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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

GOLDENSEAL

1. Exposure Data

Goldenseal (*Hydrastis canadensis* L.) was traditionally used by native Americans as a medicinal remedy and as a colouring agent ([Sinclair & Catling, 2000](#); [McKenna & Plotnikoff, 2010](#)). In his *Collections for an Essay Towards a Materia Medica of the United States,* the American botanist Benjamin Smith Barton first mentioned the medicinal use of *H. canadensis* by the Cherokee ([Hobbs, 1990](#)). Today, the main application of *H. canadensis* is for the prevention and treatment of skin disorders, dyspepsia, gastritis, peptic ulcer, colitis, anorexia, menorrhagia, dysmenorrhoea, sinusitis, mucosal inflammation, and other inflammatory conditions or infectious diseases ([BHMA, 1983](#); [BHMA, 1992](#); [NTP, 2010](#); [Sun et al., 2009](#); [McKenna & Plotnikoff, 2010](#)).

1.1 Identification of the agent and its major constituents

1.1.1 Botanical data

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 84603-60-1
Chem. Abstr. Name: Golden seal root
Botanical name: *Hydrastis canadensis* L. ([Fig. 1.1](#))
Family: *Ranunculaceae*
Genus: *Hydrastis*
Plant part: Root

Common names: Hydrastis; Golden seal; Yellow Indian plant; Yellow seal

(b) Description

Goldenseal is a perennial plant, which grows naturally in eastern USA and Canada ([AHP, 2001](#); [Zieger & Tice, 1997](#)). Goldenseal has one long-trunked basal leaves, a single stem, and two smaller leaves attached to the flowering stem. Usually, the plant has two rounded, lobed, and double-toothed leaves on a forked branch, with one being larger than the other. The plant has a knotted yellow rhizome and a solitary terminal flower with three white sepals and many greenish-white stamens in clusters, while the fruit is small and red raspberry-like ([Zieger & Tice, 1997](#); [AHP, 2001](#)). The plant grows up to about 1 foot in height [30.5 cm] ([Palmer, 1975](#); [Duke, 2002](#)).

1.1.2 Chemical constituents and their properties

The major constituents of goldenseal root are isoquinoline alkaloids such as hydrastine (1.5–4%), berberine (2.5%), canadine (0.5%), and other alkaloids (see [Fig. 1.2](#); [BHMA, 1992](#)). Berberine is usually found in the roots of goldenseal as a sulfate with a yield of 5000–60 000 ppm ([HSDB, 1997](#)). Hydrastine is also found in goldenseal in concentrations of 15 000–40 000 ppm ([NTP, 2010](#)). [Junio *et al.* (2011)](#) have also identified sideroxylin, 8-desmethyl-sideroxylin, and

Fig. 1.1 Goldenseal (*Hydrastis canadensis* L.), showing leaves, root, flower and seed

From Koehler's Medicinal-Plants (1887)

6-desmethyl-sideroxylin. Chemical Abstract Registry (CAS) number, International Union of Pure and Applied Chemistry (IUPAC) names and some physical properties of goldenseal major alkaloids are presented below (American Chemical Society, 2014; Zieger & Tice, 1997).

(a)   Berberine

Chem. Abstr. Serv. Reg. No.: 2086-83-1

IUPAC name: 7,8,13,13α-Tetradehydro-9,10-dimethoxy-2,3-(methylenedioxy) berbinium

Description: Yellow solid with a melting point of 145 °C and slowly soluble in water (O'Neil, 2013).

(b)   Hydrastine

Chem. Abstr. Serv. Reg. No.: 60594-55-0

IUPAC name: 1(3*H*)-Isobenzofuranone, 6,7-dimethoxy-3-(5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl)-

Description: Solid with a melting point of 132 °C and highly soluble in acetone and benzene; insoluble in water (O'Neil, 2013).

(c)   Canadine

Chem. Abstr. Serv. Reg. No.: 522-97-4

IUPAC name: 6*H*-benzo[*g*]-1,3-benzodioxolo[5,6-α]quinolizine, 5,8,13,13α-tetrahydro-9,10-dimethoxy-(13α*S*)

Description: Yellow pale solid with a melting point of 172 °C and soluble in methanol (O'Neil, 2013).

(d)   Others

Other goldenseal components of the flavonoid class include:

- sideroxylin (CAS No., 3122-87-0; IUPAC name, 4',5-dihydroxy-7-methoxy-6,8-dimethylflavone)
- 8-desmethyl-sideroxylin (CAS No., 80621-54-1; IUPAC name, 4*H*-1-benzopyran-4-one, 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-6-methyl-)
- 6-desmethyl-sideroxylin (CAS No., 1194721-03-3; IUPAC name, 4*H*-1-benzopyran-4-one, 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-8-methyl-).

For structural and molecular formulae and relative molecular mass, see Huang & Johnston (1990), Junio *et al.* (2011), Li *et al.* (2011), and Fig. 1.2.

1.1.3 Technical and commercial products

Official technical products are goldenseal root, powdered goldenseal root, and powdered goldenseal root extract (NTP, 2010; USP, 2013). Other minor technical products may include powdered goldenseal leaf and its derived extracts, as well as fluid extracts (Mikkelsen & Ash, 1988; Oldfield, 2005; NTP, 2010).

Powdered goldenseal root and leaf products are available as capsules and teas in combination with other herbs, in some over-the-counter (OTC) herbal supplements (Mikkelsen & Ash, 1988; Zieger & Tice, 1997; AHP, 2001). Goldenseal is also found in eardrops, feminine cleansing products, cold/flu remedies, allergy relief products, laxative products, and aids to digestion (Zieger & Tice, 1997; AHP, 2001). Chemical derivatives of purified major components of goldenseal, such as berberine hydrochloride and berberine bisulfate, are found in some commercial eyewash formulations (Zeiger & Tice, 1997). Hydrastine, another derivative, is commercially available in the form of (–)-hydrastine and used as an ingredient in some decongestant nose sprays and feminine hygiene products (Zeiger & Tice, 1997; Huang & Johnston, 1990).

As a consequence of the high cost of genuine goldenseal, some commercial products contain little or no goldenseal plant material (Govindan & Govindan, 2000). *Coptis chinensis* has been sold in place of "Chinese goldenseal" and has been found as an adulterant of goldenseal (Brown & Roman, 2008). Moreover, other species that contain berberine, such as *Coptis japonica*, *Berberis aquifolium*, *Berberis* spp., *Rumex* spp., and *Xanthorhiza simplicissima,* have been used to adulterate goldenseal (Brown & Roman, 2008; Pengelly, 2012).

1.2 Analysis

Botanical identity and composition are confirmed by macroscopic and microscopic examinations of rhizome and root, as well as by thin-layer chromatography (TLC) (USP, 2013). TLC identification tests for the major goldenseal alkaloids are reported in the United States Pharmacopeia: a dry extract from roots and rhizomes contains at least 2% hydrastine and 2.5% berberine calculated on a dried basis; a standardized developing solvent system includes ethyl acetate, butyl alcohol, formic acid, and water (5:3:1:1); chromatogram analysis with ultraviolet (UV) light at 365 nm demonstrates a lemon-yellow fluorescence for berberine and a blue-white fluorescence for hydrastine (USP, 2013). Content of the most active alkaloids is determined by high-performance liquid chromatography (HPLC) using a mobile phase composed of 0.1 M monobasic potassium phosphate and acetonitrile (60:40) (USP, 2013). An official HPLC/UV method for hydrastine and berberine in goldenseal has been published (Brown & Roman, 2008), but other HPLC methods have also been developed (Abourashed & Khan, 2001).

Additional analyses report on the use of goldenseal and illicit drugs. Goldenseal may prevent the detection of illicit drugs (such as tetrahydrocanabinol and barbiturates) in urine by inducing their rapid elimination (Mikkelsen & Ash, 1988; Hamon, 1990; Schwarzhoff & Cody, 1993).

1.3 Use

### 1.3.1 Indications and applications

(a) Medicinal use

Native Americans used goldenseal to treat common conditions such as wounds, ulcers, digestive disorders, cancer, and skin and eye ailments (Hamon, 1990; Hobbs, 1990). Over the years, goldenseal has been used to treat a variety of digestive and haemorrhagic disorders. When

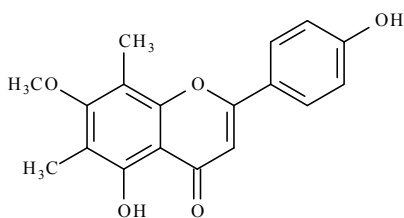**Fig. 1.2 Selected alkaloids and flavonoids from goldenseal**
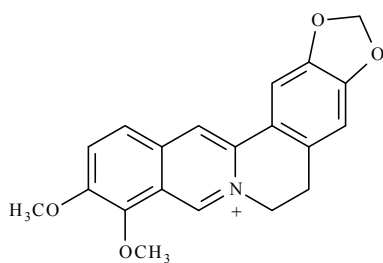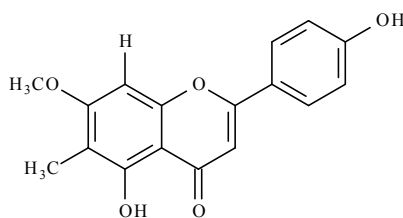

Berberine
$C_{20}H_{18}NO_4$
RMM = 336
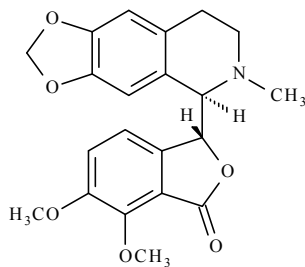
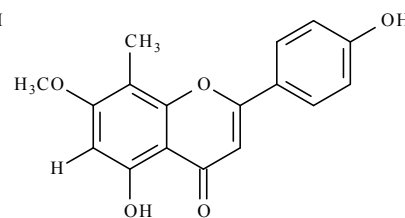Hydrastine
$C_{21}H_{21}NO_6$
RMM = 383
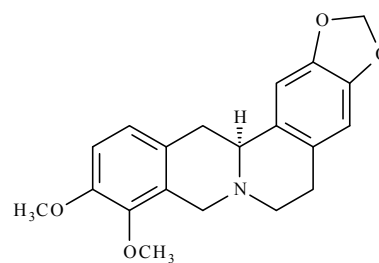
Canadine
$C_{20}H_{21}NO_4$
RMM = 339

Sideroxylin
$C_{18}H_{16}O_5$
RMM = 312.32

8-Desmethyl-sideroxylin
$C_{17}H_{14}O_5$
RMM = 298.29

6-Desmethyl-sideroxylin
$C_{17}H_{14}O_5$
RMM = 298.29

RMM, relative molecular mass
Compiled by the Working Group

applied topically, it is thought to possess slight antiseptic, astringent, and haemostatic qualities (NTP, 2010).

Some OTC dietary supplements containing goldenseal are used as to treat menstrual disorders, minor sciatica, rheumatic and muscular pain (Hamon, 1990), allergy symptoms, cold and flu symptoms, motion sickness and nausea, earaches and ear infections, and chronic diarrhoea from protozoal, fungal, and bacterial infections (Zieger & Tice, 1997; AHP, 2001; Hwang et al., 2003). Goldenseal has also been used in combination with dietary vitamins and minerals in an attempt to treat symptoms of AIDS (Zieger & Tice, 1997). It is also claimed to have the ability to cleanse the body from mucus, toxins, and waste (Zieger & Tice, 1997).

Berberine, a major goldenseal alkaloid, has been used as a bitter tonic, diaphoretic, and antipyretic (Kulkarni et al., 1972), for the treatment of skin diseases, liver diseases, eye infections, diarrhoea, cholera, giardiasis, amoebiasis, and dermal leishmaniasis (Choudhry et al., 1972; Kulkarni et al., 1972; Martin et al., 1978; Sabir et al., 1978; Khin-Maung-U et al., 1985; Vennerstrom et al., 1990; Chi et al., 1994; Müller et al., 1995). Berberine appears to control psoriasis through its ability to inhibit hyperproliferation (Müller et al., 1995). The evidence on the efficacy of berberine in treating peptic ulcers and hyperacidity, and malaria, is conflicting (Sabir et al., 1978; Vennerstrom & Klayman, 1988).

Some other pharmacological properties that have been identified for berberine include antiplatelet, anticerebral ischaemia, vasodilation, and

anti-arrythmia (Peng *et al.*, 1997). Berberine is thought to increase ileal contractility and acetylcholine retention by cholinesterase activity and is believed to be the active ingredient of *Coptis* rhizome, used to treat amnesia (Peng *et al.*, 1997).

A clinical trial using berberine suggested that it is effective in improving cardiac performance in patients with heart failure (Marin-Neto *et al.*, 1988). Berberine appears to exercise a direct depressive effect on myocardial, vascular, and smooth musculature (Sabir & Bhide, 1971; Creasey, 1977, 1979). Berberine may also have anticholinesterolase activity (Sabir & Bhide, 1971). It is also suggested that berberine exerts anticancer activities both in vitro and in vivo through different mechanisms (Sun *et al.*, 2009).

Hydrastine, another major goldenseal alkaloid, is claimed to be an abortifacient, antibiotic, antiuterotic, antivaginitic, bactericide, central nervous system depressant, choleretic, convulsant, haemostat, hypertensive, hypotensive, pesticide, sedative, uterotonic, and vasoconstrictor (NTP, 2010).

(b) Medical research

In medical research, berberine is used as a fluorescent stain for cells, chromosomes, and energized mitochondria (Borodina *et al.*, 1979; Ridler & Jennings, 1983; Mikeš & Dadák, 1983; Mikeš & Yaguzhinskij, 1985; Kim *et al.*, 1990).

1.3.2 Dosage

As a dietary supplement, goldenseal can be given at a wide range of doses: decoction of dried roots, 0.5–10 g three times per day; alcoholic tincture, 2–4 mL three times per day; and fluid extract, 0.3–10 mL three times per day (Newall *et al.*, 1996; Zieger & Tice, 1997; AHP, 2001; McKenna & Plotnikoff, 2010). OTC preparations of goldenseal are available in doses of 100 mg up to 470 mg (Zieger & Tice, 1997; AHP, 2001).

1.4 Production, sales, and consumption

1.4.1 Production

No data on production processes or volumes were available to the Working Group.

1.4.2 Sales

According to data from 2012 IMS Health MIDAS, worldwide sales of goldenseal root (*Hydrastis canadensis*) as a dietary supplement in pharmaceutical outlets totalled US$ 25 million. Appreciable sales occurred in Germany (US$ 8 million), France (US$ 5 million), and the United States (US$ 5 million) (IMS Health, 2012). Other countries known to sell products containing goldenseal include Canada.

According to the 2012 Nutrition Business Journal Report, goldenseal was the 37th best-selling herb in the USA in 2011. Following a decline from US$ 40 million in 2003, sales have remained constant (NBJ, 2012a, b). According to data from the United States National Health and Nutrition Examination Survey, there has been a decline in the prevalence of goldenseal use as follows: 1999–2002 (0.6%), 2003–2006 (0.3%), and 2007–2010 (0.2%), with similar data for males and females. These data showed a large coefficient of variation and caution should be used in interpretation (CDC, 2013).

1.4.3 Consumption

Goldenseal is consumed orally as a tea and in capsules, applied dermally as a skin lotion, applied to the eye as eyewash, to the ear as eardrops, and applied as a vaginal douche (Zieger & Tice, 1997; Hamon, 1990; AHP, 2001; NTP, 2010). Exposure to hydrastine also occurs when used in decongestant nose sprays and feminine hygiene products (Zeiger & Tice, 1997).

1.5 Occupational exposure

No data were available to the Working Group. Workers on goldenseal plantations and in goldenseal processing plants are probably exposed.

1.6 Regulations and guidelines

According to the 1994 Dietary Supplement Health and Education Act (DSHEA) in the USA, goldenseal is considered a dietary supplement under the general umbrella of "foods" (FDA, 1994). In the USA, dietary supplements put on the market before 15 October 1994 do not require proof of safety; however, the labelling recommendations for dietary supplements include warnings, dosage recommendations, and substantiated "structure or function" claims. The product label must declare prominently that the claims have not been evaluated by the Food and Drug Administration, and bear the statement "This product is not intended to diagnose, treat, cure, or prevent any disease" (Croom & Walker, 1995).

The Natural Medicines Comprehensive Database ranks goldenseal as a "possibly safe" dietary supplement when used at a single dose, and at recommended oral dosages for short-term administration (NCCAM, 2012; NMCD, 2013).

In Canada, goldenseal is a Natural Health Product (a form of OTC drug) and requires a product license (pre-market authorization) to be sold (Goldenseal Buccal, 2010). Goldenseal (*H. canadensis*) is also regulated as an endangered species under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2006).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

Goldenseal (*H. canadensis*) root powder was tested for carcinogenicity by oral administration in one study in mice and one study in rats.

3.1 Mouse

In a 2-year study of carcinogenicity, groups of 50 male and 50 female B6C3F$_1$ mice (age, 5–6 weeks) were fed ad libitum with diet containing well-characterized goldenseal root powder (*H. canadensis*) shown to contain all the major alkaloids characteristic of goldenseal (berberine, hydrastine and canadine) at a concentration of 0 (control), 3000, 9000, or 25 000 ppm. Concentrations were equivalent to average daily doses of goldenseal root powder of approximately 0, 375, 1120, or 3275 mg/kg body weight (bw) for males and 0, 330, 1000, or 2875 mg/kg bw for females (NTP, 2010). Survival of the females at 9000 ppm was lower than that of controls. At 105–106 weeks, goldenseal root powder caused a significant positive trend in the incidence of hepatoblastoma, and of hepatocellular adenoma, in males. There were no significant increases in the incidence of tumours in female mice. The incidence of liver eosinophilic foci was significantly increased in males at the intermediate and highest doses, and in all exposed females.

3.2 Rat

In a 2-year study of carcinogenicity, groups of 50 male and 50 female F344/N rats (age, 5–6 weeks) were fed ad libitum with diet containing a well-characterized goldenseal root powder (*H. canadensis*) shown to contain all the major alkaloids (berberine, hydrastine and canadine) characteristic of goldenseal at a concentration of 0 (control), 3000, 9000, or 25 000 ppm. Concentrations were equivalent to average daily

Table 3.1 Studies of carcinogenicity with goldenseal root powder in mice and rats

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 105–106 wk NTP (2010)	Goldenseal root powder (*H. canadensis*): 0 (control), 3000, 9000, or 25 000 ppm in feed, corresponding to 0, 375, 1120, or 3275 mg/kg bw (M); 0, 330, 1000, or 2875 mg/kg bw (F) 50 M and 50 F/group (age, 5–6-wk)	Hepatoblastoma: 1/50 (2%)*, 2/50 (4%), 1/50 (25), 6/50 (12%) (M)[a] Hepatocellular adenoma: 22/50 (44%)**, 16/50 (32%), 23/50 (46%), 29/50 (58%) (M) 3/50 (6%), 6/50 (12%), 7/50 (14%), 7/50 (14%) (F) Hepatocellular carcinoma: 8/50 (16%), 14/50 (28%), 15/50 (30%), 12/50 (24%) (M)	*P = 0.030 (trend test) **P = 0.016	Major alkaloids content: berberine, 3.89%; hydrastine, 2.8%; and canadine, 0.17%
Rat, F344/N (M, F) 105–106 wk NTP (2010)	Goldenseal root powder (*H. canadensis*): 0 (control), 3000, 9000, or 25 000 ppm in feed: 0, 135, 400, or 1175 mg/kg bw (M); 0, 150, 470, 1340 mg/kg bw (F) 50 M and 50 F/group (age, 5–6 wk)	Hepatocellular adenoma: 1/50 (2%)*, 1/50 (2%), 2/50 (4%), 10/50 (20%)** (M) 0/50 (0%)*, 0/50 (05), 1/50 (2%), 8/50 (16%)** (F)[b] Hepatocellular carcinoma: 0/50 (0%), 0/50 (0%), 0/50 (0%), 1/50 (2%) (M)[c] Hepatocellular adenoma or carcinoma (combined): 1/50 (2%)*, 1/50 (2%), 2/50 (4%), 11/50 (22%)*** (M)[d]	*P < 0.001 (trend test) **P ≤ 0.004 ***P < 0.001	Major alkaloids content: berberine, 3.89%; hydrastine, 2.8%; and canadine, 0.17%

[a] Historical incidence of hepatoblastoma (includes multiple) (mean ± standard deviation) for feed studies in untreated control male mice: 1/250 (0.4% ± 0.9%); range, 0–2%; all routes: 48/1447 (3.3% ± 6.4%); range, 0–34%.

[b] Historical incidence of hepatocellular adenoma (mean ± standard deviation) for feed studies in untreated control female rats: 4/250 (1.6% ± 2.2%); range, 0–4%; all routes: 16/1350 (1.2% ± 2.6%); range, 0–12%

[c] Historical incidence of hepatocellular carcinoma in untreated control male rats: 1/1250 (all routes)

[d] Historical incidence of hepatocellular adenoma or carcinoma (mean ± standard deviation) for feed studies in untreated control male rats: 7/300 (2.3% ± 2.3%); range, 0–6%; all routes: 22/1399 (1.6% ± 1.7%); range, 0–6%

bw, body weight; F, female; M, male; wk, week

doses of goldenseal root powder of approximately 0, 135, 400, or 1175 mg/kg bw to males and 0, 150, 470, or 1340 mg/kg bw for females (NTP, 2010). Survival of the females at 9000 ppm was greater than that of controls. At 105–106 weeks, goldenseal root powder caused increased incidences of hepatocellular adenoma in males and females at the highest dose. One male rat at the highest dose also developed a rare hepatocellular carcinoma. There was a treatment-related statistically significant increase in the incidence of liver eosinophilic foci in male and female rats. [The Working Group noted that hepatocellular tumours are uncommon tumours in F344/N rats and that evidence from studies reported in the literature showed that hepatocellular adenomas may progress to malignant tumours in F344/N rats.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Goldenseal contains berberine and hydrastine (see Section 1). Most data on the carcinogenicity of goldenseal came from studies with berberine. In 11 healthy subjects treated orally with a single dose of goldenseal (2.7 g) containing 78 mg of hydrastine and 132 mg of berberine, both berberine and hydrastine were absorbed from the gastrointestinal tract, and their phase I and II metabolites were rapidly detected in the plasma and urine (Gupta *et al.*, 2010). The maximal plasma concentration (C_{max}) of hydrastine was 225 ± 100 ng/mL, the time to C_{max} (T_{max}) was 1.5 ± 0.3 hours, and the area under the curve (AUC) was 6.4 ± 4.1 ng•h/mL•kg. The elimination half-life ($t_{1/2\beta}$) of hydrastine was 4.8 ± 1.4 hours. Corresponding values for

berberine were 1.1 ± 1.2 ng/mL, 3.0 ± 3.3 hours and 0.15 ± 0.09 ng•h/mL•kg, respectively. Phase I metabolites of hydrastine underwent extensive glucuronidation, but not sulfation, while the phase I metabolites of berberine were primarily sulfated (Gupta *et al.*, 2010). The area under the curve of hydrastine in plasma is significantly higher than that of berberine, suggesting that the oral bioavailability of hydrastine is also higher. There was enterohepatic recycling of berberine, which also had a high volume of distribution (Gupta *et al.*, 2010).

In humans given berberine orally, plasma concentrations of berberine were very low and variable (Li *et al.*, 2000a; Hua *et al.*, 2007). For example, the plasma C_{max} of berberine was only 0.4 ng/mL after a single oral dose of 400 mg of berberine (Hua *et al.*, 2007). This suggested that berberine is poorly absorbed by the gastrointestinal wall. [The Working Group noted the discrepancy between pharmacokinetic parameters for purified berberine compared with those for goldenseal.]

The metabolites of berberine are mainly 1,3-dioxolane ring-opened, demethylated, demethylenated, glycosylated, and sulfonated products. Several clinical studies have been performed to identify these metabolites in the plasma and urine (Pan *et al.*, 2002; Qiu *et al.*, 2008).

In one study, five healthy male volunteers (aged 21–28 years) were given berberine chloride orally (900 mg/day; 100 mg tablets) for 3 days (Pan *et al.*, 2002). Urine samples were collected and the metabolites isolated and purified by polyporous resin column chromatography. Three metabolites were recovered and identified via electrospray ionization mass spectroscopy (ESI-MS) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy: jatrorrhizine-3-sulfate, demethylene berberine-2-sulfate, and thalifendine-10-sulfate. The amounts of these purified metabolites in the urine were 250, 17, and 2 mg, respectively (Pan *et al.*, 2002).

In a later study with 12 healthy male volunteers (aged 22–26 years; body weight, 60–80 kg), additional metabolites were discovered (Qiu *et al.*, 2008). In this study, subjects were given an oral dose of berberine chloride of 300 mg, three times per day for 2 days, and urine samples were collected in the 72 hours after dosing. Using nuclear magnetic resonance spectroscopy in addition to liquid chromatography–mass spectrometry, the study identified the above three conjugated metabolites, plus previously unseen conjugates: demethyleneberberine-2-*O*-sulfate, jatrorrhizine-3-*O*-β-D-glucuronide, thalifendine-10-*O*-β-D-glucuronide, berberrubine-9-*O*-β-D-glucuronide, jatrorrhizine-3-*O*-sulfate, 3-10-demethylpalmatine-10-*O*-sulfate, and columbamin-2-*O*-β-D-glucuronide (40 mg, 6 mg, 4 mg, 6.1 mg, 2.2 mg, 1.2 mg, and 1 mg, per 16 L of urine, respectively) (Qiu *et al.*, 2008). In this study, both sulfates and glucuronides of berberine were observed.

Using the human intestinal Caco-2 model, Zhang *et al.* (2011) examined the intestinal absorption mechanisms of berberine. They found that the cellular uptake of berberine was 30.13 ± 0.57 µmol/g protein, at a substrate concentration of 10 µM, and that the apparent permeability (P_{app}) of berberine was 0.66 ± 0.04 (absorptive direction) and 15.91 ± 0.61 (secretory direction) × 10^{-6} cm/s, with an efflux ratio of 24.28 (Zhang *et al.*, 2011). Transport inhibition studies using cyclosporin A [ciclosporin] and verapamil [both potent inhibitors of P-glycoprotein 1 (P-gp/multidrug resistance protein 1)] indicated that berberine was a substrate of this transporter.

4.1.2 Experimental systems

The absorption of berberine in rats is very poor; in rats given berberine at an oral dose of 100 mg/kg bw, the plasma C_{max} of berberine was estimated to be only 4.0 ng/mL (Liu *et al.*, 2009, 2010).

The metabolism of berberine in vitro and in vivo has been well studied in rats (Qiu *et al.*, 2008; Liu *et al.*, 2009, 2010). In rat liver microsomes, or following intravenous administration, berberine was metabolized by several cytochromes P450 (CYPs) and UDP-glucuronosyltransferases. Oxidative demethylenation was the major metabolic pathway and the metabolite obtained can subsequently undergo glucuronidation (Liu *et al.*, 2009). In one study in vivo, male Wistar rats (age, 8–10 weeks) were given berberine at a dose of 100 mg/kg bw, and urine samples were collected for 48 hours; the five urinary metabolites of berberine isolated and identified included berberrubine-9-*O*-β-D-glucuronide, demethyleneberberine-2,3-di-*O*-β-D-glucuronide, demethyleneberberine-2-*O*-sulfate, 3,10-demethylpalmatine-10-*O*-sulfate, and thalifendine (Qiu *et al.*, 2008).

Berberine was found to undergo extensive first-pass metabolism in the rat intestine. After intragastric dosing, approximately half of the intact berberine passed through the gastrointestinal tract and another half was biotransformed by the small intestine, resulting in an extremely low absolute oral bioavailability in rats (0.36%) (Liu *et al.*, 2010).

[The above findings indicate that berberine is poorly absorbed in both rats and humans due to extensive first-pass disposition. Berberine is extensively metabolized by intestinal and hepatic phase I and II enzymes in rats and humans.]

4.1.3 Alterations in enzymes and metabolic capacity

Goldenseal strongly inhibits CYP2C9, CYP3A4, CYP2D6, and CYP2C19 in vitro (Budzinski *et al.*, 2000; Chatterjee & Franklin, 2003; Foster *et al.*, 2003). In one experiment, Etheridge *et al.* (2007) examined the effects of goldenseal on the activities of human CYPs in human liver microsomes, finding that goldenseal inhibited CYP2C8, CYP2D6, and CYP3A4 by ≥ 50% (Etheridge *et al.*, 2007).

In humans, goldenseal did not significantly affect the metabolism and pharmacokinetic of indinavir, a protease inhibitor, which is a substrate of CYP3A4, (Sandhu *et al.*, 2003). However, goldenseal has been shown to increase the plasma concentration of cyclosporine [ciclosporin] in healthy volunteers (Xin *et al.*, 2006) and in renal transplant patients (Wu *et al.*, 2005). In another study, single time-point phenotypic metabolic ratios of CYP probe drugs were administered to 12 healthy subjects (6 males and 6 females, all extensive metabolizers of CYP2D6) to determine whether a 28-day supplementation of goldenseal root extract (900 mg, three times per day, 2.70 g/day; no standardization claim) affected the activity of CYP1A2, CYP2D6, CYP2E1, or CYP3A4/5. The following probe cocktails were administered before and after supplementation: midazolam (CYP3A4) and caffeine (CYP1A2), followed 24 hours later by chlorzoxazone (CYP2E1) and debrisoquine (CYP2D6). The phenotypic traits of CYP3A4, CYP1A2, CYP2E1, and CYP2D6 activities were assessed using the 1-hydroxymidazolam/midazolam serum ratio at 1 hour, the paraxanthine/caffeine serum ratio at 6 hours, the 6-hydroxychlorzoxazone/chlorzoxazone serum ratio at 2 hours, and debrisoquine urinary recovery ratio after an 8-hour collection. Phenotypic ratios, taken before and after supplementation, show remarkable inhibition of CYP2D6 (~40%) and CYP3A4/5 (~40%) activities due to goldenseal supplementation. CYP1A2 and CYP2E1 activities were virtually unaffected (Gurley *et al.*, 2005). For CYP3A4/5, this clinical observation was confirmed by a more comprehensive study in which goldenseal caused a mean increase in midazolam $AUC_{0-\infty}$ and C_{max} by about 63% and 40%, respectively, while the $AUC_{0-\infty}$ and C_{max} of clarithromycin (an inhibitor of CYP3A4) increased about 5.5-fold and 2-fold, respectively (Gurley *et al.*, 2008a). However, goldenseal root extract is only a moderate inhibitor of CYP2D6 in humans (Gurley *et al.*, 2008b).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

See also Table 4.1.

(a) Mutagenicity

Goldenseal root powder (1000–10000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98 or TA100, or *Escherichia coli* strain WP2 *uvrA*/pKM101, with or without metabolic activation from rat liver S9 (NTP, 2010). Berberine was also not mutagenic in *S. typhimurium* strains TA97, TA98, TA100 and TA1535, with or without metabolic activation from rat or hamster liver S9. In three tests in TA98, berberine gave negative results in two tests and equivocal results in one.

(b) Chromosomal damage

No increase in the frequency of micronucleated normochromatic erythrocytes or polychromatic erythrocytes was observed in blood from male or female mice exposed to diets containing goldenseal root powder at up to 50 000 ppm for 3 months. No increases in the frequency of micronucleated polychromatic erythrocytes were observed in bone marrow from male B6C3F₁ mice treated with berberine chloride at a dose of up to 658 mg/kg bw via three intraperitoneal injections at intervals of 24 hours (NTP, 2010).

(c) Interaction with DNA

Data on the interaction of some goldenseal constituents with DNA are presented below (see Section 4.3.2).

Table 4.1 Genetic and related effects of goldenseal root and berberine

Test system	Results		Concentration or dose (LED or HID)	Reference
	Without exogenous metabolic system	**With exogenous metabolic system**		
Goldenseal root				
Salmonella typhimurium TA100, TA98 reverse mutation	–	–	10 000 µg/plate	NTP (2010)
Escherichia coli WP2 *uvrA*/pKM101 reverse mutation	–	–	10 000 µg/plate	NTP (2010)
Micronucleus formation in peripheral blood erythrocytes of male and female B6C3F$_1$ mice in vivo	–	NT	50 000 ppm [50 g/kg] in diet for 3 months	NTP (2010)
Berberine				
Salmonella typhimurium TA100, TA98, TA97, TA1535 reverse mutation	(–)[a]	–	1000 µg/plate	NTP (2010)
Inhibition of DNA repair activity, topoisomerase II inhibition, plasmid pUL402DNA	(+)[b]	NT	2.5 µM [0.9 µg/mL]	Kobayashi *et al.* (1995)
Inhibition of DNA repair activity, topoisomerase II inhibition,plasmid pBS DNA	(+)[b]	NT	50 µM [18 µg/mL]	Kim *et al.* (1998)
Micronucleus formation in bone-marrow polychromatic erythrocytes of male B6C3F$_1$ mice in vivo	–	NT	658 mg/kg bw, i.p. × 3	NTP (2010)

[a] One out of three tests in TA98 gave equivocal results.

[b] The test gave positive results with berberrubine, but weakly positive results with berberine.

+, positive; (+), weakly positive; –, negative; ?, inconclusive; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; NR, not reported; NT, not tested

4.3 Other mechanistic data relevant to carcinogenicity

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

Berberine alkaloids found in goldenseal root powder inhibited topoisomerases I (Topo I) and II (Topo II) (Krishnan & Bastow, 2000; Li et al., 2000b; Kettmann et al., 2004). The ability of berberine to disrupt Topo I and Topo II is related to its antitumour activity (Mazzini et al., 2003). While the role of drug–DNA interactions in the inhibition of Topo I is unclear, the binding of berberine to DNA has been considered as the cause for inhibition.

It has been shown by nuclear magnetic resonance spectroscopy that berberine binds selectively to AT-rich sequences, interacting with several oligonucleotides (Mazzini et al., 2003). Using a two-dimensional nuclear Overhauser effect assay, several contacts were detected between protons of the self-complementary oligomer d(AAGAATTCTT)$_2$ and the protons of berberine. Berberine was found on the convex side of the helix groove, the minor groove of the nucleotide (at the level of the A$_4$–T$_7$ and A$_5$–T$_6$ base pairs), presenting its positive nitrogen atom to the negative ionic surface of the oligonucleotide. The aromatic protons H-11 and H-12 are close to the ribose of cytidine C$_8$, while the methylenedioxy and ring A group are more external to the helix (Mazzini et al., 2003).

Electrospray ionization-mass spectrometry (ESI-MS) has been used to study noncovalent complexes of protoberberine alkaloids, berberine, palmatine, jatrorrhizine, and coptisine with double-strand DNA oligonucleotides, showing that berberine has the lowest binding affinity and palmatine the highest (Chen et al., 2004). These preliminary data suggested that berberine

exhibited some sequence selectivities. Additional studies using ESI-MS to examine noncovalent complexes have produced new information (Chen et al., 2005a, b). In one study, berberine exhibited different binding affinities to different nucleotides (Chen et al., 2005b); however, ESI-MS and fluorescence titration experiments with these four alkaloids indicated that sequence selectivity was not significant and no specific AT- or GC-rich DNA binding preferences were observed, in contrast to the aforementioned reports (Mazzini et al., 2003; Chen et al., 2004).

Pang et al. (2005) suggested that berberine and its derivatives, especially those with a primary amino group, are able to bind strongly with calf-thymus DNA via an intercalation mechanism. Substitution at the C-9 position is an important determinant of the biological activity, as Park et al. (2004) demonstrated in these studies on the structure–activity relationships of the berberine analogues (Park et al., 2004).

When berberine units are bridged at the C-9 position with different linker lengths (termed "bridged berberine derivatives"), they exhibit the highest binding affinity to DNA when they form a compound berberine dimer with a propyl chain (Chen et al., 2005a; Qin et al., 2006). DNA and RNA triplexes can be bound and stabilized better than their respective parent duplexes when they bind with the sanguinarine and berberine alkaloids (Das et al., 2003).

4.4 Susceptibility

No data were available to the Working Group.

4.5 Mechanistic considerations

Goldenseal root powder gave negative results in several standard bacterial assays for mutation in the absence or presence of metabolic activation systems. The principal alkaloid in goldenseal root powder, berberine, also gave negative results

in many of these assays. Goldenseal root powder gave negative results in the test for mouse peripheral blood micronucleated erythrocytes (normochromatic and polychromatic) after 3 months of dietary exposure. Berberine also gave negative results for induction of micronuclei in mouse bone-marrow polychromatic erythrocytes after three doses.

Goldenseal produced mostly negative results in assays for bacterial mutation (NTP, 2010). However, berberine, its metabolite, berberrubine, and several protoberberines have been shown to inhibit the activity of topoisomerases (Kobayashi *et al.*, 1995; Makhey *et al.*, 1995; Kim *et al.*, 1998; Li *et al.*, 2000b; Krishnan & Bastow, 2000). Etoposide, an inhibitor of topoisomerase II, was classified by the IARC *Monographs* as *carcinogenic to humans* (Group 1) (IARC, 2012).

5. Summary of Data Reported

5.1 Exposure data

Goldenseal (*Hydrastis canadensis* L.) is an endangered plant that is widely consumed in several countries. The root has been used traditionally in herbal medicine. Reported uses include the treatment of skin disorders, digestive disorders, anorexia, menstrual disorders, and mucosal inflammations. Currently, the main applications for this plant include the prevention and reduction of inflammation and related diseases. Goldenseal is available in the form of tea, capsules containing the crude drug or the extract, skin lotion, eyewash, and eardrops. In 2011, goldenseal ranked 37th among top-selling dietary supplements in the USA. Other countries that reported significant sales of goldenseal included Canada, France, and Germany.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

A well-characterized goldenseal root powder shown to contain all the major alkaloids characteristic of goldenseal was tested for carcinogenicity by oral administration in one study in mice and one study in rats.

In male mice fed a diet containing goldenseal root powder, there was a significant positive trend in the incidence of hepatoblastoma and of hepatocellular adenoma. There were no significant increases in the incidence of tumours in female mice.

In male and female rats fed a diet containing goldenseal root powder, there was an increased incidence of hepatocellular adenoma, which in F344/N rats is an uncommon tumour that is known to progress to malignancy. In addition, one rare hepatocellular carcinoma was observed in the group of males given the highest dose.

5.4 Mechanistic and other relevant data

The major alkaloid components of goldenseal, berberine and hydrastine, are absorbed from the gastrointestinal tract into the circulation and extensively metabolized in the liver after oral administration of goldenseal.

Goldenseal root powder gave negative results in several standard bacterial assays for mutation in the absence or presence of exogenous metabolic activation systems. Berberine also gave negative results in many of these assays. Likewise, goldenseal root powder gave negative results in the mouse peripheral blood micronucleated erythrocyte test (normochromatic and polychromatic erythrocytes) after dietary exposure. Berberine also gave negative results for the induction of

micronuclei in mouse bone-marrow polychromatic erythrocytes.

Berberine and its metabolite, berberrubine, have been shown to inhibit DNA topoisomerases. Such inhibition may account for the carcinogenicity of goldenseal in experimental animals.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of goldenseal.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of goldenseal root powder.

6.3 Overall evaluation

Goldenseal root powder is *possibly carcinogenic to humans (Group 2B)*.

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