Draft Report on Carcinogens (RoC) Concept: Goldenseal Root Powder

1 Background and rationale

1.1 Background

Goldenseal (*Hydrastis canadensis)*, a member of the plant family Ranunculaceae, is a slow-growing perennial herb that spreads via root-like, yellow underground stems (rhizomes) that are dried and ground into a powder for use as a medicinal remedy. It is used as a digestive aid and anti-inflammatory to treat a variety of ailments, including gastrointestinal and urinary disorders and for skin, mouth, and eye infections. Goldenseal root powder has been tested for carcinogenicity in rodents in 2-year feed studies in rats and mice (NTP, 2010).

In September 2013, the National Toxicology Program (NTP) solicited information on goldenseal root powder and other nominated substances (78 FR 57868, http://ntp.niehs.nih.gov/go/rocnom). One public comment was received which raised an issue regarding the use of concurrent and historical control data in evaluating the significance of carcinogenic activity associated with goldenseal root powder exposure in the NTP animal 2-year bioassay. The commenter also provided information on current use patterns and human exposure, identified a knowledgeable expert, and cited two recent publications for review.

1.2 Rationale

Goldenseal root powder is proposed as a candidate substance[1] for the RoC because it is one of the most widely used herbal products in the United States, thus has potential for substantial human exposure, and because there is an adequate database by which to evaluate its potential carcinogenicity. The International Agency for Research on Cancer (IARC) recently classified goldenseal root powder as *possibly carcinogenic to humans* (Group 2B) (IARC, 2013).

2 Overview of data related to human exposure

There is significant exposure to goldenseal to the general population of the United States through its use as an herbal remedy. Goldenseal root powder (prepared from the rhizome) is ingested as a capsule or tablet, as tea or in drinking water, or made into a paste for

[1] If selected as a candidate substance, the scientific evaluation of goldenseal root powder will be captured in a draft RoC monograph (for more details, see http://ntp.niehs.nih.gov/go/rocprocess). The proposed approach, delineated in this concept document, for preparing the cancer evaluation of the draft monograph, is tailored to the nature, extent, and complexity of the scientific information on goldenseal root powder. This concept document also discusses information supporting the rationale and the proposed approach for the substance review, including data on human exposure, an overview of the nature and extent of the scientific information for evaluating carcinogenicity in humans and/or animals, scientific issues and questions relevant to the evaluation of carcinogenicity of goldenseal root powder, and the proposed approach for conducting the scientific evaluation, including literature search strategy, scope and focus of the monograph, and approaches for obtaining scientific and public input to address the key scientific questions and issues.

external use. Historically, goldenseal has been used to treat a variety of health conditions including skin diseases, ulcers, colds and other respiratory tract infections, infectious diarrhea, on wounds and canker sores, and as a mouthwash for sore gums, mouth, and throat, or as an eyewash (Natural Standard, 2009; NCCAM, 2012). It can be found in dietary supplements, eardrops, feminine cleansing products, allergy remedies, laxative products, and digestive aids. Goldenseal is sometimes combined with other herbals to boost their effects, e.g., it is marketed with echinacea to treat upper respiratory infections (Natural Standard, 2009). According to published databases, there are currently around 150 products on the market containing goldenseal (DSLD, 2013; EWG, 2013) and echinacea-goldenseal was recently ranked 14th in the 20 top-selling botanical dietary supplements in the United States (Blumenthal *et al.*, 2012). Goldenseal is considered a dietary supplement under the Dietary Supplement Health and Education Act (DSHEA) of 1994 (USFDA, 1994, as cited in NTP, 2010. Dietary supplements that were on the market prior to October 15, 1994 have no requirement for proof of safety.

Although goldenseal grows wild in many parts of the United States, it's popularity as an herbal remedy has resulted in overharvesting and it is considered an endangered species (NCCAM, 2012). It is now grown commercially in the United States and the variability in sources may result in differences in chemical composition among preparations available to the public.

The active ingredients in goldenseal preparations include several isoquinoline alkaloids: hydrastine, berberine, berberastine, hydrastinine, tetrahydroberberastine, canadine, canalidine, and palmatine (Dunnick and Nyska, 2013; Hermann and von Richter, 2012). However, most of the actions of goldenseal have been attributed to only two, berberine (0.5-6%) and hydrastine (1.5-4%) (Hamon, 1990, as cited in Natural Standard, 2009). Berberine has been described as having antimicrobial, antifungal, and antimalarial properties, as well as anti-inflammatory, cytostatic, antiproliferative, and antioxidative activities (see NTP, 2010 review). Hydrastine has various reportedly beneficial heath properties, but no clinical trials validating the claims have been identified.

3 Overview of the carcinogenicity data

3.1. Human cancer studies

No epidemiological studies have been identified that examined the relationship between human cancer and exposure specifically to goldenseal.

3.2. Cancer studies in experimental animals

One report on the carcinogenic effects of goldenseal root powder in experimental animals was identified from the peer-reviewed literature. Goldenseal root powder was tested for carcinogenicity in 2-year feed studies conducted by the National Toxicology Program in both sexes of F344/N rats and B6C3F$_1$ mice (NTP, 2010). In those studies increased incidences of hepatic neoplasms (e.g., hepatocellular carcinoma, hepatocellular adenoma, and hepatoblastoma) were reported in male and female rats and male mice treated with goldenseal root powder.

3.3. Mechanistic information

The database of available metabolism and pharmacokinetic studies in humans and rodents is primarily limited to the alkaloid berberine, one of the main active ingredients of

goldenseal (NTP, 2010). The few studies identified that specifically evaluated goldenseal root powder or extract attributed the effects to berberine. A few studies have investigated effects *in vivo* of oral or intravenous exposure to berberine in rats and rabbits and by ingestion by human volunteers, and *in vitro* on metabolism and genotoxicity. These evaluations include absorption, distribution, identification of metabolites, and investigations of the substance's effects on enzymatic or transporter pathways.

Studies on the absorption and distribution of berberine in rodents and humans indicate that it is absorbed from the gastrointestinal tract, actively transported to the liver and rapidly metabolized, then excreted in the urine and bile (Tsai and Tsai, 2004). In rats and humans, the metabolism of berberine occurs initially via (1) *o*-demethylation at the C9 or C10, resulting in berberrubine and thalifendine or (2) by methylenedioxy ring opening and catechol formation, resulting in demethyleneberberine. These substances undergo further metabolism, culminating in glucuronide or sulfate conjugation; demethyleneberberine-2-*O*-sulfate was identified as the major human metabolite. Comparative experiments using germ-free (antibiotic treated) rats demonstrated the importance of the initial digestive metabolism of berberine; in these studies, glucuronide conjugates were hydrolyzed by gut microflora, resulting in aglycone circulation in the liver. The metabolism of berberine is qualitatively similar in rats and humans and results in similar metabolic profiles; however, there is preferential conjugation of berberine-derived metabolites with glucuronic acid in rats and sulfate in humans.

Several *in vitro* and a few *in vivo* studies have been conducted to investigate potential mechanisms by which goldenseal root powder, or its active alkaloid components berberine and hydrastine, may cause cancer. The studies have primarily focused on the inhibition of essential enzymatic reactions for topoisomerases and cytochrome P450s; there are also some indications of modulatory effects on cell signaling, receptors, and as a transporter inhibitor (Abidi *et al.*, 2006; Etheridge *et al.*, 2007; Kulkarni and Dhir, 2010) and genotoxicity.

Of special interest is a potential mode of action for the carcinogenicity of goldenseal/berberine involving the function of topoisomerase enzymes, which help regulate the DNA winding and unwinding process by binding to and cutting the phosphate backbone of the DNA. Since both berberine and its potent metabolite berberrubine are topoisomerase inhibitors, they can disrupt the DNA repair process and induce chromosomal damage, resulting in malignancies (Malik *et al.*, 2006). DNA damage, observed in berberine-treated human HepG2 cells, was associated with inhibition of topoisomerase II (Chen *et al.*, 2013). Goldenseal root powder and berberine hydrochloride have been tested *in vitro* for mutagenicity in bacterial cells (with and without metabolic activation) and for mutagenic effects and mitotic recombination in yeast. Both chromosomal and DNA damage have been evaluated *in vivo* in mammalian cells.

Anti-carcinogenic and cardioprotective effects of berberine have been evaluated *in vitro* (Saha *et al.*, 2013; see NTP, 2010 for review); *in vivo* studies in mice have assessed goldenseal extract for anti-carcinogenic and chemopreventive effects (Karmakar *et al.*, 2010).

4 Issues and key scientific questions relevant for the cancer evaluation

The key questions and issues for the review of goldenseal root powder concern the evaluation of studies in experimental animals and mechanistic data.

- What is the level of evidence (sufficient or not sufficient) for the carcinogenicity of the goldenseal root powder from the studies in experimental animals?
  - If sufficient, what are the target tissue sites?
- What are the potential mechanisms by which goldenseal root powder may cause cancer?
  - What is the strength of the evidence for these mechanisms?
  - Is there evidence to support biological plausibility for cancers of the liver in experimental animals and humans?
- Are the active components directly responsible for the observed results known?

5 Proposed approach for conducting the cancer evaluation

5.1 Establishment of a RoC monograph planning team

The ORoC will consult with appropriate technical advisors (external or internal to government) who will become part of the RoC monograph team, which will also consist of ORoC staff (government and contractor), as well as key NTP personnel. Sources for identifying these advisors include, but are not limited to, peer-reviewed literature databases and recommendations from the scientific community and the public. Chemists, metabolism experts, and those with relevant expertise on human exposure and uses of goldenseal root powder will be consulted to advise the ORoC on the assessment of this substance.

5.2 Protocol development

The RoC monograph team will develop a draft protocol for goldenseal root powder that will outline the methods for preparing the monograph, including the literature search strategy, the methods for evaluating the quality of the human and experimental animal cancer studies and guidelines for integrating this information to reach a listing recommendation[2], and a preliminary outline for the monograph. The public will be able to provide input on the protocol via an input box on the candidate substance webpage.

The ORoC will create a webpage for a goldenseal root powder which will include (1) RoC documents related to the review of the substance (e.g., concept document, protocol for the review, draft RoC monograph), (2) public comments, (3) an input box for the public to provide information (such as new literature) or comment (such as the identification of additional scientific issues), and (4) information on public meetings or listening sessions. The NTP will communicate when new information is added or updated (such as updated literature searches) to the website via the NTP listserv. Additional scientific issues may be identified during the preparation of the monograph.

In addition to the input box on the candidate substance webpage, other opportunities for public comments on during the review process include a request for information on the nomination, for comment on the draft concept when it has been approved, and for comment on the recommended protocol for achieving the review of goldenseal root powder. The

[2] A listing recommendation can be not to list, to list as *reasonably anticipated to be a human carcinogen*, or to list as *known to be a human carcinogen*.

ORoC will consider the information suggested by the public[3] in drafting the cancer evaluation component of the draft monograph. Future forums, such as a listening session, for receiving public comment on any additional scientific issues may be considered depending on public interest; these would be announced via the *Federal Register* notice and NTP listserv[4] and posted on the RoC website.

5.3 Development and peer review of the draft RoC monograph

The NTP will convene a peer-review panel[5] of scientific experts to review the draft RoC monograph on goldenseal root powder in a public meeting. The NTP Office of Liaison, Policy and Review will manage the NTP expert panel peer review. Members of the panel will be from the public and private sectors with expertise in disciplines related to the cancer evaluations of goldenseal root powder, particularly those with relevant knowledge of human exposure to the substance, animal carcinogenesis, pathology (particularly liver tumors), chemistry, toxicology, genotoxicity and mechanisms of carcinogenesis. The NTP will set aside time at the peer-review meeting for a discussion of specific scientific issues raised in the public comments.

6 References

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[3] Federal Register notice and public comments are available at *http://ntp.niehs.nih.gov/go/rocnom*.
[4] Persons can subscribe to the NTP listserv free-of-charge at *http://ntp.niehs.nih.gov/go/getnews.*
[5] NTP panels are federally chartered technical and scientific advisory groups convened as needed to provide advice on specific scientific issues and peer review. Members of NTP panels are scientists with relevant expertise and knowledge from the public and private sectors. The final selection of membership is based upon providing a balanced and unbiased group of highly qualified individuals and is made in accordance with Federal Advisory Committee Act and HHS implementing guidelines; *http://ntp.niehs.nih.gov/go/166*.

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Appendix A: Preliminary literature search strategies

This document identifies the data sources, search terms, and preliminary search strategies for identifying literature for the draft RoC monograph on goldenseal root powder. The literature search will be updated approximately every three months, and prior to submitting the draft monograph for interagency review. Additional literature searches will be conducted as needed to identify information to address scientific issues that arise during the review. Citations retrieved from literature searches will be uploaded to web-based systematic review software and screened using inclusion and exclusion criteria. Multi-level reviews of the literature are conducted with initial reviews based on titles and abstracts only and subsequent reviews based on full-text searches.

1. Data Sources

Identification of synonyms and metabolites for goldenseal [and active ingredients berberine and hydrastine]

- NTP Technical Report (TR-562) and Nomination Background: *Goldenseal and Two of Its Constituent Alkaloids, Berberine and Hydrastine: Review of Toxicological Literature* (both available at http://ntp.niehs.nih.gov/go/TS-M980070)
- National Library of Medicine databases (e.g., ChemIDplus)
- The National Center for Complementary and Alternative Medicine (NCCAM) (http://nccam.nih.gov/health/goldenseal)
- IARC Volume 108, in preparation [reported in *The Lancet Oncology* 14(9): 807-808 in news article by Grosse *et al*. 2013.]

Citation databases (searches titles, abstracts, and key words)

- PubMed
- Web of Science
- Scopus

Additional data sources:

- Authoritative reviews or general sources for exposure and other information (e.g., Toxnet; U.S. Government agencies websites, publications and databases; International Agency for Research on Cancer monographs)
- Citations in authoritative reviews, and primary references located by literature search
- QUOSA library of occupational case-control studies (full-text search for substance)

2. Preliminary Literature Searches

The approach for conducting the literature search in the three major databases (see Data Sources, Section 1) consists of a combination of general searches (for all literature on goldenseal and synonyms, chemical class, etc.) and topic-specific searches for information related to the carcinogenicity (see Table 1). Topic-specific searches are constructed to answer key questions in the monograph; as a result, not all chemical-specific searches are

combined with all topics covered by the monograph. Search terms for specific topics have been developed in consultation with an information specialist.

Table 1: Preliminary literature search approach

Substance	Search terms	Topics (combined with)[a]
Synonyms	Goldenseal, golden seal, extract of goldenseal, CASRN 84603-60-1, *Hydrastis canadensis*, others (e.g., common names for *H. canadensis*)	Human Exposure ADME/Toxicokinetics Human Cancer Studies Cancer Studies in Experimental Animals Genetic Toxicology Mechanisms, Toxicity
Chemical class	Isoquinoline alkaloids	Cancer Studies in Experimental Animals (for the mechanistic section) Genetic Toxicology Mechanisms, Toxicity
Active ingredients or components of goldenseal and their synonyms	Berberine, hydrastine	Human Exposure ADME/Toxicokinetics Human Cancer Studies Cancer Studies in Experimental Animals (for the mechanistic section) Genetic Toxicology Mechanisms, Toxicity
Metabolites and their synonyms	Berberine metabolites: berberrubine, thalifendine, demethyleneberberine, jatrorrhizine Hydrastine metabolites: none identified	ADME/Toxicokinetics Human Cancer Studies Cancer Studies in Experimental Animals (for the mechanistic section) Genetic Toxicology Mechanisms, Toxicity

[a] Search terms for each topic were developed in consultation with an information specialist.