Comfrey
[72698-57-8]

and One of Its Constituent Alkaloids

Symphytine
[22571-95-5]

Review of Toxicological Literature

Prepared for

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EXECUTIVE SUMMARY

The nomination of comfrey and symphytine by the NIEHS for testing is based on the potential for chronic human exposure and the limited amount of carcinogenicity data.

Comfrey is a member of the plant family Boraginaceae. The major constituent of the comfrey plant is mucilage; other constituents include allantoin, polyphenols, amino acids, phytosterols, triterpenoids, saccharides, and pyrrolizidine alkaloids. The major unsaturated pyrrolizidine alkaloids in comfrey are lycopsamine, intermedine, 7-acetyllycopsamine, 7-acetylintermedine, and symphytine. One kilogram of comfrey contains 1.7-2.5 grams of symphytine.

Comfrey is commercially available as a root or leaf, and in capsule form. In 1993, it was reported that some suppliers had removed comfrey from the U.S. market in response to adverse toxicological findings. Celestial Seasonings, a major producer of herbal teas, removed comfrey from its product line in 1981.

Symphytine can be extracted from the dried roots of comfrey using methanol. Allantoin is removed from the resulting crude extract and the residue can then be purified by reduction of the *N*-oxide with zinc dust and hydrochloric acid, followed by column chromatography.

Production and import volumes were not available for comfrey; symphytine is not produced commercially.

Comfrey has become popular as a vegetable in salads, as an herbal tea, and as an additive in specialty soft drinks. There is also one report of its use as an ingredient in cereal. Comfrey has been used as an analgesic, antidiarrhetic, astringent, cicatrizant (wound healer), demulcent (anti-irritant), diuretic, expectorant, sedative, stimulant, sudorific (sweat enhancer), and hemostat (anti-hemorrhagic), and to treat cancer, circulation problems, hemoptysis (bronchial or pulmonary hemorrhage), inflammation, swelling, lung conditions, and sores. Comfrey has also been used in skin creams and hair products; claims have been made that it is able to rejuvenate skin. No information was available on the intentional use of symphytine.

Exposure to comfrey occurs orally via ingestion of teas or capsules, or dermally following application of poultices or cosmetics containing comfrey. There are no reports of intentional exposure to or consumption of symphytine. In 1993, the American Herbal Products Association recommended that comfrey only be used externally. No U.S. regulations were found for comfrey or symphytine. However, a number of other countries, including Canada, Brazil, Australia, and the United Kingdom, have severely restricted or banned the use of comfrey.

Although there was no evidence for liver damage in a group of people who regularly consumed comfrey, there are a number of case reports that implicate consumption of comfrey in the development of liver diseases, predominantly hepatic veno-occlusive disease.

The concern over the health effects of comfrey is based on the toxic pyrrolizidine alkaloid constituents. Unsaturated pyrrolizidine alkaloids are metabolically activated to toxic compounds in the liver by mixed function oxidases. In experimental animal studies, pyrrolizidine alkaloids are almost completely excreted within 24 hours. Sprague-Dawley rats treated orally by gavage or dermally with reduced crude comfrey alkaloids or unreduced crude alkaloid *N*-oxides of comfrey extract excreted a number of pyrrolizidine alkaloids in urine collected over the first 24 hours; the urine of rats treated dermally with the reduced alkaloid extract contained about 20 times less total alkaloids than did urine from rats dosed by gavage.

No acute exposure data on comfrey were located. In mice, the intraperitoneal (i.p.) LD_{50} for symphytine is 300 mg/kg (0.786 mmol/kg); in rats the i.p. LD_{50} is 130 mg/kg (0.341 mmol/kg).

Rats fed a 10 or 30% comfrey diet for 21 days exhibited an increase in hepatic aminopyrine *N*-demethylase activity, but had no change in hepatic glutathione *S*-transferase or epoxide hydrolase activities.

The incidence of hepatocellular adenoma was increased in rats fed comfrey leaves in the diet daily for 480-600 days or comfrey roots in the diet daily for 245 or 280 days, or until death. The incidence of liver tumors were also increased in rats injected i.p. with symphytine once or twice per week for 56 weeks.

Comfrey did not induce X-linked recessive lethal mutations in *Drosophila melanogaster*, and an acetone extract of comfrey was not mutagenic in *Salmonella typhimurium* strains TA98 and TA100 with or without metabolic activation. However, incubation of human lymphocytes with an extract of comfrey induced an increase in sister chromatid exchanges in both the absence and presence of metabolic activation. Symphytine induced gene mutations in *S. typhimurium* strain TA100 with metabolic activation and in V79 Chinese hamster cells (metabolic activation condition not provided) and was positive for the induction of somatic mutations or recombination in the *D. melanogaster* wing spot test. Symphytine did not induce morphological transformation in Syrian hamster embryo cells. Extracts of comfrey suppressed the mutagenic activity of benzo[*a*]pyrene and heated extracts suppressed the mutagenic activity of 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-ß]indole in *S. typhimurium* (metabolic activation condition not provided), while a crude comfrey extract suppressed the genotoxic activity of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine in *Bacillus subtilis*.

An ethanol extract of comfrey roots strongly inhibited both the classical and alternative pathways of complement *in vitro*. An aqueous extract of comfrey root and 2 of its fractions inhibited the *in vitro* proliferation of human peripheral blood lymphocytes stimulated with phytohemagglutinin. The same comfrey extract and one of its fractions enhanced the generation and release of O_2^- by unstimulated and stimulated granulocytes and increased the total respiratory burst of unstimulated granulocytes, but had an inhibitory effect on the respiratory burst of stimulated granulocytes.

An aqueous extract of comfrey and 2 of its fractions had no effect on the *in vitro* proliferation of Ehrlich tumor cells. In contrast, in mice inoculated i.p. with EL-4 or Ehrlich ascites cells, the *in vivo* proliferation of the tumor cells was stimulated by an aqueous extract of comfrey and by one of its fractions, but was inhibited by another fraction. Symphytine exhibited cytotoxic activity towards Ehrlich ascites carcinoma *in vitro*.

An unsaturated pyrrolizidine ring is necessary for the pyrrolizidine alkaloids to exert toxic effects. The hepatotoxic pyrrolizidine alkaloids have a 1,2-double bond in the pyrrolizidine ring and the primary OH-group esterified with a branched alkyl-acid. The genotoxicity of 16 pyrrolizidine alkaloids were evaluated in *D. melanogaster* using the wing spot test. In general, macrocyclic diester-type pyrrolizidine alkaloids were the most genotoxic, 7-hydroxy C9-monoester types were the least genotoxic, and open diesters were intermediate in genotoxic activity. Stereoisomeric pyrrolizidine alkaloids sometimes exhibited similar mutagenic activity. It was also noted that as the number of hydroxy groups increased, the genotoxicity of the pyrrolizidine alkaloids decreased.

No data was found on the teratogenicity or embryotoxicity of comfrey or symphytine.

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TABLES

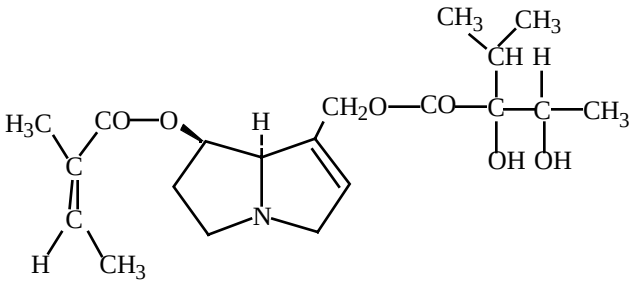
1.0 BASIS FOR NOMINATION

The nomination of comfrey and symphytine by the NIEHS for testing is based on the potential for human exposure and the limited amount of carcinogenicity data.

2.0 PROPERTIES

Comfrey (*Symphytum officinale*)
[72698-57-8]

Symphytine
[22571-95-5]



2.1 Chemical Identification

Symphytine ($C_{20}H_{31}NO_6$, mol. wt. = 381.52) is also called:

Butenoic acid, 2-methyl-, 7-((2,3-dihydroxy-2-(1-methylethyl)-1-oxobutoxy)methyl)-2,3,5,7a-tetrahydro-1*H*-pyrrolizin-1-yl ester

7-Tiglylretronecine viridiflorate

7-Tiglyl-9-viridiflorylretronecine

2.2 Physical-Chemical Properties

2.2.1 Comfrey

Comfrey is a member of the plant family Boraginaceae (Brauchli et al., 1982). It has broad, rough-hairy, lanceolate leaves that flow into a winged stem. Its flowers are bell-like, yellow, whitish, or pinkish fading to bluish, and occur in a 1-sided curled cluster accompanied by a pair of winglike leaves. Comfrey grows to a height of 2-3 feet (Peterson and McKenny, 1968).

As reported by Winship (1991), the major constituent of the comfrey plant is mucilage. Allantoin has been detected at a concentration of 0.8% in roots and 0.4% in leaves. Other major constituents include polyphenols, amino acids, phytosterols, triterpenoids, saccharides, and pyrrolizidine alkaloids .

Comfrey roots and leaves may contain as many as 9 pyrrolizidine alkaloids (Snider, 1991). The major unsaturated pyrrolizidine alkaloids in comfrey are the monoesters lycopsamine and intermedine, their acetyl derivatives, 7-acetyllycopsamine and 7-acetylintermedine, and symphytine (Vollmer et al., 1987; Stengl et al., 1982).

The concentrations of pyrrolizidine alkaloids in comfrey plants varies considerably (Awang, 1987); young leaves generally have much higher levels than old leaves, and roots tend to have much higher levels than leaves. Roitman (1981) noted that dry leaves may contain from 0.003 to 0.2% pyrrolizidine alkaloids, whereas dry roots may contain from 0.2 to 0.4%. Man'ko et al. (1970) reported that the pyrrolizidine alkaloid content was highest in above-ground parts during flowering (0.06%), and in roots at the end of fruit formation (0.31%).

2.2.2 Symphytine

Symphytine is a pyrrolizidine alkaloid (Furuya and Araki, 1968).

Property	Information	Reference
Color	almost colorless	Furuya and Araki (1968)
Physical State	oil	Furuya and Araki (1968)
Optical Rotation	$[\alpha]_D^{24}$ + 3.65° (c = 4.28 in ethanol)	Furuya and Araki (1968)
Stability	readily hydrolyzed by alkali; sensitive to oxidation	IARC (1983)

2.3 Commercial Availability

Comfrey is commercially available as a root or leaf, and in capsule form in health food stores (Tyler, 1987; Mossoba et al., 1994). It was reported in Food Chemical News (Anonymous, 1993a) that some suppliers had removed comfrey from the U.S. market in response to adverse toxicological findings. No other details were given. Snider (1991) reported that Celestial Seasonings, a major producer of herbal teas, removed comfrey from its product line in 1981.

Information on the commercial production of symphytine was not located; IARC stated in 1983 that it was not produced commercially (IARC, 1983).

3.0 PRODUCTION PROCESSES

Symphytine can be extracted from the dried roots of comfrey using methanol (Furuya and Araki, 1968). A crude extract is produced, from which allantoin is removed. The resulting residue can then be purified by reduction of the *N*-oxide with zinc dust and hydrochloric acid, followed by column chromatography.

4.0 PRODUCTION AND IMPORT VOLUMES

Production and import volumes were not located for comfrey.

5.0 USES

Comfrey has been used for centuries as a wound healer and cough suppressant. In the Middle Ages, it was commonly used as a poultice for treating broken bones. Native North American herbalists have used comfrey to treat abscesses, enlarged glands, eye pain, hernias, and

ammenorrhea. Historically, many other therapeutic uses of comfrey have been reported (Awang, 1987). In recent times, comfrey has become popular as a vegetable in salads (Awang, 1987; Mossoba et al., 1994), as an herbal tea (Awang, 1987; Anonymous, 1990a,b; Mossoba et al., 1994), and as an additive in specialty soft drinks (Scase, 1990). There is also one report of its use as an ingredient in cereal (Anonymous, 1990c).

Comfrey is currently used in the United States. The Agricultural Research Service database lists comfrey as an ethnobotanical analgesic, antidiarrhetic, astringent, cicatrizant (wound healer), demulcent (anti-irritant), diuretic, expectorant, sedative, stimulant, sudorific (sweat enhancer), and hemostat (anti-hemorrhagic), and to treat cancer, circulation problems, hemoptysis (bronchial or pulmonary hemorrhage), inflammation, swelling, lung conditions (not specified), and sores (Beckstrom-Sternberg and Duke, 1997).

Comfrey is also used in a number of skin creams and hair products (Awang, 1987; Lettich, 1990; Anonymous, 1993b, 1995, 1997). Claims have been made that comfrey is able to rejuvenate skin (Awang, 1987).

No information was located on the intentional use of symphytine.

6.0 ENVIRONMENTAL OCCURRENCE

Comfrey is a naturally occurring herb native to Europe and temperate Asia and is common throughout England (Grieve, 1995) Although its North American taxonomy is uncertain, it was noted that comfrey has been naturalized in North America (Awang, 1987). Comfrey is generally found in wet areas (Awang, 1987) and along roadsides and in ditches (Peterson and McKenny, 1968).

Symphytine is a constituent of comfrey (*Symphytum officinale*) (Furuya and Araki, 1968), Russian comfrey (*Symphytum* × *uplandicum* Nyman) (Culvenor et al., 1980), and the true forget-me-not (*Myosotis scorpiodes*) (Resch et al., 1982). One kilogram of comfrey contains 1.7-2.5 grams of symphytine (Tittel et al., 1979; cited by IARC, 1983). The roots of comfrey contain more symphytine than the leaves (Winship, 1991).

7.0 HUMAN EXPOSURE

Exposure to comfrey occurs orally, via ingestion of teas or capsules, or dermally via the application of comfrey-containing poultices or cosmetics. The concern over the health effects of

comfrey is based on the presence of toxic pyrrolizidine alkaloids as constituents (D'Arcy, 1991). Oral exposure to comfrey has been associated with toxic effects in the liver, while dermal exposure is believed to pose little or no hepatotoxicity because of limited absorption (Awang, 1987) (see **Section 9.1.2**).

Huxtable et al. (1986) estimated that a person who consumes two comfrey-pepsin capsules per meal for 6 months would ingest a total of 162 mg pyrrolizidine alkaloids from a preparation made from comfrey leaves and 1740 mg alkaloids from a preparation made from comfrey roots. Bach et al. (1989) reported that comfrey-pepsin capsules may contain as much as 2.9 mg total pyrrolizidines per gram.

Roitman (1981) detected 8.5 mg total pyrrolizidine alkaloids in one cup of comfrey root tea with the gelatinous residue removed. Inclusion of the residue resulted in a total pyrrolizidine alkaloid content of 26 mg/cup. Bach et al. (1989) reported that comfrey root tea contains as much as 26 mg total pyrrolizidine alkaloids per cup. Corrigan (1987), however, reported that some commercial comfrey products (not specified) contained no detectable pyrrolzidine alkaloids.

There are no reports of intentional consumption of symphytine, but the compound is a component of a number of herbs. Ames et al. (1988) estimated that a person who consumes 1 cup of comfrey herb tea is exposed to 38 µg symphytine.

8.0 REGULATORY STATUS

A number of FDA regulatory letters have been written to manufacturers of products containing comfrey (FDA, 1984, 1991a, b, 1994, 1995). The letters contain a warning that the products are improperly labeled.

In 1993, the American Herbal Products Association issued a press release recommending that comfrey only be used externally (Anonymous, 1993c).

No U.S. regulations were found for comfrey or symphytine. However, a number of other countries have regulated the use of comfrey. In 1989, Canada banned the use of comfrey in herbal medicinal products intended for ingestion (Snider, 1991). Brazil has prohibited the use of comfrey as an oral pharmaceutical, but permits its use in topical preparations (Anonymous, 1992). Australia has banned the use of comfrey and the United Kingdom has restricted the use of comfrey roots (Burrough, 1997), withdrawing its medicinal license (Anonymous, 1993d). In 1993, a report noted that products containing comfrey extracts were withdrawn voluntarily from the British

market (Anonymous, 1993e). The withdrawal included tablets and capsules containing comfrey, but did not include comfrey tea, tinctures, or topical preparations (Anonymous, 1993f). In 1994, a warning from the U.K. Veterinary Products Committee was issued, stating that products containing comfrey root should not be administered internally to animals, and products containing comfrey leaves should not be administered internally to animals for long periods of time (Anonymous, 1994).

9.0 TOXICOLOGICAL DATA

Summary: Although, there was no evidence of liver damage in a group of people who had regularly consumed comfrey, there are a number of case reports that implicate consumption of comfrey in the development of liver toxicity, predominantly hepatic veno-occlusive disease.

Unsaturated pyrrolizidine alkaloids are metabolically activated to toxic compounds in the liver. In experimental animal studies, pyrrolizidine alkaloids are almost completely excreted within 24 hours following a single acute exposure. Sprague-Dawley rats treated orally by gavage or dermally with reduced crude comfrey alkaloids or unreduced crude alkaloid *N*-oxides of comfrey extract excreted a number of pyrrolizidine alkaloids in urine collected over the first 24 hours; the urine of rats treated dermally with the reduced alkaloid extract contained about 20 times less total alkaloids than did urine from rats dosed by gavage.

No acute exposure data on comfrey were located. In mice, the intraperitoneal (i.p.) LD_{50} dose for symphytine is 300 mg/kg (0.786 mmol/kg); in rats the i.p. LD_{50} is 130 mg/kg (0.341 mmol/kg). Rats fed a 30% comfrey diet for 21 days exhibited a decrease in body weight gain and an increase in hepatic aminopyrine *N*-demethylase activity, but had no change in hepatic glutathione *S*-transferase or epoxide hydrolase activities.

The incidence of hepatocellular adenoma was increased in rats fed comfrey leaves in the diet daily for 480-600 days or comfrey roots in the diet daily for 245 or 280 days, or until death. The incidence of liver tumors were also increased, but not significantly, in rats injected i.p. with symphytine once or twice per week for 56 weeks.

Comfrey did not induce X-linked recessive lethal mutations in *Drosophila melanogaster*, and an acetone extract of comfrey was not mutagenic in *Salmonella typhimurium* strains TA98 and TA100 with or without metabolic activation. However, incubation of human lymphocytes with an extract of comfrey induced an increase in sister chromatid exchanges in both the absence and presence of metabolic activation. Symphytine induced gene mutations in *S. typhimurium* strain TA100 with metabolic activation and in V79 Chinese hamster cells (metabolic activation conditions not provided) and was positive for the induction of wing spot mutations in *D. melanogaster*. Symphytine did not induce morphological transformation in Syrian hamster embryo cells. Extracts of comfrey suppressed the mutagenic activity of benzo[*a*]pyrene and heated extracts suppressed the mutagenic activity of 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-ß]indole in *S. typhimurium* (metabolic activation condition not provided), while a crude comfrey extract suppressed the genotoxic activity of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine in *Bacillus subtilis*.

An ethanol extract of comfrey roots inhibited both the classical and alternative pathways of complement *in vitro*. In another study, a crude aqueous extract of comfrey root and 2 of its fractions inhibited the *in vitro* proliferation of human peripheral blood lymphocytes stimulated with phytohemagglutinin. The same crude aqueous comfrey extract and one of its fractions enhanced

the generation and release of O_2^- by unstimulated and stimulated granulocytes and increased the total respiratory burst of the unstimulated granulocytes, but had an inhibitory effect on the respiratory burst of the stimulated granulocytes.

A crude aqueous extract of comfrey and 2 of its fractions had no effect on the *in vitro* proliferation of Ehrlich tumor cells. However, symphytine exhibited cytotoxic activity towards Ehrlich ascites carcinoma cells *in vitro*. In mice inoculated i.p. with EL-4 or Ehrlich ascites cells, proliferation of the tumor cells was stimulated by a crude aqueous extract of comfrey and by one of its fractions, but was inhibited by another fraction.

9.1 General Toxicology

9.1.1 Human Data

9.1.1.1 Clinical Studies

There was no evidence of liver damage in a group of 29 people who had regularly consumed comfrey (Anderson and McLean, 1989, abstr.). Twenty-nine volunteers responded to mailed questionnaires regarding duration, amount, and form of comfrey used. At the same time, liver function tests (bilirubin, transaminase, and GGT) were performed on the volunteers. Most volunteers (21/29) had used comfrey for 1-10 years (mean intake 3.0 g dry leaf/day); 5/29 used it for 11-20 years (mean intake 2.6 g dry leaf/day); and 3/29 used it for 21-30 years (mean intake 11 g dry leaf/day).

9.1.1.2 Case Reports

A number of case reports implicate consumption of comfrey in the development of liver toxicity, predominantly hepatic veno-occlusive disease.

Veno-occlusive disease was reported in a woman who took 2 comfrey-pepsin tablets per meal for 2 months (Ridker et al., 1985; cited by Huxtable et al., 1986), in a woman who took 6 comfrey-pepsin tablets containing 280 mg/kg total pyrrolizidine alkaloids for 6 months, and in a woman who took 6 comfrey-pepsin tablets containing 988 mg/kg total pyrrolizidine alkaloids for 6 months (Huxtable, 1987 abstr.). No other details were given.

In New Zealand, a newspaper article described the death of a young man from liver collapse following regular consumption of comfrey leaves (The Press, 1986; cited by Vollmer et al., 1987).

A 13-year-old boy previously diagnosed with Crohn's disease and treated first with prednisolone and sulphasalazine, and then with comfrey tea and acupuncture, presented with

hepatomegaly and ascites (Weston et al., 1987). A liver biopsy revealed hepatic veno-occlusive disease. The duration, quantity, and frequency of comfrey use were not reported.

A 47-year-old woman with no history of blood transfusion or alcohol use also developed hepatic veno-occlusive disease after a number of years of comfrey consumption (Bach et al., 1989). The patient had previously complained of vague abdominal pain, fatigue, and allergies to a homeopathic doctor who recommended comfrey. The patient then began consuming as many as 10 cups of comfrey tea per day and also took comfrey pills "by the handful" for more than a year. Four years later, she had abnormally high aminotransferase levels; 8 years later she had developed ascites. Liver biopsies performed 9 and 10 years after the start of comfrey use revealed fibrosis and proliferation of bile ductules.

A liver biopsy of a 49-year-old woman who had consumed a "large amount" of comfrey tea (amount and duration not specified) was consistent with hepatic veno-occlusive disease (McDermott and Ridker, 1990). A follow-up 7 years later revealed that her symptoms had improved after discontinuing the use of comfrey tea.

Serious liver and pulmonary disease was observed in a 77-year-old woman who took herbal remedies containing comfrey and skullcap (*Scutelleria galericulata*) for 6 months (Miskelly and Goodyear, 1992). The patient's conditions returned to normal 4 months after use of the remedies were discontinued.

A 30-year-old man experienced light-headedness, agitation, confusion, difficulty in urinating, dry mouth, rapid heart beat, and dilated pupils immediately after consuming several cups of comfrey tea (28 g in boiling water) (Routledge and Spriggs, 1989). Twenty-four hours later his symptoms had subsided. Based on previous analyses of other batches of comfrey, the authors noted that the comfrey was possibly contaminated with atropine.

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

Unsaturated pyrrolizidine alkaloids are metabolically activated to toxic compounds in the liver by mixed function oxidases (Vollmer et al., 1987). This process involves dehydrogenation of the pyrrolizidine to form the corresponding pyrrole, a potent alkylating agent (Vollmer et al., 1987; WHO, 1988; McDermott and Ridker, 1990). In experimental animals, pyrrolizidine alkaloids are almost completely excreted within 24 hours (method of administration not provided) (WHO, 1988).

In a study conducted by Brauchli et al. (1982), Sprague-Dawley rats were treated orally by gavage (single dose) or dermally (44-hour contact time) with reduced crude comfrey alkaloids or unreduced crude alkaloid *N*-oxides of comfrey. Rats treated orally or dermally with the reduced alkaloid extract excreted 7-acetyllycopsamine, 7-acetylintermedine, lycopsamine, intermedine, and retronecine in urine collected over the first 24 hours. The urine of rats treated dermally with the reduced alkaloid extract contained about 20 times less total alkaloids than did urine from rats dosed by gavage.

In the urine of rats treated with the alkaloid *N*-oxide extract and collected over the second 24-hours, 7-acetylintermedine, 7-acetyllycopsamine, intermedine, lycopsamine, and the *N*-oxides intermedine and lycopsamine were detected following oral but not dermal exposure. However, the *N*-oxides of 7-acetylintermedine and 7-acetyllycopsamine were detected following either oral or dermal treatment. In a chemical analysis of the comfrey used in this study, 7-acetyllycopsamine, 7-acetylintermedine, lycopsamine, and intermedine were detected. Only trace amounts of symphytine were present.

9.1.3 Acute Exposure

Acute toxicity values for comfrey were not available; acute toxicity values for symphytine are presented in **Table 1**.

Table 1. Acute Toxicity Values for Symphytine

Route	Species (sex and strain)	LD_{50}	Reference
i.p.	mouse (sex and strain not provided)	300 mg/kg (0.786 mmol/kg)	Culvenor et al. (1980)
	rat (male inbred ACI)	130 mg/kg (0.341 mmol/kg)	Hirono et al. (1979a)

9.1.4 Short-Term and Subchronic Exposure

The short-term exposure study described in this section is presented in **Table 2**; no information on subchronic exposure was located.

Table 2. Short-Term Toxicity of Comfrey

Species Strain, Age	Number of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
rat (Long-Evans; age n.p.)	exposed: 6 M controls: 6 M (basal diet); 6 M (basal diet with 30% alfalfa)	comfrey, harvested in prebloom vegetative state and finely ground	5, 10, or 30% in diet	21 days; rats were killed at end of treatment period	There was a significant decrease in weight gain in rats fed 30% comfrey diet. Rats fed 10 or 30% comfrey or 30% alfalfa diet exhibited an increase in hepatic aminopyrine *N*-demethylase activity, but had no change in hepatic glutathione *S*-transferase or epoxide hydrolase activities, as compared to rats fed a basal diet alone.	Garrett et al. (1982)

Abbreviations: M = males; n.p. = not provided

Male Long-Evans rats fed a 10 or 30% comfrey diet or a 30% alfalfa diet for 21 days exhibited an increase in hepatic aminopyrine *N*-demethylase activity, but had no change in hepatic glutathione *S*-transferase or epoxide hydrolase activities, as compared to rats fed the basal diet (Garrett et al., 1982). Rats fed a 30% comfrey diet exhibited a significant decrease in body weight gain, but a 5 or 10% comfrey diet or a 30% alfalfa diet did not affect body weight gains. There was no change in enzyme activities in rats fed a 5% comfrey diet. The comfrey used in this study was harvested in the prebloom vegetative state.

9.1.5 Chronic Exposure

Other than carcinogenicity studies (see **Section 9.3**), no chronic exposure studies on comfrey and symphytine were located.

9.2 Teratogenicity and Embryotoxicity

No data was found.

9.3 Carcinogenicity

The studies described in this section are presented in **Table 3**.

9.3.1 Oral Administration

Inbred male and female ACI rats were fed comfrey leaves in the diet for 480-600 days or comfrey roots in the diet for 245 or 280 days, or until death (Hirono et al., 1978). Comfrey leaves were administered as 8, 16, or 33% of the diet. Comfrey roots were administered according to one of the following 5 schedules: 8% diet; 4% diet (185 days), 2% diet (30 days), then 1% diet; 4% diet (180 days), 0.5% (65 days), then basal diet; 2% (190 days), 0.5% (90 days), then basal diet; or 1% diet (275 days), then alternating 0.5% diet and basal diet at 3-wk intervals. A control group was fed the basal diet alone. There was a dose-response increase in the incidence of liver tumors among rats fed comfrey leaves for 600 days; this relationship was only suggestive in rats fed comfrey roots. The investigators noted that the incidence of urinary bladder tumors in rats fed comfrey leaves was too small to draw any conclusion, while the incidence in rats fed comfrey roots was not significant. A very small number of tumors were detected in other organs, but these were concluded to be spontaneously occurring.

Table 3. Carcinogenicity of Comfrey and Symphytine

Species Strain, Age	Number of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.3.1 Oral Administration						
rat (1- to 1.5-mo-old inbred ACI)	exposed: Group I-1, 11 M, 8 F; Group I-2, 10 M, 10 F; Group II, 11 M, 10 F; Group III, 14 M, 14 F controls: 65 M, 64 F	comfrey leaves, dried and ground	33% diet (Group I-1) 33% diet (Group I-2) 16% diet (Group II) 8% diet (Group III)	480 days 600 days 600 days 600 days	The following tumor incidences were reported in Groups I-1, I-2, II, and III, respectively, vs. controls (statistical significance was not given): hepatocellular adenoma: 5/19, 11/20, 7/21, 1/28 vs. 0/129 hemangioendothelial sarcoma of liver: 0/19, 0/20, 1/21, 0/28 vs. 0/129 urinary bladder papilloma: 1/19, 2/20, 2/21, 0/28 vs. 1/129 urinary bladder carcinoma: 2/19, 0/20, 1/21, 0/28 vs. 0/129 There was a dose-response relationship for induction of liver tumors among Groups I-2, II, and III. The authors noted that the incidence of urinary bladder tumors was too small to draw any conclusion. A very small number of tumors were detected in other organs, but these were labeled as spontaneously occurring.	Hirono et al. (1978)
	exposed: Group IV, 12 M, 12 F; Group V-1, 12 M, 12 F; Group V-2, 12 M, 12 F; Group VI, 12 M, 12 F; Group VII, 8 M, 7 F controls: 65 M, 64 F (same group as above)	comfrey root, dried and ground	8% diet (Group IV) 4% diet (185 days), 2% diet (30 days), then 1% diet (Group V-1) 4% diet (180 days), 0.5% (65 days), then basal diet (Group V-2) 2% (190 days), 0.5% (90 days), then basal diet (Group VI) 1% diet (275 days), then alternating 0.5% diet and basal diet at 3-wk intervals (Group VII)	until death until death 245 days 280 days until death	The following tumor incidences were reported in Groups IV, V-1, V-2, VI, and VII, respectively, vs. controls (statistical significance was not given): hepatocellular adenoma: 19/24, 9/24, 7/24, 10/24, 12/15 vs. 0/129 hemangioendothelial sarcoma of liver: 0/24, 0/24, 0/24, 0/24, 2/15 vs. 0/129 There was only a suggestive dose-response relationship for induction of liver tumors among all of the groups. The incidence of urinary bladder tumors was not significant. A very small number of tumors were detected in other organs, but these were labeled as spontaneously occurring.	
9.3.2 Intraperitoneal Injection						
rat (1- to 1.5-mo-old inbred ACI)	exposed: 20 M controls: 20 M (0.9% sodium chloride solution given i.p.)	symphytine, extracted from dried milled comfrey roots and purified	13 mg/kg body weight, twice/wk for 4 wk then once/wk for 52 wk	392 days; study terminated 650 days after the start of treatment	Liver tumors were detected in 4/20 symphytine-treated rats (3 hemangio-endothelial sarcomas, 1 liver cell adenoma) and 0/20 controls. Rats treated with symphytine also had megalocytosis and proliferation of oval cells and endothelium of the sinusoids (incidences not given). It was not specified whether other organs were examined.	Hirono et al. (1979a)

9.3.2 Intraperitoneal Injection

Liver tumors were detected in 4/20 male inbred ACI rats injected i.p. with symphytine (13 mg/kg [0.034 mmol/kg]) twice per week for 4 weeks and then once per week for 52 weeks while no liver tumors were detected in a control group of 20 rats similarly administered 0.9% sodium chloride solution (Hirono et al., 1979a). The rats were observed for up to 650 days after the first injection. While suggestive, the increase in liver tumors was not statistically significant. Rats treated with symphytine also had megalocytosis and proliferation of oval cells and endothelium of the sinusoids (incidences not given). It was not specified whether other organs were examined.

9.4 Genotoxicity

The studies described in this section are presented in **Table 4**.

9.4.1 Prokaryotic Systems

An acetone extract of comfrey (0.1 mL extract per 0.1 mL culture; extract not characterized) did not induce *his* gene mutations in *S. typhimurium* strain TA98 or TA100 with or without S9 metabolic activation (White et al., 1983).

Symphytine (doses not given) induced *his* gene mutations in *S. typhimurium* strain TA100 in the presence but not the absence of S9, and was negative in strain TA98 with or without S9 (Hirono et al., 1979b).

9.4.2 Lower Eukaryotic Systems

Comfrey did not induce X-linked recessive lethal mutations in *D. melanogaster* (Canton-S wild type, *Basc* stock) (Clark, 1982). Dried chopped comfrey leaves were administered by cooking them in the food medium on which larvae were reared or by allowing adult males to feed on Kleenex tissue soaked in 10% aqueous sucrose solution containing comfrey (doses not provided).

Symphytine induced a significant increase in wing spots, indicative of somatic mutations or recombination, in *D. melanogaster* (cell marker heterozygous *mwh* flr^{+}/mhw^{+} *flr*) when administered to larva in food at a dose of 0.1 or 0.25 mM, but not at 0.05 mM (Frei et al., 1992).

Table 4. Genotoxicity of Comfrey and Symphytine

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.4.1 Prokaryotic Systems							
Salmonella typhimurium strains TA98 and TA100	*his* gene mutations	+/-	acetone extract of comfrey, purity n.p.	0.1 mL extract per 0.1 mL culture	negative		White et al. (1983)
S. typhimurium strains TA98 and TA100	*his* gene mutations	+/-	symphytine, purity n.p.	n.p.	positive (TA100 with S9) negative (TA100 without S9; TA98 with or without S9)	No other experimental details were given.	Hirono et al. (1979b)
9.4.2 Lower Eukaryotic Systems							
Drosophila melanogaster larvae and male adults (Canton-S wild type, *Basc* stock)	X-linked recessive lethal mutations	NA	dried chopped comfrey leaves cooked in food medium (larvae) or fed on Kleenex tissue soaked in a 10% aqueous solution containing comfrey (adults)	n.p.	negative	No other experimental details were given.	Clark (1982)
D. melanogaster (cell marker heterozygous *mwh* flr^{+}/mhw^{+} *flr*)	wing spot test	NA.	symphytine, purity n.p.	0.05, 0.1, or 0.25 mM	positive (0.1 and 0.25 mM) negative (0.05 mM)	Symphytine was placed in food medium for larvae. Positive response indicative of somatic mutations and/or recombination.	Frei et al. (1992)
9.4.3 *In Vitro* Mammalian Systems							
human lymphocytes	sister chromatid exchange	+/-	comfrey, alkaloid crude extract	1.4, 14, 140, or 1400 µg/mL	positive (140 or 1400 µg/mL) negative (1.4 or 14 µg/mL)		Behninger et al. (1989)
V79 Chinese hamster fibroblast cell line	hypoxanthine-guanine phosphoribosyl-transferase (HPRT) locus mutations	n.p.	symphytine, purity n.p.	n.p.	positive	Symphytine induced resistance to 8-azaguanine. No other experimental details were given.	Hirono et al. (1979b)

Table 4. Genotoxicity of Comfrey and Symphytine

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
Syrian hamster embryo cells	transformation	NA	symphytine, purity n.p.	n.p.	negative	No other experimental details were given.	Hirono et al. (1979b)

Abbreviations: n.p. = not provided; NA = not applicable

9.4.3 *In Vitro* Mammalian Systems

Incubation of human lymphocytes with 1.4, 14, 140, or 1400 µg/mL of a crude extract of comfrey increased the frequency of sister chromatid exchanges (SCE) at the two highest doses in the absence and presence of S9 (Behninger et al., 1989). The extract contained 19% lycopsamine, 13% intermedine, 19% acetyllycopsamine, 20% acetylintermedine, and 4% symphytine; the remainder was comprised of unidentified alkaloids.

Symphytine (doses not given) induced mutations at the hypoxanthine phosphoribosyl transferase (HPRT) locus in V79 Chinese hamster cells, but did not induce morphological transformation in Syrian hamster embryo cells (Hirono et al., 1979b).

9.5 Antimutagenicity

The studies described in this section are presented in **Table 5**.

Crude comfrey extract (nature of extract not specified) suppressed approximately 43% and 52% of benzo[*a*]pyrene-induced *his* gene mutations in *S. typhimurium* strains TA98 and TA100, respectively (Ham et al., 1992, abstr.). When heated, the comfrey extract suppressed approximately 75% and 76% of 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-ß]indole-induced mutations in strains TA98 and TA100, respectively (Ham et al., 1992, abstr.). No other experimental details were given.

In *B. subtilis* H17 (rec^+) and M45 (rec^-), it was reported that a crude comfrey extract (40 µL/disc) "showed strong antimutagenic effects" against *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced genotoxicity (Ham et al., 1992, abstr.). The spore *rec*-assay was used [this assay measures differential killing as a result of DNA repair or damage and is not a measure of mutagenicity]. No other experimental details were given.

9.6 Immunotoxicity

The studies described in this section are presented in **Table 6**.

In a study conducted by van den Dungen et al. (1991), an ethanol extract of comfrey roots strongly inhibited both the classical and alternative pathways of complement *in vitro*. The extract was shown to contain amino acids and sugars, but was not further characterized.

Olinescu et al. (1993) concluded that a crude aqueous extract of comfrey root and 2 of its

fractions extracted with $(NH_4)_2SO_4$ strongly inhibited the *in vitro* proliferation of human peripheral

Table 5. Antimutagenicity of Comfrey Extracts

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
S. typhimurium strains TA98 and TA100	*his* reverse gene mutations	n.p.	crude and heated comfrey extracts (no other details given)	n.p.	Crude extract suppressed ~43 and ~52% of benzo[*a*]pyrene-induced mutagenesis in TA98 and TA100, respectively. Heated extract suppressed ~75 and ~76% of 3-amino-1,4-dimethyl-5H-pyrido[4,3-ß]indole-induced mutagenesis in TA98 and TA100, respectively.	No other experimental details were given.	Ham et al. (1992, abstr.)
B. subtilis H17 (rec^+) and M45 (rec^-)	differential survival	n.p.	crude comfrey extract (no other details given)	40 µL/disc	The crude comfrey extract "showed strong antimutagenic effects" against *N*-methyl-*N*-nitro-*N*-nitrosoguanidine-induced genotoxicity.	The spore *rec*-assay was used [this assay measures differential killing as a result of DNA repair or damage and is not a measure of mutagenicity].	

Abbreviations: n.p. = not provided

Table 6. Immunotoxicity of Comfrey Extract

Test System	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
complement (other details n.p.; see Comments)	ethanol extract of comfrey roots; the extract contained amino acids and sugars, but was not further characterized	n.p.	The extract strongly inhibited both the classical and alternative pathways of complement *in vitro.*	Complement consists of a series of serum enzymatic proteins that interact and bind with antigen-antibody complexes, producing lysis when the antigen is an intact cell (Saunders, 1974).	Van den Dugan et al. (1991)
blood lymphocytes, stimulated with PHA *in vitro*, blood obtained from healthy human volunteers or volunteers with chronic staphylococcal infections, rheumatoid polyarthritis, or solid tumors	crude aqueous extract of comfrey root and 2 of its fractions extracted with $(NH_4)_2SO_4$.	1 mg/mL (extract) 100 µL (fraction V) 1 mg/mL (fraction VI)	The crude extract and its fractions strongly inhibited the proliferation of peripheral blood lymphocytes from healthy volunteers. In the blood lymphocytes obtained from volunteers with chronic staphylococcal infections or rheumatoid polyarthritis, or with solid tumors, the inhibition of proliferation was more moderate (staphylococcal infections or rheumatoid polyarthritis) or did not occur at all (solid tumors).	One of the comfrey fractions (fraction V) was rich in carbohydrates and contained no detectable proteins, whereas the other fraction (fraction VI) contained mucus, carbohydrates, and proteins. The crude extract was not characterized.	Olinescu et al. (1993)
human granulocytes, unstimulated or stimulated via F_c receptors with opsonized zymosan particles	see description above	100 µg (extract) 100 µL (fraction V) 100 µg (fraction VI)	The crude comfrey extract and fraction VI enhanced the generation and release of O_2^- by the unstimulated and stimulated granulocytes, whereas fraction V had virtually no effect. The crude extract and fraction VI also increased the total respiratory burst of the unstimulated granulocytes, but had an inhibitory effect on the respiratory burst the stimulated granulocytes. Fraction V inhibited the total respiratory burst of both unstimulated and stimulated granulocytes.	See comments above.	
unstimulated granulocytes isolated from heparinized venous blood of healthy volunteers	aqueous extract of comfrey root, chemical composition n.p.	5%	The extract enhanced the generation and release of oxygen free radicals by unstimulated granulocytes. When the granulocytes were stimulated either with opsonized-zymosan or Concanavalin A, however, the comfrey extract inhibited oxygen free radical production.	It was noted that neither the stimulatory nor inhibitory effect of the extract on oxygen free radical production was due to the presence of enzymes such as superoxide dismutase, catalase, or xantinoxidase, because the effects were apparent even after heating the reaction mixture.	Radu et al. (1994)

Abbreviations: n.p. = not provided

blood lymphocytes stimulated with phytohemagglutinin (PHA). One of the comfrey fractions (fraction V) was rich in carbohydrates and contained no detectable proteins, whereas the other fraction (fraction VI) contained mucus, carbohydrates, and proteins. The crude extract was not characterized. Doses were as follows: 1 mg/mL (extract); 100 µL (fraction V); 1 mg/mL (fraction VI). The blood lymphocytes in this first experiment were obtained from healthy human volunteers. In another experiment, when the blood lymphocytes were obtained from volunteers with chronic staphylococcal infections or rheumatoid polyarthritis, or with solid tumors, the inhibition of proliferation was more moderate (staphylococcal infections or rheumatoid polyarthritis) or did not occur at all (solid tumors).

Olinescu et al. (1993) also evaluated the effect of the comfrey extract and fractions described above on the respiratory burst of human granulocytes. The granulocytes were either unstimulated or stimulated with opsonized-zymosan particles via F_c receptors. The crude comfrey extract (100 µg) and fraction VI (100 µg) enhanced the generation and release of O_2^- by the unstimulated and stimulated granulocytes, whereas fraction V (100 µL) had virtually no effect. The crude extract and fraction VI also increased the total respiratory burst of the unstimulated granulocytes, but had an inhibitory effect on the respiratory burst of the stimulated granulocytes. Fraction V inhibited the total respiratory burst of both unstimulated and stimulated granulocytes.

Radu et al. (1994) reported that a 5% solution of an aqueous extract of comfrey root (chemical composition not provided) enhanced the generation and release of oxygen free radicals by unstimulated granulocytes isolated from the heparinized venous blood of healthy volunteers. When the granulocytes were stimulated either with opsonized-zymosan or Concanavalin A, however, the comfrey extract inhibited oxygen free radical production. It was noted that neither the stimulatory nor inhibitory effect of the extract on oxygen free radical production was due to the presence of enzymes such as superoxide dismutase, catalase, or xantinoxidase, because the effects were present even after heating of the reaction mixture.

9.7 Other Data

A crude aqueous extract of comfrey and 2 fractions extracted with $(NH_4)_2SO_4$ had no effect on the proliferation of Ehrlich tumor cells *in vitro* when treated for 24, 48, or 72 hours (Olinescu et al., 1993). One of the fractions was rich in carbohydrates and contained no detectable proteins,

whereas the other fraction contained mucus, carbohydrates, and proteins. The crude extract was not characterized.

Wassel et al. (1987) reported that symphytine was cytotoxic to Ehrlich ascites carcinoma cells *in vitro*. No other experimental details were given.

In female A_2G mice inoculated i.p. with EL-4 or Ehrlich ascites cells, proliferation of the tumor cells was stimulated by a crude aqueous extract of comfrey and by one of its fractions (fraction VI) extracted with $(NH_4)_2SO_4$, but was inhibited by another $(NH_4)_2SO_4$-extracted fraction (fraction V) (Olinescu et al., 1993). Fraction V was rich in carbohydrates and contained no detectable proteins, whereas fraction VI contained mucus, carbohydrates, and proteins. The crude extract was not characterized. The comfrey extract and fractions were administered i.p. concurrently with the tumor cells. The mice were killed 7 or 10 days after inoculation. Ascites fluid and cells from the peritoneal cavity were removed and the volume of tumor cells was determined.

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Over 200 pyrrolizidine alkaloid compounds, which by definition share a common pyrroline structure of two fused five-member rings joined by a single nitrogen atom, have been identified (Vollmer et al., 1987). A number of pyrrolizidine alkaloids cause cancer in rats (Snider, 1991). Studies indicate that an unsaturated pyrrolizidine ring is necessary for these compounds to exert toxic effects (Wassel et al., 1987; Ridker and McDermott, 1989; McDermott and Ridker, 1990; Winship, 1991). The hepatotoxic pyrrolizidine alkaloids have a 1,2-double bond in the pyrrolizidine ring and the primary OH-group esterified with a branched alkyl-acid (Wassel et al., 1987).

Frei et al. (1992) used the *D. melanogaster* wing spot test to investigate the genotoxic potency of 16 pyrrolizidine alkaloids, including symphytine. Aliquots of the test solutions containing different alkaloids were used to rehydrate the feed onto which Drosophila larvae were placed for feeding. In general, macrocyclic diester-type pyrrolizidine alkaloids were the most genotoxic, 7-hydroxy C9-monoester types were the least genotoxic, and open diesters (including symphytine) were intermediate in regard to genotoxicity. Stereoisomeric pyrrolizidine alkaloids sometimes but not always exhibited similar genotoxic activity. It was also noted that as the number of hydroxy groups increased, the genotoxicity of the pyrrolizidine alkaloid decreased.

Frei et al. (1992) also reported a positive correlation between the genotoxic activity of the 16 pyrrolizidine alkaloids in *D. melanogaster* and their hepatotoxicity in experimental rodents. A positive correlation was also observed between genotoxic activity and carcinogenicity in mammals, although it was noted that the carcinogenicity data were limited for some of the alkaloids.

11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

SANSS
TSCATS (Toxic Substances Control Act Test Submissions)

DIALOG Files

DIOGENES
Federal Register

Internet Databases

Code of Federal Regulations full text. 1996 versions of various titles via GPO Gate, a gateway by the Libraries of the University of California to the GPO Access service of the Government Printing Office, Washington, DC. Internet URL http://www.gpo.ucop.edu/

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

STN International Files

AGRICOLA (Agricultural Online Access)
BIOSIS (Biological Abstracts)
CABA
CANCERLIT
CAPLUS (Chemical Abstracts)
CEN (Chemical & Engineering News)
CHEMLIST
CIN (Chemical Industry Notes)
CROPB
CROPU
CSNB (Chemical Safety News Base)
DDFB
DDFU

DRUGLAUNCH
EMBASE (Excerpta Medica)
FSTA
LIFESCI
MEDLINE (Index Medicus)
NAPRALERT
PHIN
PROMT
REGISTRY
TOXLINE
RTECS (Registry of Toxic Effects of Chemical Substances)
TOXLINE
TOXLIT

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicology Research Projects	CRISP
NIOSHTIC7	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

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