessed at 5, 12 and 24 weeks after completion of treatment showed artemesunate to be effective against S. haematobium, but results with PZQ were consistently better (De Clercq et al. 2002).

PZQ was more efficacious than artemesunate in treating S. haematobium.

5) A randomized, double-blind, placebo-controlled trial (n = 322) was conducted in pediatric patients in Cote d’Ivoire to evaluate the prophylactic effect of oral artemether in preventing S. haematobium infections in patients who were S. haematobium negative following three weeks of two pretreatments with praziquantel given 4 weeks apart. Patients were randomized to receive 6 mg/kg artemether (n = 161) or placebo (n = 161) every 4 weeks for 6 doses. AEs were assessed 72 hours after treatment (N’Goran et al. 2003).

- The incidence of S. haematobium in artemether-treated patients was 49% versus 65% in the placebo group, protective efficacy was 0.25, 95% CI: 0.08-0.38, p = 0.007.
- The geometric mean infection intensity in the artemether group was less than the placebo group (3.4 eggs versus 7.4 eggs/10 ml urine, p < 0.001).
- No serious adverse events were noted.

Artemether and PZQ may have a prophylactic effect against S. haematobium. Further research is needed to evaluate combination therapy with PZQ. Artemether could be used for S. haematobium prevention in areas where malaria is not endemic; however, the use of artemisinin derivatives for prevention of schistosomiasis could not be recommended in areas where both diseases co-exist to prevent development of resistance in malaria parasites.

6) A study was conducted (n=145) to assess the efficacy and tolerability of two doses of oral artemesunate 6 mg/kg two weeks apart in the treatment of urinary schistosomiasis/S. haematobium in Nigerian patients. Out of the subjects that took the two doses (87/145),
61/87 (70%) were egg-negative and considered cured 4 weeks after the second dose (Inyang-Etoh et al. 2004).

*Artesunate may be efficacious against S. haematobium, but required two doses which may affect patient compliance. However, the study has shortcoming as it was non-randomized, had a small sample size and no comparisons were made to PZQ.*

7) A randomized, double-blind, placebo-controlled clinical trial (n=783) was conducted to evaluate the effects of repeat doses of oral *artemether* in preventing *S. japonicum* infection in a highly endemic area in China. After a single dose of PZQ, patients were assigned to receive artemether 6 mg/kg or placebo once every 2 weeks for 9 – 11 doses covering the entire transmission season for *S. japonicum*. One month after treatment, stool analysis showed eggs in 3/373 (0.8%) of artemether patients and 56/361 (15%) of placebo patients (p < 0.001). In the artemether group, no acute cases were seen; in the placebo group, there were 3 acute *S. japonicum* cases. No serious AEs were noted (Li et al. 2005).

*PZQ in combination with repeat doses of artemether may be efficacious in preventing *S. japonicum*, but compliance with 9-11 doses is of concern. Another issue of concern is the resistance in malaria parasites if repeat doses of artemether were to be taken in areas endemic for both malaria and schistosomiasis.*

8) An open, randomized clinical trial (n=102) was conducted to compare the safety and efficacy of oral *artesunate* (4 mg/kg) for 3 days + sulfadoxine/pyrimethamine (25 mg on day 0) versus that of PZQ (40 mg/kg) in treating *S. mansoni* infections in schoolchildren in Sudan. The cure rate at 28 days was 59% in the artesunate and sulfadoxine/pyrimethamine group and 100% in the PZQ group (p < 0.001) (Mohamed et al. 2009).

*Artesunate and sulfadoxine/pyrimethamine appeared to be less efficacious in treating *S. mansoni* compared to PZQ.*

9) A randomized, double-blind, placebo-controlled clinical trial (n=205) was conducted on Chinese patients to assess the safety and efficacy of combining *artemether* and PZQ in different dosing regimens (60 and 120 mg of PZQ) to treat acute *S. japonica*. Efficacy rates were as follows: PZQ 60 mg + artemether = 98%; PZQ 60 mg + placebo = 96%; PZQ 120 mg + artemether = 98%; PZQ 120 mg + placebo = 96%. PZQ at 60 mg/kg was as effective as PZQ at 120 mg/kg (Hou 2008).

*Combination of AM and PZQ at two different doses did not improve efficacy compared to PZQ alone.*

10) A randomized, placebo-controlled trial (n=312) was conducted to assess the effectiveness of a combination of PZQ and *artesunate* 4 mg/kg for 3 days in the treatment of urinary schistosomiasis/*S. haematobium* in a Nigeria population. The cure rates were: 73% PZQ + placebo; 71% artesunate + placebo, 89% artesunate + PZQ (Inyang-Etoh et al. 2009).
Combination of artemunate and PZQ may be more efficacious than each ingredient alone. Per Cochrane review/2014 – trial methods not adequately described; unclear risk of bias.

11) A randomized, open-label, exploratory trial (n=83) was conducted to assess efficacy and safety of oral artemunate (3 doses at 4 mg/kg), PZQ, and mefloquine + artemunate) in a population in Cote d’Ivoire. The cure rates at 26 days after treatment were: mefloquine = 21%; artemunate = 25%; mefloquine + artemunate = 61%; PZQ = 88% (Keiser et al. 2010).

Artemunate and mefloquine may be efficacious in treatment of S. haematobium.

12) A randomized, double-blind, placebo-controlled trial (n =193) was conducted to assess the prophylactic effect of artemether after pre-treatment PZQ, on S. mansoni infection and to assess safety and efficacy of PZQ vs. PZQ + artemether in an Egyptian population. Patients received two doses of PZQ four weeks apart, after which one group received 6 mg/kg of artemether every 3 weeks in 5 cycles and the other group received placebo.

Prevalence of infection in the PZQ/artemether group = 6.7 % versus 11.6 % in the PZQ/placebo group and incidence of new infection for PZQ + artemether was 2.7% versus 6.5% for PZQ + placebo (Elmorshedy et al. 2016).

PZQ and artemether may have prophylactic effects against S. mansoni.

13) Twenty four randomized, controlled clinical trials (n=6315) assessing safety and efficacy of PZQ were evaluated where metrifonate or artemisinin derivatives were used to treat S. haematobium urinary schistosomiasis (Danso-Appiah et al. 2009). The main outcome measure was failure rate (proportion of patients who were positive for eggs in urine). This paper summarizes a Cochrane systematic review conducted by the authors.

The Cochrane review concluded that most trials were conducted more than a decade ago, had numerous methodological difficulties, including being insufficiently powered, lacking standardization in assessing and reporting outcomes that affected quality control and made assessments difficult and that while PZQ and metrifonate appeared to be efficacious as monotherapy against S. haematobium, the data was inadequate to evaluate artemunate. Safety assessments were inadequate and poorly reported, suggesting that future clinical trials to assess tolerability are needed.

14) A Cochrane review evaluated randomized controlled clinical trials assessing safety and efficacy of drugs for urinary schistosomiasis, related to S. haematobium infection,(Kramer et al. 2014). Primary efficacy outcomes were parasitological failure (S. haematobium eggs in urine one month after treatment). 30 RCTs were included (n = 8165); drugs included primarily praziquantel and metrifonate.

- Artesunate versus placebo: The Bormann et al. (2001) and the Inyang-Etoh et al. (2009) trials mentioned above were included in the review. The Bormann trial showed no

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In late 1990s, metrifonate was withdrawn from the WHO list of drugs to treat schistosomiasis
difference between artesunate and placebo. The Inyang-Etoh trial appeared to indicate
that combination of PZQ and artesunate may be efficacious but trial methods were not
well described and it was unclear if the trial was at risk of bias. Both trials indicated that
artesunate reduced egg excretion compared to placebo, but the percent reduction was low
compared to placebo-controlled trials of PZQ (52% versus 69%).

- **Artesunate** versus PZQ: Three trials, Borrmann et al. (2001), Inyang-Etoh et al. (2009),
  Keiser et al. (2010) mentioned above compared artesunate 4 mg/kg/day with PZQ 40
  mg/kg single dose with mixed results. In the Bormann and Keiser clinical trials, the
  artesunate arms showed parasitological failure of over 70% at one month and two months
  respectively. In the Inyang-Etoh 2009 trial, artesunate performed similar to PZQ with
  28% treatment failure at two months.

- **PZQ versus PZQ + artesunate** – Results from the Borrmann et al. (2001) and Inyang-
  Etoh et al. (2009) clinical trials were inconsistent. The Bormann trial found that adding
  artesunate to PZQ did not substantially reduce treatment failures or percent egg reduction
  at 8 weeks compared to PZQ alone and in the trial that may have been at risk of bias
  (Inyang-Etoh et al. 2009), adding artesunate apparently improved outcomes.

The Cochrane Kramer et al. (2014) review concluded that PZQ appeared to be efficacious for
*S. haematobium* and future strategies could focus on combination of PZQ with metrifonate or
artesunate utilizing adequately powered trials with standardized outcome measures. Also, the
inadequate quality of most clinical trials provided inconsistent results regarding the
therapeutic value of artemisinin and its derivatives for treatment of schistosomiasis. While
adverse events were described as mild for all evaluated drugs, methods for AE monitoring
and reporting needed to be improved.

15) A meta-analysis was conducted on artemisinin-based therapies for the treatment and
prophylaxis of schistosomiasis. The meta-analysis included a comparison of therapeutic
efficacy of **artesunate** alone, artesunate + sulfadoxine-pyrimethamine and a combination of
artemisinin derivatives plus PZQ against PZQ alone on different types of schistosomiasis. In
addition, studies on artesunate and artemether as preventive drugs were also analyzed against
placebo. Primary outcome for treatment was ‘parasitological cure’ whereas for prophylaxis it
was ‘infection rate’ (Perez del Villar et al. 2012). Results were as follows:

- Artesunate alone showed significantly lower cure rates than PZQ (p < 0.001) and the
  combination of artesunate and sulfadoxine-pyrimethamine was also less effective than
  PZQ (p = 0.04). Artesunate may not be beneficial for monotherapy because it affects the
  early stages of the parasite.
- Artemisinin derivatives plus PZQ combination therapy were superior to PZQ alone
  (p=0.003) and may have the potential to increase cure rates as well as help avoid
  resistance given their complementary modes of action - artemisinin derivatives against
  egg and juvenile stages of *S. mansoni, S. haematobium and S. japonicum*, versus PZQ
  against adult worms. However, the estimated results could not be regarded as definitive
  because they are based on a diverse population and small sample size
- Chemoprophylaxis using artesunate (p < 0.001) or artemether (p < 0.001) was better than
  placebo; this was most relevant in *S. japonicum* infection.
• Unclear if the efficacy of artemisinin derivatives is best measured after treatment or at 3 weeks; more studies needed.
• Incorporation of artemisinin derivatives in mass PZQ administration has limitations – cost-effectiveness, sub-optimal biopharmaceutical properties (short half-life) require rapid treatments, and use of artemisinin and its derivatives could contribute to emergence of artemisinin-resistant malaria.
• No serious adverse events and no interaction between PZQ and artemisinin and its derivatives observed, but clinical trials not designed to address differences in adverse events.

16) A meta-analysis was conducted to evaluate the antischistosomal efficacy of different medication strategies including monotherapy or combination therapies (Liu et al. 2011).

• PZQ was found to be effective at treating schistosomiasis and that multiple doses improve efficacy;
• Multiple doses of artesunate or artemether in 1 to 2 week intervals may help prevent schistosomiasis.
• PZQ in combination with artesunate or artemether may be suitable for treatment as opposed to PZQ alone.

17) A meta-analysis of clinical trials was conducted to evaluate the efficacy of artesunate compared to PZQ to treat urinary schistosomiasis (S. haematobium) Wikman-Jorgensen et al. 2012).

• Cure rate for PZQ was superior to that of artesunate
• Artesunate was not superior to placebo and had a marginal role in combination therapy with PZQ
APPENDIX 7. CLINICAL TRIALS OF ARTEMISININ AND ARTEMISININ DERIVATIVES IN THE TREATMENT OF HELMINTHIC INFECTIONS OTHER THAN SCHISTOSOMIASIS

A synopsis follows the table of Clinical Trials of Artemisinin and Artemisinin derivatives in the treatment of helminthic infections other than schistosomiasis.
<table>
<thead>
<tr>
<th>#</th>
<th>Name/date</th>
<th>Title</th>
<th>Description</th>
<th>Findings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tinga/1999/Vietnam</td>
<td>Effect of oral artemisinin or PZQ on clonorchiasis</td>
<td>Pilot study (n=21) comparing PZQ o.d. to artemisinin 500 mg bid for 5 days in clonorchiasis.</td>
<td>Reduction of egg counts was from 1103 to 542 epg, p &gt; 0.05 for artemisinin versus 1632 to 37 epg, p &lt; 0.001 for PZQ. Eradication rate was 29% for PZQ versus 10% for artemisinin.</td>
<td>Artemisinin was not efficacious compared to praziquantel for clonorchiasis.</td>
</tr>
<tr>
<td>2.</td>
<td>Hien/2008/Vietnam</td>
<td>Effect of oral artesunate versus triclabendazole on Human Fascioliasis</td>
<td>Randomized, open-label trial (n=100) comparing oral artesunate 4 mg/kg/day versus triclabendazole for 10 days.</td>
<td>The artesunate group had less abdominal pain (primary endpoint), but clinical and serological response rates were lower in artesunate patients compared to triclabendazole.</td>
<td>Unclear if artesunate was efficacious in treatment of human fascioliasis.</td>
</tr>
<tr>
<td>3.</td>
<td>Adedeji/2008/Nigeria</td>
<td>Efficacy of oral artemesate &amp; amodiaquine in intestinal helminths in children with P. falciparum malaria</td>
<td>Nested trial (n=109) assessing efficacy of artemesate 4 mg/kg/day for 7 days or amodiaquine for 3 days in patients with malaria.</td>
<td>On day 3, higher proportions of patients in ASN group 40/61 vs. 21/48 in amodiaquine group were helminth carriers, p =0.03.</td>
<td></td>
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<tr>
<td>4.</td>
<td>Soukhatham savong/2011/Laos</td>
<td>Efficacy &amp; safety of oral mefloquine, artemesate, mefloquine-artesate, tribendimidine and PZQ in Opisthorchis viverrini.</td>
<td>Randomized, open-label trial (n=125), assessing efficacy and safety of mefloquine, oral artemesate (10 mg/kg in 3 split doses), mefloquine-artesate, tribendimidine and PZQ.</td>
<td>ITT group - 14/25 in the PZQ group cured compared with none in mefloquine, 1/24 in the artemesate group, 1/24 in the mefloquine-artesate group and 19/27 in the tribendimidine group. No serious AEs noted.</td>
<td>Artesunate was not efficacious in the treatment of Opisthorchis viverrini.</td>
</tr>
<tr>
<td>5.</td>
<td>Kamgno/2011/Cameroon</td>
<td>Efficacy of oral artemesate, quinine, chloroquine and amodiaquine on Loa loa microfilaria.</td>
<td>Exploratory study (n=98) on effects of artemesate 200 mg qd for 3 days, quinine, chloroquine and amodiaquine on Loa loa microfilaria.</td>
<td>No significant decrease in microfilaraemia was observed in any of the groups during the 90-day follow-up period.</td>
<td>Lack of effect of artemesate in treatment of Loa loa microfilaria may be related to its shorter half-life (&lt; 1 hour).</td>
</tr>
<tr>
<td>6.</td>
<td>Keiser/2011/Egypt</td>
<td>Efficacy &amp; safety of oral artemether in treatment of chronic fascioliasis.</td>
<td>Exploratory study (n=36) to assess efficacy and safety of oral artemether at two different malaria regimes - 6 x 80 mg over 3 days and 3 x 200 mg over 24 hours.</td>
<td>Cure rates were 35% and 6% for the 6 x 80 mg regime and 3 x 200 mg regime respectively. Treatment failures were treated with triclabendazole.</td>
<td>Artemether (as part of malaria combination treatment regimens) does not appear to be efficacious against chronic fascioliasis.</td>
</tr>
</tbody>
</table>
1) A randomized, open-label clinical trial was conducted to compare an oral artesunate treatment of 4 mg/kg/day versus triclabendazole 10 mg/kg bid for 10 days in 100 patients aged > 8 years with human fascioliasis as in-patient therapy in Vietnam. The median duration of illness was same in both groups, but there was a broader distribution in duration of illness in the triclabendazole group. While patients receiving artesunate were less likely to report abdominal pain at hospital discharge (primary endpoint), clinical and serological response rates were lower among artesunate patients compared to triclabendazole. No serious AEs noted (Hien et al. 2008).

*Effect of artesunate on human fascioliasis is plausible, but further research is needed.*

2) The antihelminthic activities of artesunate 4 mg/kg/day for 7 days or amodiaquine 10 mg/kg/day for 3 days was studied in a nested trial designed to evaluate antimalarial efficacy in 109 children with falciparum malaria in Nigeria. Results showed that a higher proportion of patients treated with artesunate compared to amodiaquine were helminthes carriers on day 3 (40/61) vs. 21/48, p = 0.03, and on day 14 (21/61 vs. 7/48, p = 0.03). Although the mean helminth ova count was lower on day 14 in the artesunate arm compared to the amodiaquine arm (p = 0.01), compared to amodiaquine, children treated with artesunate alone had a significantly higher propensity to remain helminth carriers.

Effects noted above may be related to the cidal activity of amodiaquine compared to artesunate as well as the PK profiles and half-lives of the two drugs; further studies are needed (Adedeji et al. 2008).

3) A randomized, open-label trial was conducted in 125 children infected with *Opisthorchis viverrini* in Laos assessing the efficacy and safety of mefloquine, artesunate (10 mg/kg in 3 split doses), mefloquine-artesunate, tribendimidine and PZQ. Results showed that 14/25 in the PZQ group were cured compared with none in mefloquine, 1/24 in the artesunate group, 1/24 in the mefloquine-artesunate group and 19/27 in the tribendimidine group. No serious AEs noted (Soukhathammavong et al. 2011).

*Artesunate was not efficacious in the treatment of Opisthorchis viverrini.*

4) An exploratory study was conducted to assess the therapeutic effects of artesunate (200 mg qd for 3 days), quinine, chloroquine and amodiaquine on *Loa loa* microfilaria, a nematode/helminthic infection in Cameroon. Patients (n=98) were randomly allocated in 5 groups including a control group after stratification on microfilaria load to assess effects of standard doses of these drugs. No significant decrease in microfilaraemia was observed in any of the groups during the 90-day follow-up period (Kamgno et al. 2010).

*Lack of effect of artesunate in treatment of Loa loa microfilaria may be related to its shorter half-life (< 1 hour).*

5) An exploratory Phase 2 trial was conducted in Egypt in chronically infected *Fasciola* patients (n=36) to assess the efficacy and safety of oral artemether at two different malaria regimes.
Patients were treated with artemether at 6 x 80 mg over 3 days and 3 x 200 mg over 24 hours. Cure rates were 35% and 6% for the 6 x 80 mg regime and 3 x 200 mg regime respectively. Treatment failures were treated with triclabendazole. No serious AEs were noted (Keiser et al. 2011).

*Artemether at malaria treatment regimens shows little or no effect against fascioliasis and does not represent an alternative to triclabendazole and the role of artemisinin derivatives in combination therapy for treatment of chronic fascioliasis remains to be elucidated.*
APPENDIX 8. CLINICAL REPORTS AND TRIALS OF ARTEMISININ DERIVATIVES FOR THE TREATMENT OF CANCER

A synopsis follows the table of Clinical Reports of Artemisinin and Artemisinin derivatives in the treatment of cancer.
<table>
<thead>
<tr>
<th>#</th>
<th>Name/Date</th>
<th>Title</th>
<th>Description</th>
<th>Findings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Singh/2002/India</td>
<td>Stage II laryngeal squamous cell carcinoma treated with IM and oral artesunate (+ oral iron)</td>
<td>IM <strong>Artesunate</strong> 60 mg for 15 days, followed by oral artesunate, 50 mg qd. Oral iron supplementation continued throughout therapy.</td>
<td>Reduction in hoarseness of voice, able to take solids. Reduction in cervical lymph nodes and tumor size. Treatment discontinued after 9 months; patient died.</td>
<td>IM and oral artesunate + iron; no other chemotherapeutic treatment prescribed.</td>
</tr>
<tr>
<td>2.</td>
<td>Berger/2005/Germany</td>
<td>Metastatic uveal melanoma treated with oral artesunate – 2 patients</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; patient – oral <strong>artesunate</strong> 50 mg bid in combination with Fotemustine. 2&lt;sup&gt;nd&lt;/sup&gt; patient – oral artesunate 50 mg bid with Dacarbazine + oral iron.</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; patient - After 3 months, no new mets, lung mets decreased; 2 new CNC tumors, patient died. 2&lt;sup&gt;nd&lt;/sup&gt; patient – Lung &amp; splenic mets resolved, meds discontinued, but chemo + artesunate restarted for recurrent mets.</td>
<td>Artesunate (+ iron) used in combination with chemotherapy.</td>
</tr>
<tr>
<td>3.</td>
<td>Singh/2006/India</td>
<td>Pituitary microadenoma treated with oral artemether.</td>
<td>Oral <strong>artemether</strong> 40 mg for 29 days; dose gradually reduced to twice a week for 10 months.</td>
<td>Improvement in eye movement and vision. Tumor density reduced and disease progression slowed.</td>
<td>Patient denied surgery and chemo; treated only with oral artemether.</td>
</tr>
<tr>
<td>4.</td>
<td>Michaelsen/2015/Germany</td>
<td>Progressive prostate carcinoma treated with chemotherapy + oral <em>Artemisia annua</em> followed by intravenous artesunate.</td>
<td>Short-term 14-day treatment of bacalitumide, followed by long-term oral <em>Artemisia annua</em> capsules (5 x 50 mg/day) and afterwards, intravenous artesunate (2 x 150 mg twice weekly) for 4 months.</td>
<td>Regression of carcinoma after chemo + artemisinin treatment. 7 months after being on <em>Artemisia annua</em>, tumor recurred with skeletal metastases. <em>Artemisia annua</em> substituted by intravenous artesunate; tumor and mets progressed; patient died after 4 months. Hemoglobin levels and erythrocyte counts low through treatment period.</td>
<td>Resistance seen to long-term use of artemisinin derivatives. Hematological toxicity seen.</td>
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<tr>
<td>#</td>
<td>Name/date</td>
<td>Title</td>
<td>Description</td>
<td>Findings</td>
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<td>5</td>
<td>Zhang/2008/China</td>
<td>Non-small-cell lung cancer treated with chemotherapy + intravenous artesunate.</td>
<td>Randomized, controlled trial (n = 120). 60 pts treated with standard chemo, 60 pts treated with chemo + 120 mg intravenous artesunate qd for 8 days - for 2 cycles.</td>
<td>Artesunate + chemo patients apparently showed improved short-term and one-year survival rates compared to controls.</td>
<td>Artesunate + chemotherapy used.</td>
</tr>
<tr>
<td>6</td>
<td>Jansen/2011/Ivory Coast</td>
<td>Advanced cervical cancer treated with oral artenimol.</td>
<td>Pilot, open-label, single-center study – safety &amp; efficacy of oral artenimol (n = 10), 100 mg/day for 1st week, followed by 200 mg/day for 3 weeks.</td>
<td>Decreased vaginal discharge and pain + alteration in expression of relevant tumor proteins; clinical remission of about 6 months in all patients, after which 2 patients died.</td>
<td>Oral artenimol used on its own; no other therapy used.</td>
</tr>
<tr>
<td>7</td>
<td>Ericsson/2014/Germany</td>
<td>Population PK study of oral artesunate and its active metabolite dihydroartemisinin in patients with metastatic breast cancer.</td>
<td>Total patients = 23. First, n = 6 treated with 100 mg oral artesunate. Next, one group (n=7) received 150 mg and other group (n=10) received 200 mg artesunate once daily for 3 weeks.</td>
<td>Apparently a 25% increase in elimination clearance of dihydroartemisinin suggesting potential autoinduction.</td>
<td>Autoinduction, requiring higher doses could potentially result in toxicities.</td>
</tr>
<tr>
<td>8</td>
<td>Krishna/2015/United Kingdom</td>
<td>Colorectal carcinoma (CRC) treated with pre-op oral artesunate in patients scheduled for surgical resection.</td>
<td>Pilot, R/DB/placebo-controlled trial (n=20) – pre-op 14 daily doses of oral artesunate 200 mg (n=12) or placebo (n=11). Primary outcome – proportion of tumor cells showing apoptosis.</td>
<td>Apoptosis in &gt; 7% of cells seen in 67% of artesunate patients and 55% placebo patients. Follow-up/42 months – 1 pt in artesunate group and 6 in placebo group had recurrent CRC. 2 patients at 4 mg/kg/day had leukopenia. One recovered - 24 hours after stopping artesunate, another received G-CSF and recovered. Bone marrow: drug-induced myelosuppression.</td>
<td>Hematological toxicity seen. Dose restriction considerations.</td>
</tr>
</tbody>
</table>
**Case reports:**

1) A patient in India with Stage II squamous cell carcinoma of the larynx with local metastases was started on oral iron supplementation (given that cancer cells have a higher iron influx via the transferrin receptor mechanism) together with IM **artesunate** 60 mg for 15 days, followed by 50 mg of oral artesunate qd (No other chemotherapeutic treatment was prescribed). After starting treatment, the patient’s hoarseness of voice reduced and he was able to take solids. Apparently, cervical lymph nodes decreased in size and there was about 70% shrinkage in tumor size. Treatment was discontinued after 9 months and the patient subsequently died (Singh and Verms 2002).

2) Oral artesunate was used on a compassionate basis to treat two patients in Germany with metastatic uveal melanoma who had failed standard chemotherapeutic regimens.

   In the first patient, oral **artesunate** was started at a dose of 50 mg bid in combination with Fotemustine. After 3 months, the patient’s lung metastases started decreasing in number and there were no new metastases to the skin or visceral organs. However, there were 2 new CNS tumors, the disease progressed and the patient died.

   In the second patient who had lung and splenic metastases, oral artesunate was started at a dose of 50 mg bid in combination with Dacarbazine. The patient also received oral iron to potentially increase efficacy of artesunate. The lung and splenic metastases resolved largely, after which all medications were discontinued, then combination artesunate and chemotherapy were resumed for recurrent metastases after which the patient remained stable clinically. The authors propose that artesunate may be considered for combination with standard chemotherapy with concomitant iron, but further research is needed (Berger et al. 2005).

3) A 75 year old Indian patient with a pituitary microadenoma was treated with oral **artemether** over 12 months (patient refused surgery and radiotherapy). The patient was dosed with 40 mg (0.5 mg/kg) orally for 29 days; after 2 weeks, there was improvement in eye movement and vision. Treatment was continued for 2 more weeks, then reduced to every other day for 30 days, then further reduced and given twice a week for 10 months. Tumor size did not change but tumor density appeared to be reduced and disease progression apparently slowed (Singh and Panwar 2006).

4) A patient in Germany with progressive prostate carcinoma was first treated with short-term 14-day treatment of bacalitumide after which he was put on long-term oral **Artemisia annua** capsules (5 x 50 mg/day). PSA levels decreased, tumor regression seen by imaging techniques. Seven months later, his tumor recurred and he had skeletal metastases. **Artemisia annua** capsules were substituted by intravenous artesunate injections (2 x 150 mg twice weekly), but the tumor and metastases progressed, PSA levels increased, indicating resistance to artemisinin derivatives. Patient died after about 4 months. Hemoglobin levels and erythrocyte counts were low throughout treatment period (Michaelsen et al. 2015).
**Clinical trials**

1) In a randomized, controlled clinical trial of 120 non-small-cell lung cancer Chinese patients, 60 patients were treated with standard chemotherapy (vinorelbine and cisplatin) and another 60 patients with 120 mg intravenous **artesunate** qd together with standard chemotherapy for 8 days for at least 2 cycles. **Artesunate**-treated patients apparently showed improved short-term and one-year survival rates and prolonged time to cancer progression compared to the control group (Zhang et al. 2008) (article in Chinese).

2) A pilot, open-label single-center study was conducted in Ivory Coast to assess efficacy and safety of oral **artenimol-R** (succinate ester of artenimol) in 10 patients with advanced cervical carcinoma who were treated for 28 days. The patient was treated with a dose of 100 mg/day for the 1st week, and increased to 200 mg/day if tolerated for the next 3 weeks. The drug was overall well tolerated, with no serious adverse events. Clinical improvement, i.e., decrease vaginal discharge and pain was associated with alteration in expression of relevant tumor proteins. The 28-day treatment led to clinical remission of about 6 months in all patients. The authors recommended larger, randomized controlled trials with longer dosing periods (Jansen et al. 2011).

3) A study was conducted in Germany to characterize the population PK characteristics of oral **artesunate** and its active metabolite, **dihydroartemisinin**, in patients with metastatic breast cancer over a > 3 week daily administration period. Total patients = 23; first, n = 6 treated with 100 mg oral artesunate, next, one group (n=7) received 150 mg and other group (n=10) received 200 mg artesunate once daily for 3 weeks. There was apparently a 25% increase in elimination clearance of dihydroartemisinin suggesting potential autoinduction; the authors recommended further studies (Ericsson et al. 2014).

4) A single-center, randomized, double-blind, placebo-controlled clinical trial was conducted in the United Kingdom on 20 patients with colorectal carcinoma (CRC) who were scheduled to undergo curative resection of their tumors. Patients were scheduled to receive preoperatively 14 daily doses of oral **artesunate** 200 mg (n =12) or placebo (n = 11). Primary outcome measure was proportion of tumor cells undergoing apoptosis. Results showed apoptosis in > 7% of cells in 67% artesunate pts and 55% placebo patients. At a median follow-up at 42 months 1 patient in the artesunate group and 6 in the placebo group developed recurrent CRC; artesunate may have anti-proliferative properties. Two patients at lower weight limit for inclusion (50 kg, making the dose 4 mg/kg artesunate) developed leucopenia; one case recovered within 24 hours of stopping artesunate the other received G-CSF and recovered. Bone marrow exam was suggestive of toxic effects of artesunate; i.e. drug-induced myelosuppression (Krishna et al. 2015).

Study recommended restricting daily doses to < 4 mg/kg/day and to monitor for hematologic complications; further research is needed.
APPENDIX 9. FAERS CASES REPORTED FOR ARTEMISININ DERIVATIVES OR HERBAL ARTEMISIA.

1) Among the five cases describing use of a combination antimalarial (dihydroartemisinin/piperaquine) in PK and clinical studies, three cases reported prolonged QT interval. One case described a stroke in an HIV patient receiving concomitant nevirapine-based antiretroviral therapy. One case had a fracture after an unspecified period of starting dihydroartemisinin/piperaquine.

_Dihydroartemisinin/piperaquine is not approved in the US; however, it appears that piperaquine may be associated with QT prolongation (per Eurartesim (320 mg piperaquine tetrathosphate and 40 mg dihydroartemisinin) label; (Darpo et al. 2015). Artemisinins may increase or decrease nevirapine and other anti-HIV drug levels; stroke may have been related to HIV or malaria (which would have been expected to be severe in patient with HIV)._

2) One literature report described an 82-year old Turkish patient who was stable at warfarin dose of 2.5 mg per day for several months for atrial fibrillation, and about 10 days after starting _Artemisia absinthium_ (AA) for a sore throat, she developed a high INR and GI bleeding requiring blood transfusion. Upon discontinuing AA, the INR came down and stabilized, and she was discharged on her usual dose of warfarin (Acikgoz and Acikgoz 2013).

_The positive dechallenge associated with discontinuation of AA is suggestive of causality. Concomitant use of artemisa absinthium with warfarin may have caused the unexpected rise in INR (Acikgoz and Acikgoz 2013); AA has apparently been shown to contain 6-methoxy-7,8-methylenedioxy coumarin (Yamari et al. 2004)._  

3) One 67 year-old patient with breast cancer and lung metastases had ulcerations on her hands and feet while receiving capecitabine. Concomitant medications included octreotide, hydroxyurea, cyclophosphamide, somatostatin, leuprorelin, letrozole, melatonin, vinorelbine, and _Artemisia_.

_Capecitabine is labeled for mucocutaneous and dermatological toxicity, including ulcerations under Warnings and Precautions._

4) One 54 year-old patient who was on fexofenadine and vitamins had a seizure eight hours after receiving allergy injections including standard cat hair (_Felis catus_), dog epithelia (_Canis_ spp.), standard mite (_Dermatophagoides farinae_), six tree mix, American elm (_Ulmus americana_), red maple (_Acer rubrum_), standard Timothy (_Phleum pratense_), standard short ragweed (_Ambrosia artemisiifolia_), English plantain (_Plantago lanceolata_), and common mugwort (_Artemisia vulgaris_).

_Concomitant illnesses unknown; causality unclear._
5) One 60-year old patient who was on artemether/lumefantrine (Coartem) for babesiosis developed back pain, arthralgia and neuralgia which eventually resolved, only to return on starting artemisinin (after running out of Coartem). Outcome unknown.

*Back pain, arthralgia are associated with babesiosis; causality unclear. Coartem is not indicated to treat babesiosis.*
APPENDIX 10. CAERS CASES INVOLVING ARTEMISIA AND ARTEMISININ DERIVATIVES.

1) Report #: 112443 (1/27/2009) - A retailer reported that a consumer who had Cell-Tech Hardcore (Fruit Punch) that contains Tarragon Leaf (*Artemisia dracunculus*) experienced nausea, dizziness and vertigo on day 5 of product use and visited an ER. Further details are not available.

_Data limited, past medical history and concomitant medication history are unknown; causality unclear, but there appears to be a temporal relationship between ingestion of Cell-Tech Hardcore and patient’s symptoms. Cell-Tech Hardcore contains Artemisia dracunculus (Artemesinin concentration expected to be more in Artemisia annua compared to Artemisia dracunculus) (Mannan et al. 2010)._ 

2) Report #: 114240 (6/17/2009) - A lawyer reported that his client experienced liver damage following ingestion of Hydroxycut Max containing Tarragon (*Artemisia dracunculus*) for weight loss. Previous medical history and other information, including labs are unavailable. This report was submitted after the FDA advisory on May 1, 2009 regarding liver injury being associated with Hydroxycut products.

_Past medical history and concomitant medication history is unknown. Causality unclear; appears to be a temporal relationship between ingestion of Hydroxycut Max and patient’s symptoms._

3) Report # 119347 (10/4/2009) – A patient using the product Para-Cleanse Black Walnut ATC containing Mugwort herb (*Artemisia vulgaris*) and Wormwood herb (*Artemisia annua*) experienced a seizure and respiratory arrest on the 10th day of using the product which required hospitalization and treatment for a period of 30 hours with intravenous fluids, magnesium and potassium.

_Past medical history, concomitant medication history, hospitalization history, including lab data during current episode is unknown. It is possible that Para-Cleanse, typically used as a colon cleansing product may have produced diarrhea and electrolyte abnormalities leading to the seizure; the respiratory arrest was likely associated with the seizure. Para-Cleanse contains multiple other ingredients in addition to mugwort (Artemisia vulgaris) and sweet wormwood (Artemisia annua), such as pumpkin seed, black walnut hulls, cascara sagrada bark, chamomile flowers, mullein leaf, marshmallow root, slippery elm bark, elecampane root, clove flower, garlic bulb, ginger rhizome, spearmint leaf and flower, turmeric root, and olive leaf extract. It is difficult to ascribe causality to artemisinin given multiple ingredients in the Para-Cleanse product._

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23 Cases #5, #6, #11, #12 and #14 are included for completeness. Different *Artemisia* species of plants contain variable amounts of *Artemisia*. *Artemisia annua* contains the highest concentration.
4) **Report #: 136029 (5/18/2010)** – A patient with a prior history of ‘prolonged use of alcohol’ and ‘liver problems’ saw an acupuncturist who prescribed Bupleurum and Rehmannia, two types of herbs, between 5/18-5/22/2010. The patient had severe pain and returned to see the acupuncturist on 5/26/2010 and was given additional herbs. The patient required hospitalization, where a liver biopsy diagnosed acute hepatitis and mild cirrhosis. Subsequently, the patient had sepsis and multi-organ failure and died on [b] (b) [6].

The report states that the patient consumed Evergreen *Artemisia Yinchenhao* decoction; it is unclear if this was prescribed the second time she saw the acupuncturist. While this patient had a past medical history of alcoholism, it appears that the consumption of herbal treatments may have contributed to the acute hepatitis, while the cirrhosis was likely related to the history of alcoholism. It is unclear whether the herbal treatment/active ingredient may have contributed to the acute hepatitis.

5) **Report #: 158324 (10/16/2012)** – A patent with a history of controlled hypertension and hypothyroidism took the Young Living Essential Oil (YLEO) products containing Tarragon (*Artemisia dracunculus*) and experienced lip and tongue swelling, dysphagia, tingling of hands and feet, vomiting, bowel urgency and ocular hyperemia. Patient went to the ER for the allergic reaction and was treated with epinephrine and steroids and discharged on Epipen and a follow-up course of steroids.

While there appears to be a temporal association between ingestion of the YLEO product (dose unclear) and the allergic reaction, it is unclear if this could be related to Tarragon (*Artemisia dracunculus*) given the YLEO product contains in addition, anise, fennel, peppermint, and clove oils.

6) **Report #: 181091 (11/28/2014)** – A patient took six Young Living Products – Comfortone (containing Tarragon leaf – *Artemisia dracunculus*), Juvatone, Super B, Sulfurzyme, Inner Defense and ICP in the morning, did not eat breakfast, nor drink the amount of fluids she normally did, and passed out about 45 minutes later. She was hospitalized for 24 hours, labs were normal, and she was discharged.

Past medical history unavailable, however, given the temporal association, it appears the apparent syncopial episode may have been related to ingestion of multiple medications on an empty stomach. Difficult to ascribe causality to Tarragon/Artemisia dracunculus given each of the 6 herbal products contains multiple ingredients as described below.

- **Comfortone capsules** contain cascara sagrada bark, barberry, psyllium seed, barberry bark, burdock root, fennel seed, garlic bulb, echinacea root, bentonite, german chamomile flower, licorice root, cayenne fruit, aple pectin, tarragon (*Artemisia dracunculus*) leaf, ginger root, tangerine rind, rosemary leaf, anise seed, peppermint leaf, and ocotea leaf.
- **Juvatone tablets** contain calcium, copper, choline, di-methionine, beet root, inositol, dandelion root, l-cysteine, alfalfa sprout, oregon root, grape, parsley leaf, echinacea root, bee propolis, lemon rind, german chamomile, geranium, rosemary leaf, myrtle leaf, and blue tansy.
• Super B tablets are multivitamin, multimineral tablets; also contain para amino benzoic acid and nutmeg oil.
• Sulfurzyme capsules contain methylsulfonylmethane and Ningxia wolfberry (lycium barbarum) fruit.
• InnerDefense capsules contain coconut oil, clove bud, lemon rind, eucalyptus leaf, rosemary leaf, cinnamon bark, oregano leaf, thyme and lemongrass leaf.
• ICP contains psyllium seed, flax oil, oat bran, fennel powder, rice bran, guar gum seed, yucca root, cellulose, fennel seed oil, anise seed oil, tarragon (Artemisia dracunculus) leaf oil, ginger root oil, lemongrass leaf oil, rosemary leaf oil and aloe vera juice.
APPENDIX 11. ADVERSE EVENTS FOR ARTEMISININ DERIVATIVES

1) Bethell et al. (2010) describe a dose-dependent risk of neutropenia after 7-day courses of oral artesunate monotherapy in Cambodian patients with uncomplicated P falciparum malaria. Patients in this randomized, single-center clinical trial were assigned to receive artesunate 2 mg/kg/day for 7 days (total dose 14 mg/kg), 4 mg/kg/day for 7 days (total dose 28 mg/kg) or 6 mg/kg/day for 7 days (total dose 42 mg/kg) and were observed for 42 days. The 6 mg/kg/day group had significantly lower geometric mean absolute neutrophil counts than the 2 mg/kg/day and 4 mg/kg/day groups (p < 0.01 for each). 5 of the 26 (19%) patients receiving 6 mg/kg/day became neutropenic within 14 days and 2 patients had the artesunate discontinued because their absolute neutrophil counts were < 1.0 X 10^3 cells/μL. Neutropenic patients remained clinically well and ANC values rebounded around 48 hours after discontinuation for the patient with the lowest ANC count. The trend for neutropenic patients to have higher drug exposure is suggestive of sub-groups at risk of myelosuppressive effects of artesunate; genetic polymorphisms of enzymes that may have explained these effects is unknown.

2) Konig et al. (2016) report a prospective, open, uncontrolled, single-center, Phase 1 dose-escalation study in which patients with metastatic breast cancer received up to 2 courses of artesunate as add-on therapy to their usual chemotherapy. Doses were 100 mg, 150 mg and 200 mg daily for 4 weeks and if there was no dose-limiting toxicity, a second course was allowed. An audiological assessment was performed before and 4 weeks after therapy that included audiometry, otoacoustic emissions, brainstem evoked potentials, etc. Of the 23 patients included in the study, results showed that continuous intake of artesunate for 4 weeks in doses up to 200 mg were overall well tolerated. During the test phase, 4 patients had audiological adverse events possibly related to artesunate including sub-clinical hearing loss and tinnitus they were not severe and did not require discontinuation of treatment. 4 patients had vertigo, one of which was a serious AE, but reversible upon discontinuation. Two patients had ongoing subclinical hearing loss and another ongoing tinnitus. While there was no dose-limiting toxicity, the authors recommend audiological monitoring with prolonged dose of artesunate in doses up to 200 mg daily.

3) Leonardi et al. (2001) describe 2 cases of severe allergic reactions to oral artesunate. In the first patient, about 15-20 minutes after receiving oral artesunate 4 mg/kg, the patient developed a generalized, urticarial rash, dizziness, hypotension which resolved with treatment. He was rechallenged the next day with oral artesunate with pre-treatment with antihistamines and an hour later developed pruritus, periorbital, lip and forearm swelling tachycardia and hypotension requiring appropriate treatment after which he recovered. In the second case, eleven hours after receiving artesunate 4 mg/kg, a patient developed agitation, pruritus, chest pain, dyspnea, wheezing, rash before becoming unconscious for which he received appropriate treatment; all resolved with treatment.

4) Efferth et al. (2016) discuss hepatotoxicity induced by combination treatment of temozolomide, artesunate and Chinese herbs in a patient with glioblastoma multiforme. A 65-year old patient with glioblastoma was treated with radiochemotherapy including temozolomide after surgery. Patient was on valproic acid, levetiracetam, ondansetron,
valproic acid, lorazepam and clobazam. He was considered stable and was started on artesunate 200 mg/day and a decoction of Chinese herbs (*Captis chinensis*, *Siegesbeckia orientalis*, *Artemisia scoparia*, *Dictamnus dasycarpus*).

The patient’s clinical condition deteriorated and elevated liver enzymes were noted – ALT 238 U/L, AST 226 U/L, and γ-GT 347 U/L. **Artesunate** was discontinued a month after initiation and Chinese herbs were discontinued 2.5 weeks after initiation; labs normalized and patient felt well. The authors discuss that while TMZ, valproic acid and levetiracetam are all associated with hepatotoxicity, the combination of adding artesunate and Chinese herbs to his standard chemotherapy regime likely caused the liver injury. Given the resolution of elevated liver function tests after discontinuing artesunate and Chinese herbs, it was considered that either one of them initiated the toxicity or potentiated it and that drug-drug interactions may have contributed synergistically to increase the hepatotoxic potential of both drugs. The higher doses of artesunate used in this setting, i.e., 200 mg/day for 4 weeks may have increased the risk of toxicity. Additionally, since artesunate-type drugs confer ferrous iron-mediated oxidative damage (leading to DNA damage in tumor cells), and the liver is a major iron-storing organ in the body, hepatotoxicity by artesunate and its derivatives may be of concern.

The authors caution that compassionate use of medications such as artesunate and Chinese herbs together with anticancer drugs is not recommended given the risk of severe side effects and controlled clinical trials are required to clarify incidence of hepatotoxicity in such circumstances.

5) Uhl et al. (2016) discuss fatal liver and bone marrow toxicity by combination treatment of dichloroacetate and **artesunate** in a glioblastoma patient. A 52-year old patient was treated with standard chemotherapy with temozolomide for glioblastoma. His condition worsened and the patient sought alternative therapy and was administered dichloroacetate and intravenous artesunate (2.5 mg/kg) 148 days after surgery. About 6 days later, patient showed clinical and lab signs of liver and bone marrow toxicity (markedly increased hepatic enzyme levels, leukopenia, and thrombocytopenia) and he subsequently died 3 days later (157 days after surgery).

Dichloroacetate is associated with hepatotoxicity and artesunate with bone marrow toxicity; simultaneous use of both was thought to cause liver injury and bone marrow toxicity. Authors concluded that compassionate use of DCA and artesunate outside clinical trials cannot be recommended for glioblastoma treatment given consequences of non-approved drugs or unproven drug combinations.

*It is possible that artesunate also contributed to the liver toxicity – either on its own, or potentiated the effects of dichloroacetate.*

No serious adverse reactions associated with intravenous artesunate were identified in two large clinical trials for the treatment of severe malaria in adults and pediatric subjects (Dondorp et al. 2005; 2010).
APPENDIX 12. NEUROTOXICITY CASES IN HUMANS FOR ARTEMISININ DERIVATIVES

1) Per Efferth and Kaina (2010) neurotoxicity has been most frequently investigated as a possible adverse event related to artemisinin-type drugs. Animal studies have shown that water-soluble artesunate shows less neurotoxicity than oil-soluble artemether and arteether which may be likely related to the oil vehicle acting as a depot and affecting biodistribution and half-lives of the drug. Doses used in human studies (2-8 mg/kg/day for 3 – 5 days) are lower than those used in animals (12.5 to 600 mg/kg/day); this higher dosing may explain neurotoxicity seen in animals not usually seen in humans (for more details on neurotoxicity data collected from animal studies, see Appendix 3).

2) van Hensbroek et al. (1996) conducted a randomized, unblinded comparison of intramuscular artemether vs. intravenous quinine in 576 Gambian children with cerebral malaria to assess mortality and neurological sequelae. The study showed that the coma recovery time was longer with intramuscular artemether vs intravenous quinine (median 26 hours vs. 20 hours, \( p < 0.05 \)). 38.5% patients in the artemether arm suffered seizures after treatment initiation (93.8% vs 28.1%; \( p = 0.01 \)). Following adjustment for pre-treatment seizures and coma duration, odds ratio for seizures under artemether was 1.86 (95% CI 1.28-2.71; \( p < 0.001 \)). However, it did not appear that there were long-term neurologic sequelae; over 3 to 9 months the sequelae rate was 3.3 % in the artemether vs 5.3 % in the quinine group.

Causality unclear given use in cerebral malaria.

3) Hien et al. (1996) conducted a randomized, double-blind trial in 560 adults with severe falciparum malaria comparing quinine to intramuscular artemether (4 mg/kg followed by 2 mg/kg for 8 hours) for a minimum of 72 hours. The coma recovery time was 66 hours in the artemether arm versus 48 hours in the quinine arm; \( p = 0.003 \). Treatment with artemether was associated with slower recovery but there was no difference in the incidence of neurological sequelae between the artemether and quinine groups.

Causality unclear given use in severe falciparum malaria.

4) Van Vugt et al. (2000) compared neurological outcomes, audiometry and auditory evoked potential in a single-blind comparison of 79 patients who had been treated with 2 or more courses of oral artemether or artesunate within previous 3 years and 79 age and sex matched controls in Thailand. Results showed there were no consistent differences between both groups and no evidence of neurotoxicity was detected in patients previously treated for acute malaria.

5) Miller and Panosian (1997) describes a patient in Ghana who developed ataxia and slurred speech that started within two days of taking artesunate for falciparum malaria that resolved after about a month.

This case may have been a complication of falciparum malaria; cerebellar syndrome (Kochar et al. 1999) has been described as being associated with falciparum malaria and
MRI scan confirmed cerebellar atrophy (Sriram et al. 2013) has been associated with falciparum malaria.

6) Elias et al. (1999) describes a patient who developed tremors and unsteadiness after taking two courses of artemether for falciparum malaria. The tremor was treated with propranolol and eventually resolved after about 2 months.

Causality unclear given use in severe falciparum malaria.
APPENDIX 13. HEMATOLOGIC TOXICITY AND IMMUNOTOXICITY CASES FOR ARTEMISININ DERIVATIVES

Hien et al. (1996) reported reduced reticulocyte counts in 284 malaria patients who received a dose of 4 mg/kg artemether intramuscularly followed by 2 mg/kg every 8 hours for at least 72 hours. Wootton et al. (2008) also reported reduced reticulocyte counts in healthy male volunteers and adult patients who received artesunate 4 mg/kg alone or artesunate+chlorproguanil+dapsone. These effects correlated with animal findings, were reversible and mild to moderate in severity.

Rolling et al. (2012) describe a case report regarding post-treatment hemolysis in European travelers with falciparum malaria. In three patients, delayed hemolysis was detected in the second week after the first dose of intravenous artesunate. Reticulocyte production remained low 7-14 days after the first dose of artesunate despite rapid parasite clearance, whereas usually the reticulocyte production index rises concurrently with parasite clearance. An adequate rise in reticulocyte production index was delayed until the second week – long after parasites had been cleared and approximately at the time when hemolytic anemia was diagnosed. Artemisinin and derivatives have been reported to target erythropoiesis and reduce reticulocyte counts several days after treatment. Recommend follow-up up to 1 month after treatment.

Rolling et al. (2014) describes delayed hemolysis after treatment with parenteral artesunate in African children with severe malaria in a double-center prospective study. Children 6 to 120 months with severe malaria were followed after treatment with parenteral artesunate to assess delayed hemolysis. Patients received a total of 12 mg/kg of parenteral artesunate followed by a full course of weight-adapted artemether/lumefantrine. Artesunate was given as 3 randomly allocated regimes (3 doses of 4 mg/kg iv, 3 doses of 4 mg/kg im or 5 doses of 2.4 mg/kg im). Blood transfusions were given as indicated and folic acid was given for 14 days. Of 72 children who were finally evaluated, delayed hemolysis was seen in 5 children (7%) with hemoglobin as low as 2.8 g/dL. Patients with delayed hemolysis had higher parasite counts and were younger. No correlation with sickle cell trait or glucose-6-phosphate dehydrogenase deficiency was observed.
Tab 7

Oral Solid Modified Release Drug Products That Employ Coated Systems
| Oral Solid Modified Release Drug Products That Employ Coated Systems Nominations |
March 4, 2014

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

Re: Docket No. FDA-2013-N-1523

Dear Sir or Madam:

AbbVie is a global, research-based biopharmaceutical company formed in 2013 following separation from Abbott. Our mission is to use our expertise, dedicated people and unique approach to innovation toward developing and marketing advanced therapies that address some of the world’s most complex and serious diseases.

AbbVie is pleased to have the opportunity to comment on the “Request for Nominations: Drug Products that Present Demonstrable Difficulties for Compounding Under Sections 503A and 503B of the Federal Food, Drug, and Cosmetic Act”, announced in the Federal Register on December 4, 2013 (FR 78, No. 233 pgs 72840-41).

AbbVie supports the comments submitted by the Biotechnology Industry Organization (BIO) that recommends several categories of drug products for inclusion on FDA’s ‘difficult-to-compound’ list. We also refer FDA to USP <795>, Pharmaceutical Compounding-Nonsterile Preparations for recommended guidance on applying good compounding practices when preparing nonsterile compounded formulations for dispensing and/or administration. Additionally, AbbVie respectfully requests FDA consider including the following categories of drug products on its ‘difficult-to-compound’ list. For reference, Attachment 1 includes examples of AbbVie drug products that fall within the categories listed below.

Sterile and Sterile Injectable Drug Products
This category of drug products was at the center of the meningitis outbreak that began in 2012, which was traced to contaminated medication. FDA inspections identified poor process and quality control at multiple compounding pharmacies, and a number of product recalls were initiated. These events eventually led to the passing of the Drug Quality and Security Act (DQSA) on November 27, 2013.

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The risk of sterile compounding depends on the complexity of the manufacturing process, source and control of materials, ability to control environmental conditions, and enhanced training of personnel. Compounding of sterile drugs poses a public hazard because of the potential risk for microbial and other contamination. Special testing and monitoring of these drug products are required to assure sterility both at the conclusion of the manufacturing process and until use by the patient.

**Drug Products that are Difficult to Manufacture**

Drug products within this classification utilize highly advanced manufacturing processes and sophisticated equipment and formulations. They require more complex, enabling technology to achieve product quality. These drug products require specialized formulations and excipients where any variability can have significant impact on drug performance. Due to the complex processes and equipment required to manufacture these types of drug products, it is difficult to ensure that functional excipients and/or drug is incorporated uniformly throughout a batch. Excipient and drug uniformity is critical for accurate dosing. Additionally, an understanding of the release rate of certain drug products within this category is also critical. End product testing is often required to ensure the drug performs as intended.

In many cases, it is difficult to establish stability for certain types of drug products in this category if environmental conditions are not appropriately controlled during manufacturing. In addition, processing of drug products in this category requires sophisticated monitoring and control of environmental conditions. Such controls are expected to be outside the traditional realm of compounding pharmacies and outsourcing facilities.

Drug products within this classification include:

- drugs manufactured by extrusion or nanotechnology
- drugs utilizing transdermal drug delivery systems
- modified release drug products
- hydroscopic, light sensitive, temperature-controlled drug products
- products susceptible to oxidation

**Drug Products with Special Safety Considerations**

Examples of drug products within this category include low dose drugs, potent drugs, and narrow therapeutic index drugs. These drug products require the ability to ensure product uniformity, which is critical for accurate dosing. Due to the high ratio of excipients vs. active ingredients for drug products within this category, it is difficult to ensure the drug is incorporated uniformly throughout a batch. Variability of dose uniformity and accuracy of dose can result in over- or under-dosing causing patient safety issues.
AbbVie appreciates the opportunity to share its views. Please feel free to contact Ms. Demetra Macheras at 847-938-5603 (demetra.maches@abbvie.com) should you have any questions.

Sincerely,

[Signature]

Lauren M. Hetrick
Senior Director
Regulatory Policy & Intelligence
AbbVie
### Attachment 1
Examples of AbbVie Drug Products That Fall Within the Identified Drug Product Classifications

<table>
<thead>
<tr>
<th>Drug Product Classification</th>
<th>Examples of AbbVie Drug Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile and Sterile Injectable Drug Products</td>
<td>Humira®   [sup]®\          Survanta®   [sup]®\   Lupron®   [sup]®\     Zemplar IV®   [sup]®\     Nimbex®   [sup]®\   Calcijex®   [sup]®\</td>
</tr>
<tr>
<td>Drug Products that are Difficult to Manufacture</td>
<td>Kaletra®   [sup]®\      Norvir®   [sup]®\       Tricor®   [sup]®\      AndrogeL®   [sup]®\    KTab®   [sup]®\      Simcor®   [sup]®\     Advicor®   [sup]®\     Tarka®   [sup]®\     Trilipix®   [sup]®\     Cardizem®   [sup]®\</td>
</tr>
<tr>
<td>Drug Products with Special Safety Considerations</td>
<td>Synthroid®   [sup]®\</td>
</tr>
</tbody>
</table>
Dockets Management Branch (HFA-305)  
Food and Drug Administration  
5630 Fishers Lane, Rm. 1061  
Rockville, MD 20852


Difficult to Compound: Tecfidera® (dimethyl fumarate)

Biogen Idec strongly recommends that dimethyl fumarate, including Tecfidera (dimethyl fumarate) delayed-release capsules, be placed on the FDA’s difficult-to-compound list. The Tecfidera drug product and the active pharmaceutical ingredient (API), dimethyl fumarate, both present “demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety or effectiveness of that drug product.” First, compounding of branded Tecfidera may disrupt the patented formulation of the product, resulting in a drug whose safety, efficacy, and tolerability cannot be substantiated. Secondly, compounded Tecfidera (brand) or compounded dimethyl fumarate (API) may result in a sub-potent product if the drug substance is not protected from sublimation.

There is a direct relationship between the dose and the clinical safety/efficacy profile of Tecfidera; this relationship has been supported by extensive clinical studies. Tecfidera is provided as a patented formulation of delayed-release (enteric-coated) microtablets contained in a gelatin capsule. The microtablets resist the acidic gastric environment and instead release the dimethyl fumarate once in the small intestine. The use of a multiparticulate dosage form also provides protection against the rapid release of the entire amount of drug into the small intestine—a phenomenon known as “dose dumping”. These specialized features of the Tecfidera formulation were studied in a robust clinical development program which demonstrated strong clinical efficacy and favorable safety and tolerability. For these reasons, compounded dimethyl fumarate cannot be expected to have the same clinical and safety results as Tecfidera.

The active ingredient, dimethyl fumarate, is subject to sublimation. Uncontrolled sublimation of the API could result in a sub-potent product. To protect against this, the Tecfidera formulation employs a two-layer coating on the microtablets that acts as a seal coat as well as an enteric coat. Compounding of the branded product may disrupt this protective coating and jeopardize the product’s potency. Compounded dimethyl fumarate (API) may have no protection from sublimation and result in a sub-potent product over time.
By placing Tecfidera (dimethyl fumarate) on the difficult-to-compound list, FDA will protect patients by ensuring that the only supply of dimethyl fumarate is one that is carefully formulated and manufactured to meet expectations for safety, potency, and tolerability as proven in its robust clinical development program.

Sincerely yours,

Ken Oh

Nadine Cohen, Ph.D.
Senior Vice President, Regulatory Affairs
Biogen Idec, Inc.

4 March 2014

Food and Drug Administration
Division of Dockets Management (HFA-301)
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852


Dear Sir or Madam:

Reference is made to the notice published by the Food and Drug Administration (FDA) in the Federal Register on 4 December 2013 (78 Fed. Reg. 72640), encouraging interested parties to nominate specific drug products or categories of drug products for inclusion in the Agency’s list of products that present demonstrable difficulties for compounding (the difficult-to-compound list). The purpose of this submission is to note several drug products and categories of drug products that GlaxoSmithKline (GSK) believes warrant inclusion in the difficult-to-compound list.

GSK is a research-based pharmaceutical and biotechnology company. Our company is dedicated to the discovery, development, manufacture, and distribution of medicines and vaccines that enable people to live longer, healthier, more productive lives. GSK appreciates the opportunity to provide comments on this important topic. While GSK recognizes the importance of preserving access to compounded drugs when patients cannot be treated with FDA-approved products, inappropriate compounding activities can present significant risks. The timely issuance, and rigorous enforcement, of FDA’s difficult-to-compound list is critically important to protect patients from these risks.

As described in the Federal Register notice, for a drug product to be compounded under either Section 503A or Section 503B of the Food, Drug, and Cosmetic Act (FDCA), it must (among other things) not be a drug product identified by the Secretary as one that presents demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety or effectiveness of that product, taking into account the risks and benefits to patients. After evaluating the responses to its request for nominations, and after consulting with the Pharmacy Compounding Advisory Committee, FDA has stated that it plans to develop and publish a single list for compounding under both Sections 503A and 503B, using notice-and-comment rulemaking procedures.

In its request for nominations, the Agency lists a number of factors that may be relevant in assessing whether a certain drug product or category of products should be included in the difficult-to-compound list, including factors that may impact the potency, purity, or quality of a drug product, and thereby affect its safety or effectiveness. The factors listed by FDA include those related to: the drug delivery system; drug formulation and consistency; bioavailability; the complexity of compounding; facilities and equipment; training; and testing and quality assurance. Below, we list a number of drug products and categories of products that we believe should be included in the list, based on our assessment of these and other factors. GSK reserves the right to expand upon these comments or nominate additional drug products or categories of products in the future.
I. Respiratory Drug Products

Respiratory products often incorporate sophisticated drug delivery systems, such as dry powder or metered dose inhalers, which are precisely engineered and tightly controlled to deliver their active ingredients to local sites of action within the body. In addition to their device components, the formulations of respiratory medicines are often complex, using active and inactive ingredients with defined particle size profiles and other qualities that are intended to interact with those components in specific ways. The manufacturing of respiratory products thus requires sophisticated facilities and equipment, and highly trained personnel, beyond the capabilities of drug compounding operations. Post-manufacture, ensuring the quality and performance of such drug/device combination products requires difficult-to-perform testing, such as aerodynamic particle size distribution and emitted dose assessments.

Failure in any of these numerous elements – from device design and formulation work, to manufacturing, to quality assurance – would threaten the safety and effectiveness of the drug product. Moreover, these medicines generally cannot be compounded into more common dosage forms, such as tablets or capsules, because of concerns with dosing accuracy and bioavailability at the local sites of action. For these reasons, GSK believes that respiratory drug products, including the following GSK products, should be included in FDA’s difficult-to-compound list:

- Advair Diskus® (fluticasone propionate and salmeterol) Inhalation Powder
- Advair HFA® (fluticasone propionate and salmeterol) Inhalation Aerosol
- Anoro™ Ellipta™ (umeclidinium and vilanterol) Inhalation Powder
- Beconase AQ® (beclomethasone dipropionate, monohydrate) Nasal Spray
- Breo® Ellipta™ (fluticasone furoate and vilanterol) Inhalation Powder
- Flonase® (fluticasone furoate) Nasal Spray
- Flovent Diskus® (fluticasone propionate) Inhalation Powder
- Flovent HFA® (fluticasone propionate) Inhalation Aerosol
- Relenza® (zanamivir) Inhalation Powder
- Serevent Diskus® (salmeterol xinafoate) Inhalation Powder
- Ventolin HFA® (albuterol sulfate) Inhalation Aerosol
- Veramyst® (fluticasone furoate) Nasal Spray

II. Modified Release Drug Products

Modified release products, including delayed, sustained, and extended release tablets and capsules, are generally manufactured using complex, often patent-protected, technologies. The failure of a drug compounding operation to understand, have access to, and utilize these technologies appropriately could result in products with poor dosing accuracy, bioavailability, or product-to-product uniformity – any of which may affect safety or effectiveness. The failure of a release mechanism, for example, may present a safety issue, if it leads to dose dumping, or an effectiveness issue, if the drug is not released into the circulation in a timely manner. For these reasons, GSK believes that modified release drug products, including the following GSK products, should be included in FDA’s difficult-to-compound list:

- Coreg CR® (carvedilol phosphate) Extended-Release Capsules
- Requip XL® (ropinirole) Extended Release Tablets
- Rythmol SR® (propafenone hydrochloride) Extended-Release Capsules
- Wellbutrin SR® (bupropion hydrochloride) Sustained-Release Tablets
- Zyban® (bupropion hydrochloride) Sustained-Release Tablets
- Lamictal® XR (lamotrigine) Extended-Release Tablets
III. Drug Products Presenting Increased Risks

Certain drugs and drug products, including but not limited to those subject to Risk Evaluation and Mitigation Strategies (REMS), present increased risks. Adequate mitigation of these risks requires careful and consistent manufacturing, enhanced labeling and risk communications, and even restricted distribution. Compounded products containing drugs associated with teratogenicity, mutagenicity, or carcinogenicity may also present increased occupational risks to those performing the manufacturing operations themselves, through respiratory or skin exposure. These products therefore require sophisticated facilities and equipment, and highly trained personnel, to ensure not only the potency, purity, and quality of the drug products, but also the safety of those working with them. For these reasons, GSK believes that certain increased risk drug products, including the following GSK products, should be included in FDA’s difficult-to-compound list:

A. Drug Products with Approved REMS

- Potiga® (ezogabine) Tablets [Controlled Substance – Schedule V]
- Promacta® (eltromopag olamine) Tablets
- Zyban® (bupropion hydrochloride) Sustained-Release Tablets
- Avandamet® (rosiglitazone maleate and metformin hydrochloride) Tablets
- Avandaryl® (rosiglitazone maleate and glimepiride) Tablets
- Avandia® (rosiglitazone maleate) Tablets

B. Drug Products Presenting Occupational Risks

- Avodart® (dutasteride) Capsules
- Jayln® (dutasteride and tamsulosin hydrochloride) Capsules
- Tafinlar® (dabrafenib) Capsules
- Votrient® (pazopanib) Tablets
- Soriatane® (acitretin) Capsules
- Veltin® (clindamycin phosphate and tretinoin) Gel

IV. Anti-Epileptic Drug Products

Certain drugs are characterized by narrow margins between their effective and toxic doses. Others require careful dose selection and titration, because even small differences in dose or bioavailability can have clinical consequences for patients. Anti-epileptic drugs (AEDs) are perhaps the most well-known such products. Consistency of manufacturing, dosing uniformity, and reliable bioavailability are critical for these drug products. Any potential compounding of such products is highly complex, with significant potential for

1 GSK understands that biological products, licensed under the Public Health Service Act, are not covered by the new drug application exemption provisions of Sections 503A and 503B of the FDCA. For this reason, biological products may not be compounded or distributed without an approved biologics license application. If FDA interprets Sections 503A and 503B to apply to biological products, however, such products – including the GSK products Benlysta® (belimumab) Injection, Arzerra® (ofatumumab) Injection, and rax baccumab injection – should be included in the do-not-compound list. Biological products are uniquely challenging to manufacture, handle, and distribute, and the inappropriate compounding of biological products would present significant risks to patients.

2 Section 503B(a)(7) of the FDCA prohibits the compounding by outsourcing facilities of certain drugs subject to REMS (those approved with elements to assure safe use), unless the facilities demonstrate prior to beginning compounding that they will utilize controls comparable to the controls applicable under the relevant REMS. This does not address, however, compounding under Section 503A of the FDCA, or the compounding of other drugs presenting increased risks.
errors that may affect the safety or effectiveness of the products and present unacceptable risks to patients. For these reasons, GSK believes that AEDs, including the following GSK products, should be included in FDA’s difficult-to-compound list:

- Lamictal® (lamotrigine) Chewable Dispersible Tablets
- Lamictal® (lamotrigine) Tablets
- Lamictal® XR (lamotrigine) Extended-Release Tablets
- Potiga® (ezogabine) Tablets [Controlled Substance – Schedule V]

Again, we appreciate the opportunity to provide input on this important topic. GSK looks forward to participating in FDA’s continued development of the difficult-to-compound list, including the advisory committee and rulemaking processes. Please contact me via e-mail at leo.j.lucisano@gsk.com or telephone at (919) 483-5848 with any questions or comments.

Sincerely,

Leo Lucisano
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Global CMC Regulatory Affairs
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March 4, 2014

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Dear Commissioner Hamburg and Dr. Woodcock:

Public Citizen, a consumer advocacy organization with more than 300,000 members and supporters nationwide, submits these comments in response to the Food and Drug Administration (FDA) request for nominations for Drug Products That Present Demonstrable Difficulties for Compounding Under Sections 503A and 503B of the Federal Food, Drug, and Cosmetic Act (FDCA; Docket Number FDA-2013-N-1523).

We wish to express our concern that the FDA intends to develop and publish a single list of drug products and categories of drug products that cannot be compounded because they present demonstrable difficulties for compounding. Sections 503A and 503B of the FDCA, which create exemptions from new drug approval and other requirements for compounding pharmacies and outsourcing facilities, respectively, each separately authorize the FDA to publish a distinct list identifying drug products that present demonstrable difficulties for compounding and therefore
cannot be produced under the exemptions. We believe two separate lists are necessary, because drugs compounded at compounding pharmacies under a Section 503A exemption will be subject to reduced regulatory standards and fewer enforcement mechanisms relative to drugs compounded at outsourcing facilities under a Section 503B exemption. (Although it is important to note that drugs qualifying for either type of exemption will be subject to reduced requirements relative to drugs that undergo new drug approval, and therefore in general pose greater risk to patients than FDA-approved drugs).

We urge the FDA to classify products involving nonsterile-to-sterile compounding as a category of products presenting demonstrable difficulties for compounding under 503A, but not under 503B. Production of drugs using this inherently high-risk process should be carried out only by a facility that is regularly inspected to verify compliance with current federal Good Manufacturing Practices (cGMP) requirements. Compounding pharmacies regulated under 503A are not required to follow cGMP, will rarely—if ever—be inspected by the FDA, and may or may not be regularly inspected by state officials, depending on the pharmacy regulations in each state, and any such state inspections are likely to be far less rigorous than those conducted by the FDA. By contrast, 503B outsourcing facilities, while not required to obtain new drug approval for their drug products, are nevertheless required to comply with cGMP and will be inspected by FDA officials on a risk-based schedule.

Alternatively, if the FDA chooses to proceed with its proposed plan of establishing only one list, we urge the agency to identify compliance with cGMP and the requirements of 503B as conditions necessary to prevent certain drugs or categories of drugs from presenting demonstrable difficulties for compounding, and to require such conditions for high-risk nonsterile-to-sterile compounding. Outsourcing facilities that register under Section 503B and comply fully with the FDCA will be permitted to compound such products, whereas compounding pharmacies regulated under 503A would not be allowed to compound such products.

We also recommend designation of several additional product categories as presenting demonstrable difficulties for compounding, and which therefore cannot be produced under 503B and/or 503A exemptions. A full list of product categories we urge the FDA to identify as demonstrably difficult to compound, along with our recommendations for their appropriate regulatory classification, is summarized as follows:

1. Nonsterile-to-sterile compounding (non-exempt under 503A only)
2. Metered dose inhaler (MDI) products (non-exempt under 503A and 503B)
3. Dry powder inhaler (DPI) products (non-exempt under 503A and 503B)
4. Transdermal Delivery Systems (TDSs) (non-exempt under 503A and 503B)
5. Sustained or time-release dosage forms (non-exempt under 503A and 503B)
6. Enteric-coated preparations (non-exempt under 503A and 503B)
I. Regulatory Background and Relevant Statutory Authority

Section 503A of the FDCA, created under the Food and Drug Administration Modernization Act of 1997 (FDAMA),1 describes the conditions under which a human drug product, compounded for an identified individual based on a prescription, is entitled to an exemption from the federal requirements for new drug approval, compliance with cGMP, and specific federal labeling requirements.2 Rather than follow cGMP requirements, pharmacies qualifying for a 503A exemption must produce drug products under conditions that comply with the United States Pharmacopoeia (USP) chapter on pharmacy compounding, including USP Chapter 797, addressing sterile compounding.3,4

Pharmacies may qualify for a Section 503A exemption only when producing a drug product “not . . . identified by the Secretary by regulation as a drug product that presents demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety or effectiveness of that drug product.”5 Section 503A requires that the FDA consult an advisory committee on pharmacy compounding prior to identifying such products, absent urgent public health need.6

Following passage of FDAMA, the FDA initiated an administrative process aimed at creating a list of drugs presenting demonstrable difficulties for compounding. In 2000, the FDA requested comments on a concept paper describing the agency’s preliminary thoughts on the matter (FDA Concept Paper).7 However, these preliminary efforts were suspended following a 2002 Supreme Court decision holding portions of Section 503A unconstitutional.8

Regulation under Section 503A has been revived by the Drug Quality and Security Act of 2013, which verified the constitutionality of the portions Section 503A that had not been addressed in the Supreme Court’s 2002 decision, including the relevant sections addressing the difficult-to-compound list, by removing the provisions deemed unconstitutional by the Court.9 The 2013 Act also added Section 503B to the FDCA, creating a new category of drug producers, known as

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1 Pub. Law No. 105-115.
9 Ibid.
“outsourcing facilities.”10 Like compounding pharmacies regulated under 503A, outsourcing facilities that qualify for Section 503B are exempt from new drug approval and specific federal labeling requirements, and are therefore subject to lighter federal regulation than manufacturers of FDA-approved drugs. However, unlike Section 503A compounding pharmacies, Section 503B outsourcing facilities will be required to comply with cGMP. Outsourcing facilities must also comply with additional requirements, including federal registration and periodic reporting requirements, as well as federal inspections of facilities and records, conducted on a risk-based schedule.

Like Section 503A, Section 503B excludes drugs that present demonstrable difficulties for compounding that are reasonably likely to lead to an adverse effect on the safety or effectiveness of the drug or category of drugs.11 However, rather than cross-reference the same list of products identified under Section 503A, Section 503B outlines distinct procedural steps for the FDA to follow in identifying drugs that are difficult to compound, including a specific timeline and process for creating a list of such products.12 Section 503B also requires the FDA to “tak[e] into account the risks and benefits to patients” when identifying products for the list and authorizes the agency to identify “conditions that are necessary to prevent the drug or category of drugs from presenting demonstrable difficulties [for compounding].”13

Neither Section 503A nor Section 503B require that the FDA develop and publish a single list of drug products that present demonstrable difficulties for compounding. If anything, Congress, having identified two distinct processes and two slightly different sets of requirements and authorities for each section, appears to have contemplated that the FDA would create two separate lists. Moreover, even if two separate lists are not statutorily required, the FDA can certainly exercise its discretion to promulgate two separate lists. Separate lists would represent sound public health policy because the conditions for compounding in each type of facility are markedly different, with 503A compounding pharmacies subject to significantly lower regulatory standards than 503B outsourcing facilities.

Alternatively, if the FDA proceeds with its proposed plan to promulgate only one list, the agency has the authority to identify compliance with 503B and cGMP requirements as conditions necessary to prevent certain drugs or categories of drugs from presenting demonstrable difficulties for compounding. Outsourcing facilities that register under Section 503B and comply fully with cGMP would then be permitted to compound such products, whereas compounding pharmacies that qualify for exemption under 503A that have not verified compliance with cGMP would not be allowed to compound such products.

10 Section 503B, not yet codified. Pub. Law 113-54. -
11 Pub. Law 113-54. Sec. 503B (a)(6). -
12 Pub. Law 113-54. Sec. 503B (c)(2). -
13 Pub. Law 113-54. Sec. 503B (a)(6). -
II. Specific Drug Product Categories

We propose six categories of drug products for placement on the list or lists of products presenting demonstrable difficulties for compounding under Sections 503B and/or 503A.

1. Nonsterile-to-sterile compounding

Certain drugs must be sterile (in other words, free from all living microorganisms) in order to be administered safely. These include dosage forms administered parenterally (injections, infusions, or implants), aqueous-based inhalation solutions, and ophthalmic products. As stated in the 2000 FDA Concept Paper, “[s]terility is absolute and should never be considered in a relative manner -- a product cannot be partially or almost sterile.”

Problems that develop in compounding sterile products can have serious and far-reaching consequences for patient safety. In September 2012, the Centers for Disease Control and Prevention (CDC) and the FDA announced the beginning of what would become the largest outbreak of infection linked to a medical product in more than four decades: healthcare facilities in 23 states received three lots of contaminated preservative-free injectable methylprednisolone acetate produced by the New England Compounding Center (NECC), a compounding pharmacy in Framingham, Massachusetts. Over the next year, the CDC tracked 751 cases of infection, including meningitis, paraspinal/spinal infection, stroke, and joint infection. Sixty-four of those cases resulted in death.

While the NECC-linked outbreak was by far the largest ever associated with a compounding pharmacy, it was by no means an isolated event. Table 1 contains a list of infection outbreaks linked to compounding pharmacies since 2004. Many more small-scale outbreaks or isolated infections caused by compounded products likely went undetected because the source of such infections is often not suspected or challenging to identify.

<table>
<thead>
<tr>
<th>Date of Outbreak</th>
<th>Type of Injury</th>
<th>Pharmacy</th>
<th>Source</th>
</tr>
</thead>
</table>


15 Ibid.


<table>
<thead>
<tr>
<th>Date Range</th>
<th>Description</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 2004 – Feb 2005</td>
<td>Bloodstream infections; 36 cases, including at least 13 children</td>
<td>Anonymous</td>
<td>CDC200518</td>
</tr>
<tr>
<td>Jan – Mar 2005</td>
<td>11 cases of bacteremia, including 5 cases of sepsis</td>
<td>PharMEDium</td>
<td>CDC200520</td>
</tr>
<tr>
<td>Mar 2005</td>
<td>6 cases of sepsis; 1 resulting in death</td>
<td>PharMEDium</td>
<td>FDA2007(1)21</td>
</tr>
<tr>
<td>Dec 2004 – Aug 2005</td>
<td>Eye infection resulting in permanent loss of vision; 6 cases</td>
<td>Anonymous</td>
<td>Sunenshine200922</td>
</tr>
<tr>
<td>Dec 2006</td>
<td>70 complaints indicating signs of infection</td>
<td>Med-South Pharmacy</td>
<td>FDA2007(2)23</td>
</tr>
<tr>
<td>Oct – Nov 2007</td>
<td>7 bloodstream infections</td>
<td>Anonymous</td>
<td>Maragakis200924</td>
</tr>
<tr>
<td>Mar 2011</td>
<td>19 bloodstream infections</td>
<td>Meds IV</td>
<td>FDA201125</td>
</tr>
<tr>
<td>Jul 2011</td>
<td>12 eye infections; 11 resulting in vision loss</td>
<td>Infupharma</td>
<td>Goldberg201326</td>
</tr>
<tr>
<td>Aug 2011 – Mar 2012</td>
<td>47 eye infections; 39 resulting in vision loss</td>
<td>Franck’s Compounding Lab</td>
<td>Mikosz201427</td>
</tr>
</tbody>
</table>

In addition to being free of microorganisms, injectable compounded pharmaceuticals must also be free from pyrogens (the byproducts of microorganisms that can cause reactions when introduced into humans) and particulate matter, which can cause harmful blood clots, particularly when a product is administered in large quantities.  

Sterile-to-sterile compounding, described as “low” or “medium” risk compounding by the U.S. Pharmacopeial Convention, involves manipulating sterile ingredients entirely within an ISO Class 5 or better environment (a “clean room” carefully controlled to exclude microbial growth) using only sterile ingredients, products, components, and devices.  

Depending on the number of sterile products and aseptic manipulations involved, sterile-to-sterile compounding may involve low or medium risk of microbial contamination.

Nonsterile-to-sterile compounding, described as “high” risk compounding by the U.S. Pharmacopeial Convention, involves compounding using nonsterile ingredients or materials, including nonsterile active pharmaceutical ingredients (API), finished FDA-approved products not intended for sterile routes of administration (e.g., oral), or nonsterile devices or packaging. It also includes sterile contents of commercially manufactured products that have been exposed to conditions that would render them nonsterile (e.g., exposure to air quality worse than ISO Class 5 for more than one hour). To engage in this process safely, an appropriate sterilization method must be used to ensure that such products are sterile and free of pyrogens and particulate matter prior to distribution.

The high-risk process of nonsterile-to-sterile compounding is not appropriate for compounding pharmacies exempt under Section 503A, as these entities are not held to cGMP standards and

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31 <797> Pharmaceutical Compounding—Sterile Preparations. The United States Pharmacopeial Convention. 2008. -
32 Ibid.
33 Ibid.
34 Ibid.
instead must comply with USP standards only. USP standards for sterile compounding, laid out in Chapter 797 of the USP, are set by the U.S. Pharmacopeial Convention, a private organization that sets standards for drugs, food ingredients, and dietary supplements. While USP standards have advanced over time, they remain relatively lax compared to the cGMP standards developed and enforced by the FDA. One key difference is that cGMP requires a drug manufacturer to validate and periodically re-validate each step in the production process through direct testing, whereas USP Chapter 797 routinely allows pharmacists to base production design on review of available literature and the pharmacist’s prior experience.

For example, in determining sterilization methods, cGMP requires that any sterilization process used to prevent microbial contamination be validated through appropriate direct studies, and offers detailed guidance on the design and conduct of such validation studies. Once production begins, a single contaminated product in any batch smaller than 5,000 should trigger an investigation and revalidation of the entire manufacturing process. USP, by contrast, does not generally require product-specific validation, instead allowing the pharmacist to select a method based on “experience and appropriate information sources,” stating that the sterilization method should “preferably” be verified “whenever possible.”

Similarly, federal cGMP regulations require a detailed written stability testing program to determine appropriate storage conditions and expiration dates. By contrast, USP describes the practice of establishing “beyond use dating (BUD),” and the especially high-risk practice of “theoretical beyond use dating,” both of which can be based on a review of general literature and do not require direct product testing. The USP acknowledges that “[t]heoretically predicted beyond-use dating introduces varying degrees of assumptions and, hence, a likelihood of error or at least inaccuracy,” yet USP Chapter 797 does not require direct stability testing to avoid such problems. Indeed, actual testing is only “strongly urged” to support dating periods exceeding 30 days.

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36 21 CFR 211.113(b). -
39 §797 Pharmaceutical Compounding—Sterile Preparations. The United States Pharmacopeial Convention. 2008. -
40 21 CFR § 211.166. (“There shall be a written testing program designed to assess the stability characteristics of drug products. The results of such stability testing shall be used in determining appropriate storage conditions and expiration dates. The written program shall be followed and shall include: (1) Sample size and test intervals based on statistical criteria for each attribute examined to assure valid estimates of stability; (2) Storage conditions for samples retained for testing; (3) Reliable, meaningful, and specific test methods; (4) Testing of the drug product in the same container-closure system as that in which the drug product is marketed; (5) Testing of drug products for reconstitution at the time of dispensing (as directed in the labeling) as well as after they are reconstituted.”). -
41 §797 Pharmaceutical Compounding—Sterile Preparations. The United States Pharmacopeial Convention. 2008. -
42 Ibid.
We are aware that the FDA previously issued a preliminary conclusion in its Concept Paper published in 2000, which indicated that sterile compounding could be carried out by compounding pharmacies compliant with USP requirements for sterile compounding.\textsuperscript{43} We urge the FDA to reconsider this preliminary conclusion, which addressed all sterile compounding, rather than focusing separately on, and requiring more stringent standards for, especially high-risk nonsterile-to-sterile compounding.

The FDA’s earlier preliminary conclusion was also based in part on a perceived “substantial need for compounded sterile products, especially in the area of extemporaneous compounding.”\textsuperscript{44} While a general need for extemporaneously compounded sterile products may have existed under the conditions that the FDA considered in 2000, no substantial need exists for high-risk nonsterile-to-sterile compounding to be performed in compounding pharmacies exempt under Section 503A. First, most needs for sterile compounded products can be met through modifying federally regulated commercially available sterile products, a low- to medium-risk form of sterile compounding, rather than through high-risk compounding from nonsterile-to-sterile ingredients. Second, following the passage of the Drug Quality and Security Act, any residual needs requiring nonsterile-to-sterile compounding (in other words, making products from bulk API rather than modifying FDA-approved sterile products) are more appropriately met by carrying out such high-risk compounding in outsourcing facilities compliant with Section 503B and federal cGMP requirements (as opposed to relying on 503A compounding pharmacies exempt from cGMP requirements).

Furthermore, more information is now available on the actual conditions of practice in compounding pharmacies, historically subject to minimal federal oversight. Recent FDA inspections of compounding pharmacies have revealed widespread sterility concerns, some of which may violate USP standards in addition to cGMP standards, suggesting that the safety of high-risk nonsterile-to-sterile compounding cannot be assured without increased federal oversight.\textsuperscript{45} Some of these violations are discussed in greater detail below.

Companies that have registered as outsourcing facilities under Section 503B will now be held to higher federal standards, and we hope that conditions in these facilities will improve. However, the FDA cannot reasonably expect these conditions to improve substantially in compounding pharmacies exempt from federal oversight under Section 503A, as the current regulatory environment does not provide for appropriate oversight of compounding pharmacies that qualify for this exemption. While the FDA does have authority to inspect and take enforcement action against compounding pharmacies for violations of federal law, the agency has no plans to carry


\textsuperscript{44} Ibid.

out regular inspections, leaving day-to-day oversight up to state boards of pharmacy.\textsuperscript{46} Many compounding pharmacies are not routinely monitored by state boards to verify compliance with USP Chapter 797 requirements for sterile compounding. A 2012-2013 survey of state boards of pharmacy published by the office of U.S. Rep. Edward J. Markey (now Senator Markey), indicated that 37 state boards of pharmacy do not routinely track which pharmacies are providing sterile compounding services, and only 19 state boards of pharmacy provide inspectors with special training to identify problems with sterile compounding.\textsuperscript{47}

For these reasons, as well as our comments on more specific factors below, we urge the FDA to identify nonsterile-to-sterile compounding as a category presenting demonstrable difficulties for compounding under Section 503A, but not necessarily Section 503B.

The FDA has requested comment on specific relevant factors, including the complexity of compounding, facilities and equipment, personnel training, and testing and quality assurance. We now address each of these factors in turn with regard to nonsterile-to-sterile compounding:

\textit{Complexity of Compounding}

Nonsterile-to-sterile compounding involves extremely complex production processes. As stated in the FDA’s Concept Paper:

\begin{quote}
The preparation of sterile products is often unavoidably complex, involving many steps and manipulations. Each step poses an opportunity for microbial contamination. The manipulation of a sterile drug product may contaminate it, especially when nonsterile components are used (e.g., if the product is packaged into a nonsterile syringe or vial purported to be sterile), nonsterile equipment is used, or novel, complex, or prolonged aseptic processes are employed.\textsuperscript{48}
\end{quote}

Even a relatively small change in the production process, such as a switch to new packaging material, may result in unanticipated and far-reaching consequences. The largest infection outbreak associated with a pharmaceutical product in United States history occurred as the result of one such seemingly minor change: Between April and September 1970, Abbott Laboratories began phasing in a new type of cap liner that relied on synthetic plastic, rather than natural

\begin{itemize}
\item Food and Drug Administration. Compounding and the FDA: Questions and Answers. December 2, 2013. \url{http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/PharmacyCompounding/ucm339764.htm \#regulates}. Accessed February 24, 2014. -
\item Report of the US House of Representatives. State of Disarray. How states’ inability to oversee compounding pharmacies puts public health at risk. April 15, 2013. -
\end{itemize}
rubber. The rubber previously used in the caps had antibacterial properties that synthetic liners lacked. Inadequate environmental control and sampling protocols contributed to microbial contamination of the liners, which thrived on the new synthetic medium. The result was catastrophic: Abbott Laboratories distributed approximately 45 percent of all intravenous fluids sold in the United States at the time, and the outbreak is estimated to have led to between 2,000 and 8,000 cases of infection, and between 200 and 800 deaths.

Both USP and cGMP standards have been updated dramatically over the ensuing decades, yet complex production processes remain challenging to monitor. Any change in the production process should be validated through direct testing to ensure that it does not result in unforeseen consequences. This type of direct validation can only be ensured in facilities verified as fully compliant with cGMP. Nonsterile-to-sterile compounding, therefore, presents demonstrable difficulties for compounding under any other conditions.

Facilities and Equipment

Nonsterile-to-sterile compounding requires sophisticated facilities and equipment that must be maintained to rigorous standards. As stated in the FDA’s concept paper:

To maintain the essential characteristics of sterile products (i.e., sterility and freedom from particulate matter and pyrogens), the products and their components must be manipulated in a suitable environment using aseptic techniques. ... It is important to minimize bioburden during the production process even when terminal sterilization is used. Therefore, the production facilities and associated procedures must meet exacting standards.

While USP and cGMP have developed harmonized standards regarding appropriate levels of bioburden (the accumulation of potential biological contaminants during the production process) in the environment, recent FDA inspections of compounding pharmacies have revealed repeated failures in maintaining the environmental monitoring necessary to meet these standards. In 2013, FDA inspectors cited dozens of compounding pharmacies for failing to assess airflow patterns with adequate smoke studies performed under dynamic conditions and/or failing to conduct appropriate environmental monitoring. While FDA inspectors focused on violations of cGMP

49 Centers for Disease Control. Epidemiologic notes and reports nosocomial bacteremias associated with intravenous fluid therapy – USA. MMWR Weekly. December 26, 1997/46(51);1227-1233.
50 Ibid.
51 Ibid.
52 FDA Concept Paper: Drug Products That Present Demonstrable Difficulties for Compounding Because of Reasons of Safety or Effectiveness.
standards, many of the conditions identified would be unacceptable under either cGMP or USP standards. For example, FDA inspectors also noted visible dust, stains, splatters, residue, rust, live or dead insects, and other sources of potential contamination in a disturbing number of facilities.54,55,56,57,58,59,60,61,62

Some of the pharmacies cited by FDA inspectors in 2013 have subsequently registered as outsourcing facilities.63 While we remained concerned that outsourcing facilities will not be required to undergo new drug approval or verify compliance with cGMP prior to producing sterile products, we assume that the FDA will make every effort to ensure that these facilities comply with cGMP standards moving forward. (If this assumption proves to be incorrect, then nonsterile-to-sterile compounding by outsourcing facilities will also pose unacceptable risks to patients.)

By contrast, many pharmacies that have not registered as outsourcing facilities continue to claim that their compounding facilities adequately comply with applicable state and USP standards.

54 Food and Drug Administration. Axium Healthcare Pharmacy dba Balanced Solutions Compounding. March 15, - 2013. -
55 Food and Drug Administration. 483 Inspection Report. Custom Compounding Centers, LLC. December 13, 2012. -
56 Food and Drug Administration. 483 Inspection Report: Anazaohealth Corporation. February 22, 2013. -
57 Food and Drug Administration. 483 Inspection Report: University Pharmacy, Inc. February 26, 2013. -
58 Food and Drug Administration. 483 Inspection Report: College Pharmacy Incorporated. March 15, 2013. -
59 Food and Drug Administration. 483 Inspection Report: The Compounding Shop, Inc. March 22, 2013. -
60 Food and Drug Administration. 483 Inspection Report: Pentec Health, Inc. April 1, 2013. -
61 Food and Drug Administration. 483 Inspection Report: Pallimed Solutions, Inc. April 9, 2013. -
62 Food and Drug Administration. 483 Inspection Report: Central Admixture Pharmacy Services, Inc. February 19, - 2013. -
63 Food and Drug Administration. Registered Outsourcing Facilities. Updated as of February 21, 2014. -
http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/PharmacyCompounding/ucm378645.htm. -
Accessed February 28, 2014. -
even when they have been informed by the FDA of sterility concerns, making them unlikely to adjust their practices or upgrade their current facilities. In fact, one pharmacy, NuVision, recently refused a request by the FDA to recall all sterile products after the agency identified safety concerns related to sterility during a facility inspection\(^\text{64,65}\). The pharmacy still claims on its website to adhere to USP standards for sterile compounding\(^\text{66}\). In addition, three other compounding pharmacies have responded following FDA inspections with their opinion (without citing verification by independent inspectors) that the current facilities satisfy USP requirements, in spite of the fact that federal inspectors had identified serious sterility concerns\(^\text{67,68,69}\). Regardless of whether these pharmacies do, in fact, comply with USP requirements (a claim that has not been confirmed through independent inspections), it is clear that they are unlikely to dramatically upgrade their facilities in the near future. Appropriately, at least one of these compounding pharmacies has reported that it does not engage in nonsterile-to-sterile compounding\(^\text{70}\). We urge the FDA to ensure that all compounding pharmacies exempt under 503A avoid this type of high-risk compounding, which cannot be performed safely except in a facility that has been regularly inspected for compliance with cGMP standards.

**Personnel Training**

Specialized, highly technical training is essential to ensure proper compounding of nonsterile-to-sterile drug products. As stated in the FDA’s Concept Paper:

> The processes used in pharmacies to prepare sterile products are highly personnel-intense. The contamination of pharmacy-prepared products (e.g., intravenous admixtures and prefilled syringes) by aseptic processing most likely will be caused by personnel-associated factors. These factors may include the shedding of contaminants from people into the controlled environment, improper procedures under laminar air flow, and the use of poor aseptic technique. Therefore, pharmacy personnel involved in compounding

sterile products must have sufficient knowledge, training, and experience to perform the task correctly and safely. Furthermore, a pharmacy’s quality assurance program for sterile products must include requirements that personnel consistently adhere to performance standards; that performance problems be monitored, detected, and corrected; and that personnel undergo initial and periodic certification.\(^71\)

Appropriate training is essential to ensure that sterile solutions do not become contaminated during preparation. A study of pharmacy students by Isanhart et al, published in 2008, assessed procedures performed at the beginning and end of a 16-week parenterals laboratory course offering instruction in aseptic technique.\(^72\) Prior to undergoing training, 21 of 504 syringes (4 percent) prepared by the students were contaminated during media fill tests, a number that was reduced to 0 of 498 by the end of the course.

While zero contamination is clearly possible with appropriate technique, reports from the FDA and published literature suggest that use of inadequate technique is widespread. Rates of contamination during medium and low risk compounding operations remain highly variable and unacceptably high in practice, ranging from 0 percent to over 6 percent among experienced, practicing pharmacists and technicians.\(^73,74,75,76,77\) FDA inspection reports from 2013 also document numerous examples of inappropriate aseptic technique and inadequate monitoring of pharmacy personnel. Observations included inadequate gowning that leaves skin exposed, failure to adequately monitor employees for microbial contamination during aseptic operations, uncontrolled movement of employees in and out of the ISO Class 5 clean room where sterile drugs are prepared, inappropriate use of nonsterile objects in aseptic operations, and failure to adequately clean and sanitize equipment and surfaces in the clean room.\(^78,79,80,81\) Such high-risk


\(^{72}\) Isanhart CM, McCall KL, Kretschmer D, Grimes BA, Parenteral lab course to reduce microbial contamination rates in media fill tests performed by pharmacy students. Am J Pharm Educ. 2008;72(2):27. -

\(^{73}\) Reiter PD. Sterility of intravenous fat emulsion in plastic syringes. Am J Health Syst Pharm 2002;59:1857-9. -


\(^{75}\) Trissel LA, Ogundele AB, Ingram DS et al. Using medium-fill simulation to establish a benchmark microbiological contamination rate for low-risk-level compounding. Am J Health Syst Pharm. 2003; 60:1853-5. -


\(^{79}\) Food and Drug Administration. 483 Inspection Report: FVS Holdings, Inc. dba Green Valley Drugs. March 15, - 2013. -
nonsterile-to-sterile compounding by improperly trained personnel poses unacceptable risk to patients. To avoid this risk, nonsterile-to-sterile compounding must be carried out only in facilities that are regularly inspected for compliance with cGMP.

Testing and Quality Assurance

Testing and quality assurance are especially important in nonsterile-to-sterile compounding as a means of verifying that sterility has been successfully achieved. As the FDA stated in its Concept Paper:

All compounded sterile products should be inspected prior to use in patients. Low-risk compounded sterile products (e.g., sterile products prepared from sterile components using proper techniques and equipment) should, at a minimum, be inspected physically and visually for cloudiness and particulate matter. High-risk compounded sterile products (e.g., sterile products prepared from nonsterile components using proper techniques and equipment) should undergo end-product sterility and pyrogen testing before they are dispensed from the pharmacy.82

Sterility testing is required under cGMP, with samples taken at the beginning, middle, and end of the aseptic processing operation.83 Any positive test result is considered a serious cGMP issue requiring thorough investigation.84 Under USP standards, only high-risk sterile products prepared in groups of 25 or more or that are exposed to certain temperatures for varying lengths of time must be tested for sterility prior to release, and the pharmacy need not await test results before dispensing the products to patients.85 Moreover, products intended for inhalation or ophthalmic administration need not be tested for bacterial endotoxins (pyrogens) prior to release.86

As might be expected, a disturbing number of compounding pharmacies forgo testing and quality assurance measures that would be required under cGMP. FDA inspection reports of


Food and Drug Administration. University Pharmacy, Inc. February 26, 2013. -


82 FDA Concept Paper: Drug Products That Present Demonstrable Difficulties for Compounding Because of Reasons - of Safety or Effectiveness. -


84 Ibid.
86 Ibid.
compounding pharmacies in 2013 identified widespread failure to conduct sterility, endotoxin, and potency testing on all end products. Many pharmacies also failed to document adequate investigation after identifying particulates, discoloration, microbial contamination, leaking product, or other issues with finished samples. In two cases, particulate matter was discovered in products from lots that had already been shipped to customers. Half a dozen pharmacies were also cited for failing to adequately follow up on complaints, including reports indicating mislabeling, particulate matter, and other serious concerns with drug products, including fever, injection-site redness, abscess, and other disturbing adverse events in patients.

Based on the factors identified above, high-risk nonsterile-to-sterile compounding cannot be conducted safely in compounding pharmacies that are not regularly inspected for full compliance with cGMP standards. We therefore urge the FDA to identify nonsterile-to-sterile compounding as a category of products presenting demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety and effectiveness of such drug products under Section 503A, but not necessarily Section 503B.

Alternatively, if the FDA creates a single unified list, we urge the FDA to identify nonsterile-to-sterile compounding as a category of products presenting demonstrable difficulties for

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compounding except under conditions present in outsourcing facilities compliant with Section 503B and cGMP requirements.

2. Metered dose inhaler (MDI) products

The FDA’s Concept Paper published in 2000 recommended that MDI products be identified as presenting demonstrable difficulties in compounding. Specifically, the FDA stated:

The MDI is one of the most complicated drug delivery systems currently marketed by the pharmaceutical industry ….MDI products are primarily used by patients suffering from chronic lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). Individuals suffering from asthma and COPD tend to have airways that are hyper-reactive to inhalants. It is therefore critical that the contents and the delivery characteristics of MDI products be carefully controlled to ensure that the product will be safe and effective. Even slight changes in the formulation, drug substance particle size, valve, or actuator can have a major effect on the aerosol delivery and potency characteristics. This effect can significantly alter the safety and effectiveness of the device. For example, a change in particle size distribution may lead to greater systemic absorption of a beta agonist drug, which can increase the amount of systemic side effects and may also decrease the local effectiveness of the drug in the lungs.95

The FDA concluded that MDI products present demonstrable difficulties in compounding because:

- Metered dose inhalers are sophisticated drug delivery systems that require extensive development to ensure dosing accuracy and reproducibility.
- A sophisticated formulation of the drug product is required to ensure dosing accuracy and reproducibility, and product-to-product uniformity is critical for dosing accuracy and is usually difficult to achieve.
- Reproducible bioavailability of the compounded drug product is difficult to achieve.
- The compounding of MDI products is complex.
- Sophisticated facilities and equipment are required to ensure proper compounding of the drug product.
- Specialized, technical training is essential to ensure proper compounding of the drug product.
- Sophisticated, difficult to perform testing of the compounded drug product is required to ensure potency and purity.96

96 Ibid.
We agree with the FDA’s prior analysis and conclusions with respect to MDI products and urge the agency to identify MDI products as presenting demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety and effectiveness of such drug products under Sections 503A and 503B.

3. Dry powder inhaler (DPI) products

The FDA’s Concept Paper published in 2000 also recommended that DPI products be identified as presenting demonstrable difficulties in compounding. Specifically, the FDA stated:

DPIs are complex drug products that differ in many aspects from more conventional drug products. … There is a wide array of potential DPI designs, all complex in their design and function and many with characteristics unique to the particular design.

Regardless of design, the most crucial attributes of DPIs are the reproducibility of the dose and particle size distribution. It is difficult to maintain these qualities through the expiration date and to ensure the functionality of the device during the period of patient use. The unique characteristics of DPIs must be considered in their preparation, particularly with respect to the product’s formulation, container closure system, and testing.  

The FDA concluded that DPI products present demonstrable difficulties in compounding because:

- Dry powder inhalers are sophisticated drug delivery systems that require extensive development to ensure dosing accuracy and reproducibility.
- A sophisticated formulation of the drug product is required to ensure dosing accuracy and reproducibility, and the product-to-product uniformity that is critical for dosing accuracy is usually difficult to achieve.
- Reproducible bioavailability of the compounded drug product is difficult to achieve.
- The compounding of DPI products is complex.
- Sophisticated facilities and equipment are required to ensure proper compounding of the drug product.
- Specialized, technical training is essential to ensure proper compounding of the drug product.
- Sophisticated, difficult to perform testing of the compounded drug product is required to ensure potency and purity.  

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97 FDA Concept Paper: Drug Products That Present Demonstrable Difficulties for Compounding Because of Reasons of Safety or Effectiveness.  
98 Ibid.
We agree with the FDA’s prior analysis and conclusions with respect to DPI products, and urge the agency to identify DPI products as presenting demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety and effectiveness of such drug products under Sections 503A and 503B.

4. Transdermal Delivery Systems (TDSs)

Finally, the FDA’s Concept Paper published in 2000 recommended that TDS products be identified as presenting demonstrable difficulties in compounding. Specifically, the FDA stated:

TDS products are complex to develop and may require the use of new technologies. Each system is formulated to meet specific biopharmaceutical and functional criteria. The materials of construction, configurations, and combination of the drug with the proper cosolvents, excipients, penetration enhancers, and membranes must be carefully selected and matched to optimize adhesive properties and drug delivery requirements. The equipment and the technology required for the manufacture of TDS products limit their preparation to properly equipped manufacturers.99

The FDA concluded that TDS products present demonstrable difficulties in compounding because:

• TDSs are sophisticated drug delivery systems that require extensive development to ensure dosing accuracy and reproducibility.
• A sophisticated formulation of the drug product is required to ensure dosing accuracy and reproducibility.
• Reproducible bioavailability of the compounded drug product is difficult to achieve.
• The compounding of TDS products is complex.
• Sophisticated facilities and equipment are needed to ensure proper compounding of TDS products.
• Specialized technical training is essential to ensure proper compounding of TDS products.
• Sophisticated, difficult to perform testing of the compounded product is required to ensure potency, purity, and quality of the drug product prior to dispensing.100

We agree with the FDA’s prior analysis and conclusions with respect to TDS products and urge the agency to identify TDS products as presenting demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety and effectiveness of such drug products under Sections 503A and 503B.

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100 Ibid.
5. Sustained or time-release dosage forms

Public Citizen previously submitted comments on the FDA’s Concept Paper published in 2000. In those comments, we recommended that the FDA evaluate sustained or time-release dosage forms for categorization as products presenting demonstrable difficulties for compounding. As we stated previously:

Because there is no requirement to test [compounded sustained or time-release] products, it is no known if 90 percent of the active ingredient is released within the first 30 minutes after the dose is taken, or if 90 percent of the active ingredient remains in the dosage form after the dose is taken.

Variation in rates of release of the active ingredient could impact bioavailability, potentially reducing the drug’s efficacy or increasing safety risks. Clinical testing is necessary to ensure appropriate bioavailability for sustained or time-release dosage forms. Such clinical testing is not required under either Section 503A or Section 503B and can only be required for drug products that undergo premarket approval by the FDA. We therefore urge the FDA to categorize sustained or time-released dosage forms as presenting demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety and effectiveness of such drug products under Sections 503A and 503B.

6. Enteric-coated preparations

Public Citizen also previously recommended that the FDA evaluate enteric-coated preparations for categorization as products presenting demonstrable difficulties for compounding. Enteric-coated preparations are preparations intended for drugs that are either destroyed by gastric acidity or that cause gastric irritation. As we previously stated, “enteric-coated preparations may, if not properly formulated, resist dissolution in the intestine, and very little if any of the active drug may be absorbed into the blood stream.”

As with sustained-release dosage forms, improperly formulated enteric-coated preparations could impact bioavailability, potentially reducing the drug’s efficacy or increasing safety risks. Clinical testing is necessary to prevent these problems. Because such testing is not required under either Section 503A or Section 503B, we urge the FDA to categorize enteric-coated preparations as presenting demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety and effectiveness of such drug products under Sections 503A and 503B.

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102 Ibid.
103 Ibid.
104 Ibid.
III. Conclusion

We are concerned that the FDA intends to develop and publish a single list of drug products and categories of drug products that cannot be compounded because they present demonstrable difficulties for compounding, and urge the agency to withdraw its proposal and instead develop two separate lists. Drugs compounded at compounding pharmacies under a Section 503A exemption should be treated differently than those subject to Section 503B, as the regulations governing each category of facility are different.

Alternatively, if the FDA chooses to proceed with its proposed plan of establishing only one list, we urge the agency to identify compliance with cGMP and the requirements of 503B as conditions necessary to prevent certain drugs or categories of drugs from presenting demonstrable difficulties for compounding.

Regardless of whether one or two lists is used, we urge the FDA to classify high-risk nonsterile-to-sterile compounding as a category of products presenting demonstrable difficulties for compounding under compounding pharmacies exempt under Section 503A, but not necessarily outsourcing facilities exempt under 503B. This high-risk process may be safely carried out only by a facility that is regularly inspected to verify compliance with federal cGMP requirements.

We have also recommended designation of several additional product categories as presenting demonstrable difficulties for compounding.

A full list of product categories that we urge the FDA to identify as demonstrably difficult to compound, along with our recommendations for their appropriate regulatory classification, is summarized as follows:

1. Nonsterile-to-sterile compounding (non-exempt under 503A only)
2. Metered dose inhaler (MDI) products (non-exempt under 503A and 503B)
3. Dry powder inhaler (DPI) products (non-exempt under 503A and 503B)
4. Transdermal Delivery Systems (TDSs) (non-exempt under 503A and 503B)
5. Sustained or time-release dosage forms (non-exempt under 503A and 503B)
6. Enteric-coated preparations (non-exempt under 503A and 503B)

Thank you for your consideration of these comments.

Sincerely,

[Signature]

Sarah Sorscher, J.D., M.P.H.
Attorney
Public Citizen’s Health Research Group
March 4, 2014, Comments to the FDA on Drug Products that Present Demonstrable Difficulties for Compounding

Michael Carome, M.D.
Director
Public Citizen’s Health Research Group
DATE:        April 4, 2017  
FROM:       Ahmed Zidan, Ph.D. and Muhammad Ashraf, Ph.D., Office of Testing and Research, Office of Pharmaceutical Quality  
THROUGH:    Lucinda Buhse, Ph.D., Director Office of Testing and Research, Office of Pharmaceutical Quality  
TO:         Pharmacy Compounding Advisory Committee  

I.  INTRODUCTION

The categories “modified release drug products,” “sustained or time-release dosage forms,” and “enteric-coated preparations,” as well as a number of specific modified release drug products, have been nominated for the list of drug products or categories of drug products that present demonstrable difficulties for compounding (the Difficult to Compound List) under sections 503A and 503B of the Federal Food, Drug, and Cosmetic Act (FD&C Act). At this time, and for the purposes of this review, we evaluated a subset of modified release technologies that we refer to as Modified Release drug products that employ Coated systems (or “MRC”). For the purpose of this review, we define MRC to include oral solid drug products that consist of a drug-containing core enclosed within a polymeric coating, and these coated systems are designed to release active ingredient at specified rates, patterns, and/or onsets through the gastrointestinal (GI) tract to produce systemic, enteric, or local action. For the purposes of this review, the Agency does not consider MRC to include matrix systems as described below.

The Agency has reviewed the information submitted by the nominators and other available sources on the formulation, drug delivery mechanism, dosage form, bioavailability, compounding process, and physicochemical and analytical testing of MRC and their likelihood to adversely affect safety or effectiveness. For the reasons discussed below, we believe the evaluation criteria weigh in favor of placing MRC on the Difficult to Compound List under sections 503A and 503B of the FD&C Act.

II. BACKGROUND
A. An Introduction to MRC

Oral solid modified release dosage forms have a number of therapeutic uses and are used in various populations, including pediatric and geriatric patients. The United States Pharmacopeia defines modified-release as “a dosage form with a drug substance release pattern that has been deliberately changed from that observed for the immediate-release dosage form of the same drug substance.” Most modified release oral systems fall into one of the following two categories:

1. Hydrophilic, hydrophobic or inert polymeric matrix systems: These consist of a polymer matrix that controls the release rate of an embedded active ingredient. Typically, the finished dosage form is prepared by filling or compressing the active ingredient loaded matrix into a capsule or tablet. We did not evaluate these systems in this review.

2. MRC: These consist of an active ingredient containing core enclosed within a polymeric coating. The polymeric coating may affect the pattern, onset, and/or rate of active ingredient release. An example of a polymeric coating that affects the onset is an enteric coating. There are two types of MRC that affect the rate of active ingredient release: diffusion and osmotic systems. Figures 1 and 2 below depict general schematics of MRC diffusion and osmotic pump systems, respectively.

a) Diffusion systems: Figure 1 illustrates diffusion systems’ basic physical attributes, which include a hydrophilic and/or water-insoluble polymeric coating enclosing a whole tablet core (Figure 1a) or a subunit core of active ingredient and excipient (Figure 1b). Various subunits with varying, but specified, release rates may be packaged into a single dosage form. The dosage form may be a single coated tablet or a multiparticulate tablet or capsule consisting of a number of smaller coated discrete subunits (often called beads, pellets, or spheres). Release of the active ingredient is achieved by diffusion of the active ingredient through the polymeric coating.

b) Osmotic systems: Figure 2 illustrates the basic physical attributes of osmotic systems. These attributes include a semi permeable polymeric membrane coating which encloses a compressed core composed of active ingredient, osmotic agent, and other excipients. Osmotic systems have one or more mechanical or laser drilled release orifices through which solutions or suspensions of active ingredient are released at a specific rate over time. Release is achieved by an osmotic pressure differential generated within the core.
Figure 1: Whole coated (a) and multiparticulate (b) modified release systems

Figure 2: a) The elementary osmotic pump (EOP); and b) the push-pull osmotic pump (PPop) oral modified release systems

As explained above, the types of modified release systems evaluated in this review are limited to those manufactured using coating technologies that control active ingredient release characteristics. Hydrophilic, hydrophobic, or inert polymeric matrix systems that do not employ coating technologies (e.g., simple physical mixtures) are not evaluated in this review.

The critical performance characteristics related to MRC include the release of active ingredient at specific rates, patterns, and onsets through the GI tract. The rate and pattern of active
ingredient release are dependent on, but not limited to, active ingredient containing core design; type and concentration of the polymer, pore forming agent, and osmogen used; percent of active ingredient loading; and the overall characteristics of the system.

In some MRC, pore forming agents are added to a hydrophobic polymeric coating to form a microporous structure of the coating membrane. In other instances, plasticizers are added to modify the physical properties and improve film-forming characteristics of polymers. Plasticizers alter the workability, flexibility, and permeability of the coating polymer by significantly changing the viscoelastic behavior of polymers, thereby converting a hard and brittle polymer into a softer, more pliable material, which makes it more resistant to mechanical stress.

**B. Common Examples**

Common examples of MRC employing diffusion systems include: metoprolol succinate extended-release tablets (e.g., Toprol XL), dexamethasone hydrochloride extended-release capsules (Focalin XR), and diltiazem hydrochloride extended release capsules (Tiazac). Common examples of MRC employing osmotic pumps include: methylphenidate hydrochloride extended-release capsules (Concerta), and glipizide extended release tablets (Glucotrol XL).

**III. EVALUATION CRITERIA**

FDA has considered the following criteria in developing a recommendation as to whether drug products or categories of drug products present demonstrable difficulties for compounding that reasonably demonstrate and are reasonably likely to lead to an adverse effect on the safety or effectiveness of the drug product or category of drug products:

1. Complex formulation
2. Complex drug delivery mechanism
3. Complex dosage form
4. Complex bioavailability issues
5. Complex compounding process
6. Complex physicochemical or analytical testing issues
IV. ANALYSIS

A. MRC Have Complex Formulations.

MRC have complex formulations in order to release a specified amount of active ingredient to a specific region of the GI tract over a specified period of time, for a given therapy. To achieve a desired release rate and pattern, MRC must maintain a specified mechanism of diffusion of active ingredient throughout its GI tract residence. The complex characteristics of, and the complex relationship among, the active ingredient (including the active ingredient’s polymorphic forms, stability in the GI fluids, solubility, compatibility with excipients, and purity) and excipients (e.g., polymeric coating materials, plasticizers, pore forming agents, osmotic agents, cushioning agents, antitack agents, disintegrants, and lubricants) influence the manner in which MRC release active ingredient throughout the GI tract. The ability of MRC to maintain its intended performance throughout its residence in the GI tract is related to its safety profile.

1. Active Ingredient

Several properties of the active ingredient used in an MRC formulation may affect product performance characteristics, including the active ingredient’s polymorphic form, solubility, physicochemical in vivo and in vitro stability, purity, and the active ingredient-excipient compatibility.

a. Polymorph

Some MRC require the active ingredient to exist in an amorphous state or a specific polymorphic form for compatibility within the MRC and/or for achieving the desired dissolution rate in GI media. When an amorphous state or a specific polymorphic form of the active ingredient is required, the manufacturing process should be controlled to maintain the intended state or form and yield minimal variability. Moreover, storage conditions, levels of excipients and impurities, residual moisture, and the overall compatibility with the functional polymeric coating should be evaluated because they may influence the rates and patterns of active ingredient release. All of these factors have the potential to induce polymorphic changes. The introduction of, or interconversion to, undesired polymorphs may lead to varying dissolution rates in the GI tract, permeation rates through the GI mucosa, or unexpected interactions with other ingredients, which may impact the safety or effectiveness of the drug product.

b. Solubility

In general, for MRC, the active ingredient must be in a dissolved state to permeate across the GI mucosa. The concentration gradient of active ingredient from the core across the release modifying polymeric coating must be maintained (i.e., sink conditions) throughout the GI
residence to ensure adequate therapeutic levels. The dissolution rate of the active ingredient may vary due to a vast array of excipients, including different grades (e.g., monomer ratios) of release limiting polymer, solubilizers, pore forming agents, lubricant, and osmotic agent. Further, challenges in maintaining sink conditions may arise with active ingredient that exhibit low solubility because of its propensity to remain in a solid state within the GI tract. Conversely, highly soluble active ingredient may exhibit unspecified release rates if the concentration, type, and viscosity grade of the release limiting polymer and pore forming agents, or the diameter of the release orifice of an osmotic system, are improperly selected or controlled.

c. Compatibility Between Active Ingredient and Excipient

Unlike immediate release oral products, where excipients are often used as bulking agents or processing aids, excipients in MRC directly impact the performance of the system. Active ingredient-excipient combinations are meaningfully selected based on specific quality and performance attributes. Physical and/or chemical interactions between active ingredient and excipient may affect stability, performance, safety, and therapeutic activity of the drug product. For example, excipient-containing tannate salts or cross-linked polystyrene may form undesirable complexes with certain active ingredients.

When selecting an excipient for the purposes of modifying the release rate of a certain active ingredient, the excipient’s stability during manufacture, the location where the active ingredient is to be released within the GI tract for absorption, and the excipient’s shelf life are critical attributes to be considered. Further, slight variations in the selected excipient’s loading concentrations, grade or properties may not only lead to greater degradation of the active ingredient, but also enhanced delivery of impurities in vivo.

d. Purity

The purity of the active ingredient is a critical quality attribute that may affect the safety and effectiveness of the product. The presence of degradants or other impurities within the active ingredient containing core can influence the performance characteristics of the MRC. The interactions between impurities and active ingredient and the interaction’s effect on the performance of the MRC cannot be known without extensive studies.

2. Excipients

The characterization and control of key functional excipients are critical to the safety, efficacy, quality, and performance of MRC. Excipients used in MRC can include various hydrophilic and lipophilic release limiting polymers, pore forming agents, stabilizers, osmotic agents, anti-tack agents, cushioning agents, plasticizer, permeation enhancers, diluents, disintegrants, lubricants, and glidants, all of which can influence the performance characteristics of MRC.
Different release limiting hydrophilic and hydrophobic polymers for coating in various grades are commercially available, such as celluloses, acrylates, pyrrolidones, and styrenes. Further, each have individualized raw material characteristics (e.g., viscosity profiles, impurity profiles, solvent/dispersant systems, molecular weight distribution, selected cross linkers, percentage of crosslinking, and functional groups). The performance of MRC can vary widely depending on the selected release limiting polymeric coating and release modifier.

Polymeric components and release modifiers must be adequately qualified for use in diffusion systems in order to carry out their intended function. This often includes an assessment of the polymer at three different stages: (1) as a raw material, (2) as a coating over the subunits (that is, in the absence of the other tableting excipients), and (3) in the finished dosage form. During the first two stages, identifying the impurity profiles of the polymer and other functional excipients can be performed. Many raw material polymers also contain neurotoxic, genotoxic, or mutagenic residual catalysts or impurities generated during the polymerization process.

When determining whether a polymer is suitable as a component for use in MRC, one must consider the potential variability that may exist between manufacturers or within batches produced from a single manufacturer. Examining the polymeric coat on the tablet or subunit can verify the functional parameters of their active ingredient release rate. Finally, assessing the polymer in the final product can help identify unanticipated manufacturing deformations of the coating and any undesirable interactions between the polymeric coats and other processing excipients. For multiparticulate systems, the compression process may alter the modified release characteristics of the subunits and cause a change of release rate or pattern, which may lead to “dose dumping.”

In addition to the polymeric coating, osmogens play an essential role in the performance of osmotic pump systems. Selection of the appropriate semipermeable polymeric membrane coating and osmogen is critical to achieve the desired active ingredient delivery characteristics. Osmogens are dissolved into the biological fluid once the fluid penetrates the osmotic pump through the semipermeable polymeric membrane coating, creating an increase in osmotic pressure within the pump. This leads to a release of active ingredient through the delivery orifice. Various inorganic salt and carbohydrate osmogens can be selected, such as potassium chloride, sodium chloride, and mannitol. Oftentimes, combinations of osmogens are used to achieve a specified osmotic pressure inside the system.

3. Conclusion

MRC formulations are complex because they are required to release a specified amount of active ingredient to a specific region of the GI tract over a specified period of time, for a given therapy. Developed properly, MRC must be physically stable, and exhibit consistent functional properties of active ingredient release rate, pattern, and location within the GI tract. Raw material selection
may influence these attributes. If MRC are not produced correctly, taking into account the principles discussed above, sub or supra-therapeutic release, GI mucosa irritation, and variability in performance within and across batches may occur. Based upon the considerations discussed above, the complexity of MRC formulations presents demonstrable difficulties for compounding.

B. MRC Have a Complex Drug Delivery Mechanism.

The complexity of the mechanism by which MRC release active ingredient throughout the GI tract presents a demonstrable difficulty for compounding. The release rate and pattern of the active ingredient are impacted by several factors. The qualitative and quantitative characteristics of active ingredient and excipient, physical design, and the intended performance at various regions of the GI tract all influence the release rate and pattern of active ingredient of MRC.

Physical design varies based on the thickness of the release modifying polymeric coating, distribution of the pore forming agents, thickness and surface area of the active ingredient containing core, layering pattern of polymer, core and cushioning agent, final surface area of the coated subunits, design and components of the extragranular matrix, compaction characteristics of the finished dosage form, dimensions and distribution of the delivery orifices, distribution of osmogens and wicking agents, and design of the pushing matrix of osmotic pump, among other things. Performance in the GI tract is influenced by pH, enzymes, salt concentrations, bile salts, mucous, transit time, gastric emptying rate, and fed and fasted states, among other things.

As previously discussed, the selected components and their interactions with one another can significantly impact active ingredient release from the MRC and permeation through the GI mucosa to the target site. For example, each of the hundreds of different grades of commercially available release limiting polymers, pore forming agents, osmogens, and wicking agents have unique characteristics that impact active ingredient release rate and pattern.

In the diffusion systems, the polymeric coat of the finished tablet or subunits permits the entry of GI fluid to dissolve the active ingredient containing core. The dissolved active ingredient then diffuses through the network of pores and channels within the polymeric coating at a specified onset and rate. The specified rate is determined by the physicochemical characteristics of the active ingredient and the polymeric coating components, such as the type and concentration of the coating polymer, plasticizer, and dissolution rate modifiers. In this mechanism, the membrane is intended to stay intact during the passage of the coated subunits through the GI tract.

Release of active ingredient may also occur after erosion of the polymeric coating. The polymeric coating gradually softens and then completely erodes when exposed to specific conditions present at certain regions of the GI tract. For example, enteric coatings are designed
to dissolve at a predetermined pH, releasing the active ingredient into the intestine rather than the stomach.

In the osmotic systems, a semi-permeable polymeric membrane coating covers the entire tablet and an orifice is drilled though the coating. Osmotic systems are capable of delivering active ingredient at zero-order kinetics through the pressurized process described above. The rate is determined by characteristics of the active ingredient containing core, the excipients, the coating, and the diameter of the orifice. Osmotic gradients may also influence the release of active ingredient if solution moves outside the system through pores and channels within the network of the semi-permeable polymeric coat.

**Conclusion**

The mechanism by which active ingredient is released from the MRC throughout the GI tract is complex because it requires the design and formation of a system that delivers a specific amount of active ingredient per unit time and in some cases in specific regions of the GI tract (e.g., enteric coated MRC). In addition, because the dose release profile is impacted by several factors, precise control of raw materials, the manufacturing process, and the final product are necessary for safety and efficacy. Based upon the considerations discussed above, the complexity of the drug delivery mechanisms of MRC presents demonstrable difficulties for compounding.

**C. MRC Are Complex Dosage Forms.**

MRC are complex dosage forms for the reasons discussed above in sections A and B. Specifically, MRC have complex formulations and mechanisms by which amount of active ingredient is released per unit time, and in some cases in specific regions of the GI tract. They require well designed controls of component attributes and process parameters for predictable active ingredient release. In addition, MRC are designed to maintain their integrity *in vivo* to minimize local irritation to the GI tract and to ensure that dose dumping does not occur. As mentioned above in section A, various components play a critical role in the dosage form performance. Extensive product development and precise control over raw material selection and the production process are essential to evaluating the active ingredient release mechanism and profile, and overall MRC performance characteristics. Based upon these considerations the complexity of MRC dosage forms presents demonstrable difficulties for compounding.

**D. Complexity of Characterizing and Controlling Drug Bioavailability of MRC.**

FDA defines bioavailability as “the rate and extent to which the active ingredient…is absorbed from a drug product and becomes available at the site of action.” 21 C.F.R. § 320.1. Small changes in the performance characteristics of MRC can significantly impact bioavailability, and
in turn the safety and effectiveness of the product. For example, quick onset or an extended duration of active ingredient release from MRC products may be critical to treat sleep disorders, attention deficit disorders, and other conditions. Characterizing pharmacokinetic behavior of such MRC formulations is critical. In addition, MRC formulation and production processes need to be well controlled to achieve an optimal rate and extent of active ingredient absorption at the site of action.

In addition, for both locally and systemically acting products, several physiological factors within the GI tract impact the bioavailability of the active ingredient. Factors include fed and fasted states, mucosal secretions, salt concentrations, pH, bile acid and phospholipid secretions, gastric emptying rates, and variations in the anatomical sites of the GI tract. For locally acting products, which may have little to no systemic uptake, drug activity is often assessed using in vitro testing and pharmacodynamic studies, or by evaluating therapeutic endpoints.

In developing MRC for the purposes of seeking FDA approval, sponsors typically perform a multitude of studies, including in vitro, pharmacokinetic, and other in vivo assessments to demonstrate and ensure their performance. These measurements are necessary to ensure consistent product is produced with a release rate of active ingredient falling uniformly within predetermined and specific acceptable ranges.

**Conclusion**

Characterizing and controlling bioavailability of MRC is complex. Subtle changes to any of its components or manufacturing processes could significantly impact its performance characteristics, which may in turn influence local, systemic, and enteric bioavailability. In general, for MRC, in vitro assessments, such as in vitro dissolution testing, alone are insufficient to accurately predict bioavailability and overall clinical effect. Rather, in vivo assessments are needed. Based on these considerations, characterizing and controlling MRC bioavailability is complex and presents a demonstrable difficulty for compounding.

**E. MRC Involve Complex Compounding Processes.**

MRC require complex and specialized manufacturing processes, including the use of specialized equipment, to reproducibly yield predictable drug delivery. For example, mixing of active ingredient with excipient must be done with specialized mixers under appropriate controls to ensure blend uniformity.

MRC diffusion systems are often prepared with specialized layering/coating equipment to produce coats around the active ingredient containing cores. Other times, inert subunits are coated with active ingredient followed by release limiting excipient. In order to achieve proper coating, the release limiting polymeric layer should be of uniform thickness and continuous,
without gaps, to prevent uncontrolled active ingredient leaks and dose dumping (refer to Figure 1b). The finished dosage form is manufactured either by filling the coated subunits of specific design into capsules or compressing the subunits into tablets. This process requires specialized equipment under appropriate controls to ensure integrity and uniformity of the subunits within the system.

Osmotic systems must also be prepared with specialized tablet coating equipment under appropriate controls to lay a semipermeable polymeric membrane coating around the tablet matrix. Afterwards, a precision drilled orifice with specific dimensions is added to control the active ingredient release rate.

1. Mixing

The overall unit-to-unit and batch-to-batch blend uniformities are dependent on the physical characteristics of the MRC components. Such characteristics include flow, density, surface charge, particle size, particle shape, and volume fractions. Blend uniformity is also dependent on various processing parameters, such as mixing time, mixing principle, mixer dimensions and shape, and propeller speed. For diffusion systems, the variability of the blend for filling into capsules or compression into tablets is in turn dependent on the variability of the coated subunits of the active ingredient and the extragranular excipients mixture. In addition to minimizing the raw material variability discussed in sections A and B, the mixing step itself is critical for achieving a uniform mixture of active ingredient and excipient before further processing. For MRC with FDA-approved applications, it is common practice to test for various performance attributes, such as blend uniformity, viscosity, and particle size distribution, as part of “in-process” testing during the mixing process.

Incomplete mixing can result in a fluctuation in the amount of active ingredient available for release or can lead to variable release rates in regions throughout the GI tract. Over-mixing or the use of an excessive propeller speed during mixing can result in air being trapped within the mixture, which leads to non-uniformity of the powder blend. Further, variable mixing, hold or transfer times can lead to unintended powder segregation.

2. Coating and drying

For conventional manufacturing of coated subunits, coating is typically performed on automated equipment with precise controls for coat thickness, weight gain, drying rate, temperature, and residual solvent/moisture. This is to ensure uniform thickness of continuous coats around the solid particles. Varying thickness, weight gain, residual solvent, and moisture level of each coating layer directly impact active ingredient content and release characteristics.
As seen in Figure 1b, subunit coating may involve the application of a release modifying polymeric coating, or other release retardant material, to an active ingredient containing core. The active ingredient containing core is produced by granulating a blend of active ingredient and excipient to a specific size, followed by coating with the release retardant material. This is often performed using fluidized bed coating, wurster coating, and similar coating approaches. Subunit coating may also involve the application of active ingredient to inert cores of specialized size, such as nonpareil beads (sugar spheres). A release retardant material is then applied to the inert cores that are coated with active ingredient.

The coating process involves a process of successive layering and drying. This is done according to a specified design to achieve the desired release rate and pattern. Uniform processing conditions are achieved by passing a gas (usually air) through the bulk particulates under controlled velocity conditions to create a fluidized state. During the layering process, a coating material solution is sprayed onto the surface of fluidized solid particulates. The fluidizing gas is also used to dry the deposited solution, which forms a coat on the surface of the particulate. The process is terminated after achieving the predetermined coating weight, layers, and thickness. The residual materials are also accounted for to ensure proper coating on the particulate.

Numerous release retarding and other modifying materials are commercially available with various physical and chemical properties. These commercially available products have specific requirements for efficient and reproducible coating characteristics. Coated particulates may be prepared with different layers or thicknesses of release retarding materials. This is important to modulate the predetermined release rate and pattern of active ingredient.

The drying process during fluidized bed processing is a critical step in controlling residual solvents and volatile polymer impurities. Some polymers are often solvent-based or mixed with solvents for further processing. Drying processes are utilized to reduce these residual solvents and impurities to acceptable levels that are qualified through non-clinical testing. Further, if drying temperature and fluidization air flow are not adequately optimized and controlled, product performance may be adversely impacted.

3. Compression and filling

After the coated subunits are produced, the finished dosage form is fashioned by filling the subunits into capsules or compressing them into tablets. In some cases, to achieve the intended release pattern, subunits of varying release rates of active ingredients may be packaged together to form the finished dosage form.
For subunits that are compressed into tablets, compaction is typically performed on an automated tablet press. Tablet presses are equipped with precise controls for applying compression and ejection forces that define the dimensions and weight of the tablet. Use of this technology is intended to ensure uniform tablets with consistent active ingredient release patterns. Increasing the applied compression force above certain predefined limits can lead to fractures in layers of the release regulating polymer, which may result in dose dumping. Conversely, a lower compression force can lead to fragile, friable, and/or non-consistent tablets.

For subunits that are filled into capsules, any variation in the loading fractions of varying particulates can result in undesirable release rates and patterns. Generally, variations in the compression or filling parameters directly impact the efficacy and safety profiles of the product.

The most widely used osmotic pump systems are the Elementary Osmotic Pump (EOP) and the Push-Pull Osmotic Pump (PPOP) (Figure 2). EOP systems are produced by compressing a blend of active ingredient, osmogen, and other processing excipients into tablets followed by coating with a semipermeable polymeric membrane. The PPOP system resembles a standard bilayer coated tablet. One layer contains a blend of active ingredient in a polymeric matrix with the other excipients. The other layer contains polymeric matrix of the osmotic agent and excipient. These layers are formed and bonded together by tablet compression to create a single bilayer core. The tablet core is then coated with a semipermeable polymeric membrane. As previously discussed, for both EOP and PPOP systems, a small orifice is drilled through the membrane coating for active ingredient release when pressure inside the system increases. Similar to diffusion systems, the compaction process is typically performed on an automated tablet press to produce uniform tablets with consistent performance characteristics. Appropriate compression is critical for maintaining a consistent wicking action of osmotic agent, which is crucial to product performance, effectiveness, and safety.

4. Creation of a Delivery Orifice

An osmotic pump system contains at least one delivery orifice in the coating for active ingredient release. The size of the delivery orifice must be optimized in order to control the predetermined release rate and pattern of release. Size of the delivery orifice must not be too large; otherwise, diffusion of osmogen from the orifice may take place, leading to a decrease in osmotic pressure within the system. If the size of the delivery orifice is too small, hydrostatic pressure within the core will increase. These phenomena result in unpredictable active ingredient release and may cause dose dumping.

Delivery orifices in osmotic systems are often created using a mechanical drill or by laser. Laser drilling is one of the most commonly used techniques. A laser beam is focused onto the surface of the tablet, which becomes heated and ultimately pierces the semipermeable polymeric
membrane, causing a precise orifice to form. The size and depth of the orifice are controlled by the laser beam parameters, such as laser power, firing pulse and duration. These parameters are individually selected based on the specific osmotic system being drilled. Consideration is given to polymeric coat thickness and dimensions of the beam at the coating. Appropriate drilling parameters are critical to ensure precise dimensions of the release orifice, which is crucial to product performance, effectiveness, and safety.

**Conclusion**

The compounding processes for MRC are complex because specialized equipment under appropriate controls is critical for the automated processing and precise control over the manufacturing process. Steps include technically complex mixing, fluidization coating and drying, compression, filling, and orifice drilling. Poor technique or control at any of these steps will likely result in variability in system performance. For these reasons, manufacturing of MRC involves complex processes that present demonstrable difficulties for compounding.

**F. MRC Necessitate Complex Physicochemical or Analytical Testing.**

A number of complex tests are critical to ensure predetermined MRC performance characteristics. Additionally, extensive characterization and developmental studies on the specific formulation, functional properties, and manufacturing processes are necessary to develop the specifications and in-process controls that should be used to ensure that the product will perform at a predetermined level.

1. **Raw Materials Testing**

As discussed in sections A and B, rigorous qualification of the release controlling materials, osmogens, pore forming agents, as well as other key components, is important to the safety and effectiveness of MRC. For example, raw material properties, such as inherent viscosity, swelling ratio, dissolution, and impurity content, can often impact irritation potential of MRC. Manufactured raw materials could vary from manufacturer to manufacturer. Without independently conducting complex testing of raw materials received, the safety and effectiveness of MRC may be adversely affected.

2. **Product Quality Testing**

Due to the complex and intricate relationship among active ingredient, release limiting polymer, pore forming agent, osmogen, and other excipients, MRC require *in vitro* dissolution tests to ensure adequate, predictable, and reproducible dissolution of active ingredient within the GI tract. *In vitro* dissolution tests may be utilized to assure similar batch-to-batch characteristics throughout its shelf life.
In vitro dissolution testing is just one important quality/performance test for oral solid dosage forms. USP General Chapter <2>, *Oral Drug Products-Product Quality Tests*, highlights universal tests related to description, identification, assay and impurities for oral dosage forms. Specifically for tablets, Chapter <2> identifies tests for volatile contents, disintegration, friability, breaking force and uniformity, as well as specific tests for coated tablets. However, precise methods for each of these tests are not articulated; only general methods for testing the dissolution rate and pattern are described. There are multiple compendial and non-compendial methods and technical nuances for dissolution testing. Nuances include a specific apparatus design, volume, composition of the medium, stirring rate, temperature, and sampling schedule. Variations in any of these parameters can significantly impact the meaningfulness of the result, particularly for MRC. For example, in diffusion systems, surfaces of whole tablets and beads in multiparticulate systems may be coated to dissolve at varying pH levels. Tests must typically be designed to verify performance at multiple relevant physiological pH levels to ensure appropriate release rates.

Other important active ingredient release tests for MRC include assay, content uniformity, disintegration, friability, impurity, and residual solvent testing. Similar to *in vitro* dissolution testing, sophisticated equipment and specialized methods need to be developed and utilized for these tests. Developing a method that can first extract a particular component, like an active ingredient, from a unique polymeric matrix and then precisely quantify it is complex. Further, proper testing requires the quantitation of polymer impurities and residual monomers and solvents for each batch produced.

### 3. Stability Testing

MRC require both product quality and product performance testing to determine appropriate shelf-life. This should be studied throughout their in-use period, after dispensing to the patient, to help ensure quality and performance is maintained during storage and administration. The effect of storage time, storage conditions, and in-use storage condition (e.g., open or sealed containers, presence or absence of desiccants, and fill volume) can impact quality and performance. The same tests utilized to measure release of the product typically need to be conducted throughout the shelf life and in-use period to ensure product quality and performance at the time of administration.

One quality concern that may arise with MRC is polymorphism. Under certain conditions, active ingredient can convert into different polymorphs. Polymorphic changes may impact the release rate and pattern of the active ingredient. Solid state characterization studies, such as X-ray diffraction and differential scanning calorimetry, are typically necessary and performed under
varying conditions to establish appropriate acceptance criteria for polymorphic purity of the active ingredient and appropriate limits for undesired polymorph impurities.

4. Conclusion

MRC require complex physicochemical and analytical testing of raw material, product quality/performance, and stability because evaluating the physical and chemical properties of the raw materials and finished dosage form, as well as the product-critical performance parameters, require specialized analytical devices and procedures for accurate measurement. Furthermore, chemical impurities must be quantitated through various sensitive analytical techniques developed specifically for these impurities. For these reasons, the physicochemical and analytical testing required for production of the desired MRC is complex and therefore presents demonstrable difficulties for compounding.

V. RISKS AND BENEFITS TO PATIENTS

MRC have grown in popularity since their introduction in the 1950s, and FDA-approved MRC are currently used for the management of severe pain, hypertension, diabetes, attention deficit hyperactivity disorder, Parkinsonism, epilepsy, and schizophrenia. The safety profile for these products is monitored by FDA to identify drug safety concerns and recommend actions to improve product safety and to protect the public health. There is currently an adequate supply of approved MRC on the market and thus there is limited, if any, benefit to expanding the market to compounding MRC.

As discussed above, MRC design and the relationship between excipient and active ingredient directly impacts release rate and pattern and performance. These in turn affect drug product effectiveness and safety. Substituting or removing excipients, such as release retarding polymers, plasticizers, solubilizers, and permeation enhancers, would likely change the release characteristics of the product, and, in turn, may adversely impact product performance. Also, precise and consistent quality controls of raw materials, the manufacturing process, and final product are essential for predictable and reproducible active ingredient release, performance and safety profiles. Any potential benefit that may be derived would be outweighed by the risks, discussed above, associated with allowing a compounder to attempt to produce these complex drug products.

VI. RECOMMENDATION

We have evaluated the category of MRC as candidates for the Difficult to Compound List. Based on an analysis of the evaluation criteria, we believe that MRC present demonstrable difficulties for compounding that reasonably demonstrate and are reasonably likely to lead to an adverse effect on the safety or effectiveness of this category of drug products. Taking into
account the risks and benefits to patients, we believe oral MRC should be included in the Difficult to Compound List under sections 503A and 503B of the FD&C Act.
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18