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Risks Associated with Consumption of Herbal Teas

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Contents

I. Introduction

Plants have been used for medicinal purposes for centuries (Larkin 1983). The writings remaining from the ancient civilizations of Sumer, Assyria, Egypt, Greece, China, and Rome describe the use of plants believed to possess medicinal qualities. The first comprehensive list, or *Materia Medica*, of all known medicinal herbs dates to the days of the Roman Empire (Larkin 1983). Tea, originating in China, is one of the world's oldest known prepared beverages. In the early years of the twentieth century, many herbals were tested for activity and eliminated as ineffective or replaced by synthetic products. However, during the past 20 years there has been a resurgence of interest in "natural" products such as herbal teas and supplements with medicinal or nonmedicinal purposes. With concerns about the possible ill effects of consuming beverages containing caffeine, health-oriented individuals are turning to herbal teas as alternatives to traditional low calorie, caffeinated beverages such as coffee, cocoa, and tea. The popularity of herbal tea consumption has acquired such dimensions that during

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1985 the sales of herbs and herbal teas in health food stores exceeded $190 million in the United States (Tyler 1987). Hundreds of different herbal teas are sold in health food stores as varied mixtures of roots, leaves, seeds, barks, or other parts of shrubs, vines, or trees. Although chemists have been characterizing toxic plant constituents for over 100 yr (Ames 1983), toxicological studies of herbal teas have been scarce, and therefore the safety of many of these products is unknown. Increased use of herbal teas could present a health hazard to the consumer who is exposed to large quantities of plant extracts containing compounds of unknown toxic potential.

In nature, plants are known to synthesize – in addition to carbohydrates, amino acids, purines, pyrimidines, and other primary metabolites – a large group of compounds known as secondary metabolites (Street and Cockburn 1972). Secondary plant metabolites, unlike primary metabolites, are not essential in the production and use of chemical energy by plants. Rather, they have an important role in defining plant–plant, plant–herbivore, and plant–parasite interactions (Kingsbury 1983). Plant toxins, a subset of this complex, heterogeneous array of secondary compounds, are present (sometimes in large quantities) in most plant species, where they serve as defense mechanisms against microbiological and herbivorous invasions (Kingsbury 1983). Plants used as part of the human diet are not exempted from these metabolic products.

Pyrrolizidine alkaloids (PAs) were among the first naturally occurring carcinogens found in products of plant origin. Their wide distribution, both geographical and botanical, makes their probable presence in herbal tea preparations a matter of public health significance.

In 1954, Bras et al. introduced the term veno-occlusive disease (VOD) to designate a condition prevalent in Jamaica, particularly among children. The essential pathology of the disease included portal hypertension with severe ascites due to obliteration of centrilobular and sublobular hepatic veins caused by newly formed connective tissues. These changes, reported to occur naturally in animals that had ingested PA-containing plants (Bras et al. 1957; Van Dam et al. 1995), supported the theory that human disease could have the same etiology. It was also postulated that PAs present in "bush tea" prepared from *Crotalaria* and *Senecio* plants and ingested by the Jamaican population constituted the etiological factor in hepatic VOD. Since then, VOD of the liver caused by the consumption of herbal teas has been recognized in other parts of the world (Culvenor et al. 1986; Fox et al. 1978; Huxtable 1980a,b; McGee et al. 1976; Ridker et al. 1985).

Cases of PA intoxication caused by consumption of herbal teas or supplements have appeared in the literature. In 1983, the development of hepatic VOD in four young women who had taken herbal tea as a treatment for psoriasis was reported (Kumana et al. 1983). Analysis of the herbal mixture revealed the presence of PAs (0.47% dry wt) whose source, after germination of seeds present in the mixture, was identified as *Heliotropium lasiocarpum* (Culvenor et al. 1986).

An area of high incidence of esophageal cancer with rates as high or higher than those seen in Curacao has been localized in Coro, located close to Curacao on the northwestern coast of Venezuela (Morton 1986). A link with the consumption of "bush tea" was later established when a Venezuelan survey revealed that 30% of the population of Coro and the arid state of Falcon habitually ingest infusions from *Krameria ixina* (Merino et al. 1979).

There are numerous cases of intoxication from the consumption of herbal teas. In cases of acute poisoning, linking of the condition with dietary habits is likely to occur. However, when herbal preparation sources of carcinogenic or teratogenic compounds are ingested, symptoms or chronic diseases developing after a long latent period are unlikely to be attributed to consumption of herbal products, particularly if the condition developed as a result of a single exposure to the toxicant. Such insidious actions have only recently come to be recognized and can only be detected by screening plant material for toxic, mutagenic, and teratogenic potentials using a battery of tests, including animal feeding studies. The screening of plants (herbal teas) for toxic potential is a very expensive and time-consuming process. Commercially available herbal tea preparations have generally been screened for toxic, mutagenic, and teratogenic potentials using short-term bioassays that included the brine shrimp (*Artemia* sp.), mouse acute toxicity, *Salmonella*/microsomal mutagenicity, and chicken embryo bioassays. Herbal mixtures have also been screened for toxic PAs by chromatographic techniques.

II. Occurrence and Distribution

Plants containing PAs are ubiquitous in nature. The main sources are plants in the families Boraginaceae (all genera, especially *Cynoglossum officinale*), Compositae (tribes Senecioneae and Eupatorieae), and Leguminoseae (genus *Crotalaria*) (Smith and Culvenor 1981; Van Dam et al. 1995). Other families containing plant sources of PAs are listed in Table I. An extensive compendium of plant sources of PAs was published by Smith and Culvenor in 1981.

The distribution and accumulation of PAs in plants have been studied in a number of species of *Senecio*, *Amsinckia*, and *Crotalaria* (Hartmann and Zimmer 1985; Johnson et al. 1985; Liddel and Logie 1993; Stelljes et al. 1991; Were and Benn 1991). Although considerable inter- and intraspecies variation in alkaloid content was observed, in most species the total alkaloid concentration in leaves reached the maximum at the preflower or early bud stage, with a drop to its minimum level immediately after flowering, at which time most of the alkaloid content of the plant was concentrated in the reproductive organs (Johnson et al. 1985). By contrast, Hartmann and Zimmer (1985) reported a constant alkaloid content for the vegetative organs (roots, stems, and leaves) of two annual *Senecio* species. They also found the reproductive organs (flower heads) to be the major sites of alka-

Table 1. Plant families and genera containing pyrrolizidine alkaloids.

Family	Genera
Apocynaceae	*Fernaldia, Personsia*
Boraginaceae	*Alkanna, Amsinckia, Anchusa, Asperugo, Borago, Caccinia, Cynoglossum, Echium, Hackelia, Heliotropium, Lappula, Lindelophia, Lithosperum, Macrotomia, Messerschimidtia, Myosotis, Paracaryum, Paracynoglossum, Rindera, Solenanthus, Symphytum, Tournefortia, Trachelanthus, Trichodesma, Ulugbekia*
Compositeae	*Adenostyles, Brachyglottis, Cacalia, Conoclinium, Crassocephalum, Doronicum, Echinacea, Emilia, Erechtites, Eupatorium, Farfugium, Gynura, Lingularia, Petasites, Senecio, Syneilesis, Tussilago*
Leguminosae	*Crotalaria*
Ranunculaceae	*Caltha*
Scrophulariaceae	*Castilleja*

Source: WHO Task Group on Pyrrolizidine Alkaloids (1988).

loid accumulation, with concentrations exceeding those of the vegetative organs 5- to 10-fold.

Assuming that the leaves are the main site of alkaloid formation, an intensive translocation of alkaloids into the reproductive organs would explain the pattern of alkaloid content in leaves, as reported by Johnson et al. (1985). This assumption is supported by the diurnal rhythm of alkaloid n-oxides accumulation in leaves and flower heads described by Hartmann and Zimmer (1985). More recently, Van Dam et al. (1995) studied the occurrence, relative distribution, and biosynthesis of PAs in Boraginaceae (*Cynoglossum officinale*), due to their wide distribution and toxicity to livestock in the temperate regions of western Europe, Asia, and Canada. All PAs were found predominantly as N-oxides, and the process of biosynthesis took place exclusively in the shoots (Van Dam et al. 1995). It has been generally accepted that plants synthesize, translocate, and store PAs in the form of N-oxides (Hartmann and Witte 1994).

The biological role of PAs in the plant is not well understood. Some propose that PAs occurring in plants as both free alkaloids and alkaloidal N-oxides may provide a redox system in the plant (Huxtable 1980b). Others have proposed the role of a defense mechanism against herbivory (Hartmann and Zimmer 1985). This role is well supported by the fact that reproductive organs are the main sites of alkaloid accumulation, with the flower heads in mature plants accounting for 70%–80% of total plant alkaloid

content (Hartman and Zimmer 1985; Johnson et al. 1985). Similarly, PAs are used as a defense mechanism against predators by adult ithomiine butterflies, which sequester PA from larval food plants and concentrate them in the tegument (where the predators will immediately sense them), reproductive tissues, and eggs (Brown 1984). In contrast to reproductive organs, young leaves were found to contain the highest PA level in rosette plants, reflecting the plant's effort to protect its future photosynthetic output against herbivores (Van Dam et al. 1995). In addition, *Creatonotos* male moths have been reported to depend on the availability of dietary PAs to biosynthesize pheromones used in mating and as territory markers (Schneider et al. 1982).

III. Chemical Characteristics

A. Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids (PAs) are an important class of natural plant carcinogens that are widely distributed, both botanically and geographically. Interest in this group of compounds originated from economic losses resulting from the grazing of animals in pastures infested with pyrrolizidine-containing plants. In eastern North America, serious stock losses from consumption of pasture contaminated with *Senecio jacobaea* have been reported since 1860. In 1972, an estimated $20 million of horses and cattle were lost in the state of Oregon alone (Huxtable 1980b). Economic losses to the livestock industry have been reported in Australia and other countries throughout the world (Culvenor 1985; Porter 1994). Livestock animals reported to be sensitive to PA poisoning include cattle, horses, sheep, chickens, turkeys, and pigs. Symptoms of intoxication differ from species to species and are dependent on factors such as sex, age, time of exposure, type of alkaloid involved, and plant species consumed.

Livestock toxicosis caused by *Acremonium* (endophyte) infected grasses has a pronounced negative economic effect on animal production due to the presence of ergopeptine alkaloids (Hoveland 1993). Acute liver damage has been observed in sheep, horses, pigs, and dogs, and lung damage in sheep, poultry, and pigs. In addition, horses suffer from neurological disturbances, sheep exhibit a hemolytic syndrome, and cattle suffer a fatal gastrointestinal disorder (McLean 1970). PA toxicosis in humans is manifested as hepatic VOD, a condition characterized by the obstruction of the central and sublobular veins of the liver. The acute disease, associated with high mortality and a subacute or chronic onset, may lead to cirrhosis.

Chemically, PAs are complex, aliphatic, hydroxylated fatty acid esters that exist as monoesters, diesters, or cyclic diesters (Williams and Weisburg 1986). The pyrrolizidine nucleus is composed of two five-membered rings sharing a common nitrogen at position 4. Esterifications at positions 1 and 7 are commonly observed (Fig. 1). The nitrogen atom of the nucleus readily

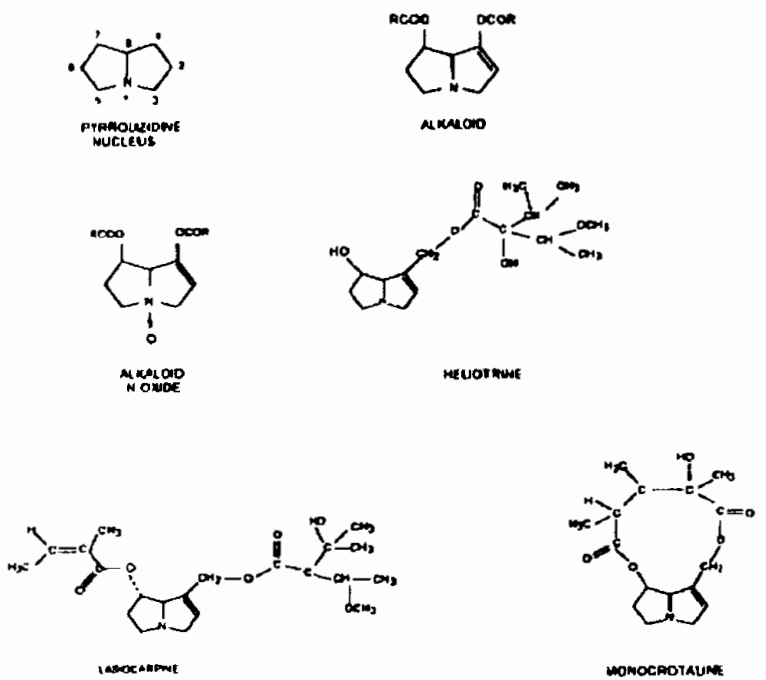

Fig. 1. Chemical structures of selected pyrrolizidine alkaloids.

undergoes oxidation, and N-oxides of the alkaloids are commonly found together with the parent alkaloid in plants (McLean 1970).

Only about one-half of the approximately 250 known PAs are hepatotoxic (Peterson and Culvenor 1983). PAs that are derived from saturated amino alcohols or are not esters are not hepatotoxic (Bruggeman and Van der Hoeven 1985). Thus, it is well established that the structural requirements for toxicity are the presence of an unsaturation in the 1,2 position of the pyrrolizidine nucleus, esterification at the 1 or 7 position, and branching of the ester side chain (McLean 1970). The unsaturated nucleus is essential in permitting metabolic activation to a pyrrolic derivative, while the ester groups are necessary for high reactivity in the primary pyrrolic metabolite. The substituted acids are essential in protecting the parent alkaloid or the pyrrole metabolite against hydrolysis by esterases or water. Therefore, the acid moieties, as postulated by Mattocks (1970), modify the toxic response by influencing the amount of parent alkaloid being metabolized to pyrrole derivatives and by modifying the stability of the reactive metabolites in liver cells.

Formation of highly reactive pyrrolic metabolites in the liver and their role in the development of pathological effects associated with pyrrolizidine alkaloid toxicosis were first recognized by Mattocks (1968). Metabolic pyrroles were detected in the urine and several organs of rats dosed with different types of PAs. These metabolites were detected primarily in the liver and to a lesser extent in the lungs, heart, spleen, and kidneys. Formation of pyrroles *in vitro* from added PAs was shown after incubation with liver slices from normal rats. However, pyrroles were not formed by portions of lung tissue under similar conditions. These "pyrrolic metabolites" gave positive reactions as alkylating agents using 4-(*p*-nitrobenzyl)-pyridine (Mattocks 1969). In addition, the hepatotoxicity of the alkaloids was related to the amount of pyrroles to which they gave rise *in vivo*. This experimental evidence supported the idea that PAs are metabolized to highly reactive metabolites in the liver, where their alkalyting properties cause them to bind to nucleophilic centers in the hepatocytes and give rise to the described effects. Under certain conditions, pyrrolic metabolites are excreted in the urine or feces as "soluble pyrroles" or are transported in the bloodsteam to other organs, where they react with nucleophilic centers.

Transport to other organs is dependent on the stability of the metabolite. The extensive lung damage caused by anacrotaline has been related to the high stability of its pyrrolic metabolite, which is sufficiently stable to escape decomposition or reaction with liver tissue and survives to reach the lungs in relatively large amounts (Mattocks and Driver 1987).

The mechanism of action of PAs, as proposed by Mattocks (1968), is represented in Fig. 2. The first step in biotransformation includes metabolism of the parent alkaloid (I) in the liver by the action of mixed-function oxidases (MFOs), more recently known as polysubstrate mono-oxygenases (PSMOs), of the microsomal fraction to the corresponding pyrrole metabolite (II). In contrast to the structure (II), the ester groups in (II) are highly reactive and can react with nucleophiles such as X′ and Y′. The alkalyting capacity of structure (II) depends on rearrangement of the nitrogen electrons over the ring structure (III), leading to ionization of the ester group to form a putative carbonium ion, which reacts with the nucleophile X′ (IV). Further reorganization of the nitrogen electrons (V) causes the second ester group to ionize, creating a carbonium ion (VI), which is stabilized by resonance to structure (VII), a difunctional alkalyting agent, which reacts with the nucleophile Y′. PAs of similar structure, i.e., monocrotaline and trichodesmine (Huxtable et al. 1996), exhibit marked differences in toxicity.

Unsaturated PAs are metabolized to both pyrroles and n-oxide metabolites by the action of MFOs in the liver (Swick 1984) (Fig. 3). Evidence shows that each type of metabolite is formed through parallel noncompetitive pathways (Mattocks and Bird 1983). While N-oxides are water soluble, show decreased toxicity, and may be visualized as detoxification products, pyrrolic metabolites can bind to tissue *in vivo* and are associated with

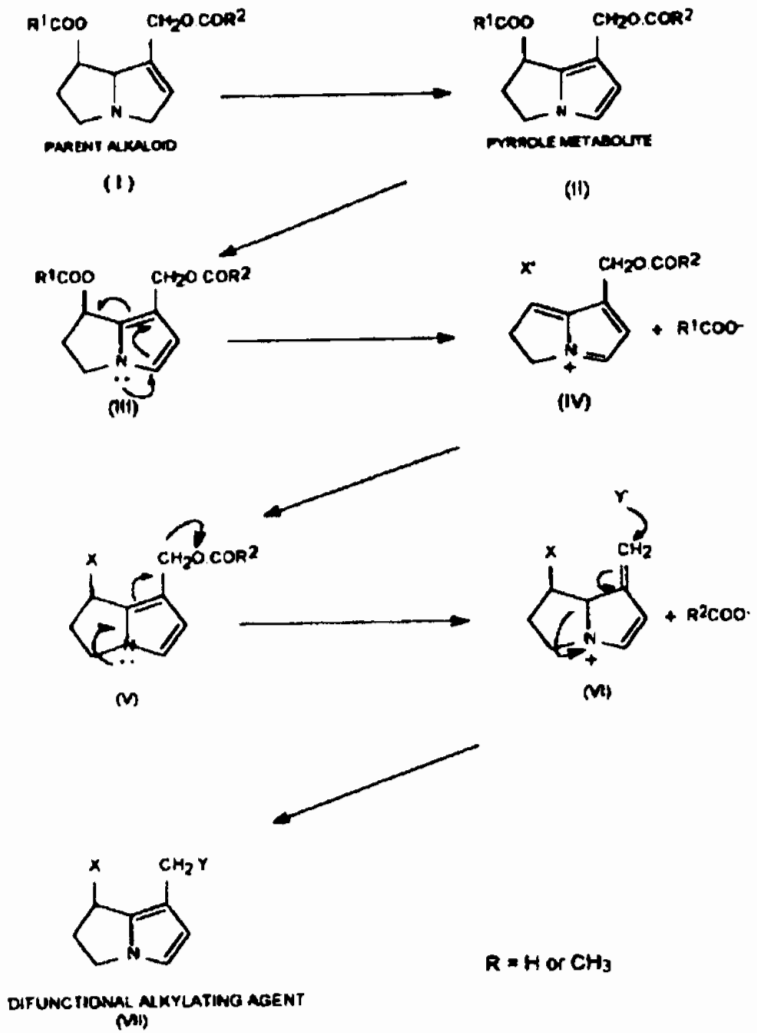

Fig. 2. Mechanism of action of pyrrolizidine alkaloids.

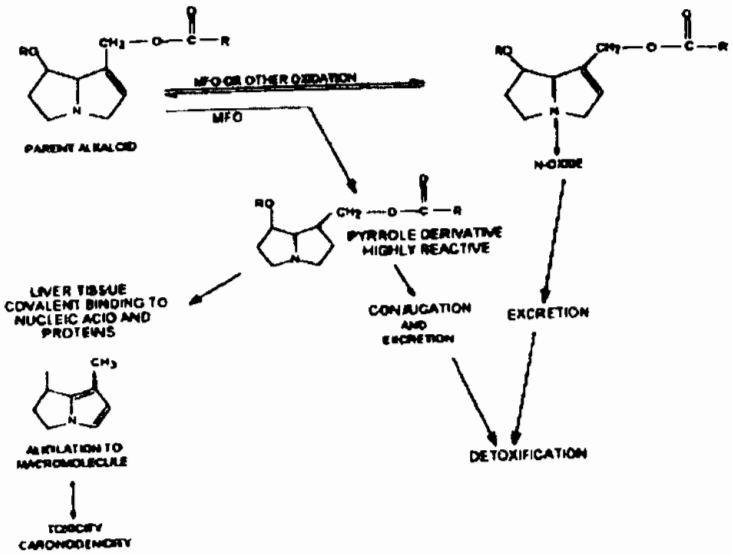

Fig. 3. Suggested metabolic pathway for pyrrolizidine alkaloid free basis and N-oxides and their possible role in toxicity.

hepatotoxic effects. Consequently, the extent to which a PA is metabolized to its pyrrole derivative strongly influences the toxicity of the specific alkaloid.

Mattocks and Bird (1983) studied the rates of formation of N-oxides and pyrrolic metabolites *in vitro*. While formation of both metabolites was enhanced by lipophilicity, the relative rate of production of each species was determined by the structure of the esterified acid. The ratio of pyrrolic metabolites to N-oxides was highest for macrocyclic diesters and monoesters and lowest for open diester alkaloids. These differences between rates of formation of each species were accounted for by the stearic hindrance offered by the acid moiety at the site of each respective reaction. Stearic hindrance at C-8, where hydroxylation could lead to pyrrole formation, is exerted greatly by noncyclic diesters, and this correlates with low pyrrole production; however, macrocyclic diesters, which are held away from the amino alcohol moiety by their relatively rigid conformation, offer less stearic hindrance and give a high pyrrole yield.

B. Tannins

Tannins are secondary plant metabolites (not in metabolic pathways providing energy for growth and reproduction) that are characteristically rich in phenolic hydroxyl groups (Butler et al. 1984). They are divided into two

major classes on the basis of structure and reactivity to hydrolytic agents: (1) hydrolyzable tannins, which are hydroxyl carboxylic acids esterified to sugars such as glucose; and (2) condensed (nonhydrolyzable) tannins, chemically known as proanthocyanidins, which are polymers of flavin-3-ols linked by carbon-carbon bonds (Butler et al. 1984).

Tannins (condensed and hydrolyzable) are widely distributed in plants. Although hydrolyzable tannins are uncommon in human food, condensed tannins are found abundantly in beverages (coffee, cocoa, tea, red wine), fruits (persimmon, banana), and vegetables (spinach) (Singleton 1981). The carcinogenic activity of tannins was first recognized by Korpassy after postmortem examination of burned patients treated with tannic acid during World War II (Korpassy 1961). Histological examination of tissues revealed central necrosis of the liver and hemorrhagic focal necrosis of the adrenal cortex in burned patients who had been treated with tannic acid. Burned patients receiving other treatments did not show such effects. These observations prompted Korpassy to suspect, as early as 1943, that the tannic acid was absorbed from the region of its application and caused damage to the liver (Korpassy 1961).

Korpassy's suspicion of the carcinogenic potential associated with tannic acid has been widely confirmed in laboratory animals. Tannic acid, a hydrolyzable tannin from the galls of many *Quercus* species, was shown to produce cirrhosis and hepatomas in rats on prolonged subcutaneous administration (Korpassy and Moyonsi 1950). Kirby (1960) showed that parenteral administration of extracts of both condensed and hydrolyzable tannins was carcinogenic for rats and mice. While extracts of condensed tannins produced liver tumors as well as sarcomas at the site of injection, extracts of hydrolyzable tannins induced only liver tumors (Kirby 1960).

Carcinogenic effects of a variety of tannin-containing extracts from plants, including a fraction from tea (*Camelia sinensis*), have been demonstrated in laboratory animals (Kapadia et al. 1976). Plants such as *Krameria ixina* (Cadia Del Pero), *Krameria* triandra (Rhatani), and *Acacia villosa* (Watanapa Shimaron), commonly consumed by the inhabitants of Curacao, were shown by Pradhan et al. (1974) to induce carcinoma in National Institutes of Health (NIH) black rats after subcutaneous injections of aqueous extracts. The carcinogenic potential of these plants was associated with the tannin content, as tannin-free fractions did not show any carcinogenic activity when administered subcutaneously (Pradhan et al. 1974). The carcinogenicity of the aqueous extracts obtained from *Acacia villosa* was also reported by O'Gara et al. (1974), who observed development of sarcomas at the site of injection in 100% of injected NIH black female rats. Similarly, the failure to induce sarcomas after repeated injection of tannin-free extract was observed (O'Gara et al. 1974).

Although there are no reports on the induction of tumors in humans by the dietary intake of tannins, a high consumption of tannin-rich plants was linked to the high incidence of esophageal cancer in Curacao (West Indies),

South Carolina (U.S.), and other geographical regions of the world (Morton 1970, 1972, 1973, 1980; Segi 1975). A number of widely used tannin-containing herbal preparations, such as *Arctostaphylos uva ursi, Ephedra viridis, E. nevadensis, Myrica cerifera*, and *Ilex paraguariensis*, have been cited for possible carcinogenic activity (Morton 1980).

C. Safrole

Safrole, 4-allyl-1,2-methylenedioxybenzene, is the major constituent (80% by weight) of the aromatic oil present in the root bark of the sassafras tree (*Sassafras albidum*) (Borchert et al. 1973) and a major constituent of the essential oils of nutmeg, star anise, and cinnamon leaf (Hirono 1981).

Sassafras and its essential oil or synthetically manufactured safrole were widely used in soft drinks such as root beer in the U.S. In 1960, the use of safrole as a food additive was banned by the U.S. Food and Drug Administration (FDA) (Federal Register 1960) after a chronic rat feeding study indicated that safrole was a weak hepatocarcinogen in this species (Long et al. 1963). Since then, the findings of Long and colleagues have been confirmed by many other investigators. Hepatic damage in rats fed for 2 yrs on a diet containing less than 1000 μg/g safrole was reported by Hagan et al. (1965). The development of benign and malignant esophageal tumors in rats fed a diet containing 5000 μg/g dihydrisafrol, a monohydroxylated derivative of safrole, was also reported.

Safrole is extensively metabolized in the liver by two major pathways: oxidation of the allyl side chain and oxidation of the methylene-dioxy group (Ioannides et al. 1981). Both routes involve the enzymatic action of the hepatic microsomal MFOs (Hodgson and Philpot 1974). As expected, administration to rats of phenobarbital and 3-methylocolenthrene, typical inducers of the MFOs, resulted in increased urinary excretion of safrole metabolites produced by each of the metabolic routes mentioned (Janiaud et al. 1977).

The monohydroxylated derivative of safrole, 1′-hydroxysafrole, is believed to be the proximate carcinogen of safrole (Borchert et al. 1973). This metabolite, formed by oxidation of the allyl side chain of safrole, is more hepatotoxic and hepatocarcinogenic to animals than is the parent alkaloid when fed at the same dietary level (Wislocki et al. 1976). Administration to rats of tritium-labeled 1′-hydroxysafrole gives rise to tritium-labeled DNA, RNA, and protein, including the covalent binding of 1′-hydroxysafrole or a further metabolite to biological nucleophiles.

Swanson and colleagues (1979) reported an increase in the mutagenic potential of 1′-hydroxysafrole after incubation with a microsomal activation system, indicating further metabolism of 1′-hydroxysafrole to a more potent mutagen(s) that could act as the ultimate carcinogen(s) responsible for safrole toxicity. Borchert et al. (1971) reported the conversion of 1′-hydroxysafrole in animals and man to 3′-hydroxysafrole after enzymatic

action of β-glucoronidase. Other metabolites formed from the further metabolism of 1′-hydroxysafrole include 3,4-methylenedioxyphenyl vinyl ketone (1′-oxosafrole) (Peele and Oswald 1978) and 1′-hydroxy-2′,3′-epoxide (Wislocki et al. 1976). It was also reported that 1′-hydroxysafrole epoxide, derived from 1′-hydroxysafrole, induces formation of skin papillomas in mouse after repeated applications of the tumor promoter croton oil; in addition, 1′-hydroxysafrole epoxide as well as all epoxides investigated formed from safrole were found to be directly mutagenic in the Ames test (Dorange et al. 1977). This pointed to 1′-hydroxysafrole epoxide as the possible ultimate carcinogen of safrole.

In view of the accumulated evidence, FDA extended the ban on safrole use to the interstate commerce of sassafras tea or any other safrole-containing products (Federal Register 1974). However, in spite of the legal restrictions, sassafras continues to be freely available in "health food" stores and similar outlets in the U.S.

IV. Analytical Methodology

A. Sample Preparation and Extraction

Herbal teas can exist as mixtures and single-ingredient herbal teas such as weightless, female toner, PMS, and chapparal (creosote bush, *Larrea tridentata*). These teas were analyzed for toxic/mutagenic potentials because some of their ingredients have been implicated in cases of animal or human intoxication in the U.S. (Manteiga 1991). These are commonly used herbal teas available in health food stores and are registered products. The composition of each tea preparation, as indicated by the manufacturer, is shown in Table 2.

Sample preparation prior to extraction for chromatographic and bioassay testing includes processes such as grinding, homogenization, and collection of the test portion. Extraction of herbal mixtures can include sequential extraction with solvents of decreasing polarity, e.g., deionized distilled water, absolute methanol, and chloroform, followed by a steeping process for a given period (20 min) and then storage at 0 °C until analysis or lyophilization.

Volimer et al. (1987) described a method for the isolation of PAs. In this method, ground herbal mixtures were extracted in a Soxhlet apparatus for 5 hr with 300 mL of methanol. After extraction, the methanol was removed on a rotary evaporator, and the oily residue was dissolved in 200 mL of a 2 N HCl + ethyl ether (1 : 1) solution. The mixture was partitioned in a separatory funnel and the ether layer discarded. The acidic solution was washed three times with 100 mL ether to remove additional constituents that were not alkaloidal. To regenerate the dissolved alkaloids, the solution was made basic by adding concentrated NH_4OH until the pH reached 8, followed by extraction of the basic solution four times with 50-mL portions of CH_2Cl_2. The solvent was removed on a rotary evaporator, and the oily residue was dissolved in 2 mL of chloroform for storage at 4 °C until further analysis.

Table 2. Composition of herbal tea mixtures.

Common name	Scientific name	Weightless	PMS	Toner
Angelica root	*Angelica* sp.			x
Barley (roasted)	*Hordeum* sp.		x	
Blessed thistle	*Cnicus* sp.			x
Borage	*Borago* sp.		x	
Buchu leaves	*Barosma* sp.	x		
Carob (roasted)	*Ceratonia* sp.		x	
Chamomile flower	*Matricaria* sp.			x
Chickweed	*Stellaria* sp.		x	
Chicory (roasted)	*Chicorium* sp.		x	
Cleavers herb	*Galium* sp.	x		
Cornsilk	*Zea mays*		x	
Crampbark	*Viburnum* sp.			x
Dandelion root[a]	*Taraxanum officinale*		x	
Flax seeds	*Linum* sp.	x		
Fennel seeds	*Foeniculum* sp.	x		
Ginger root	*Zingiber* sp.			x
Hibiscus flowers	*Hibiscus* sp.	x		
Lemon grass	*Cymbopogon* sp.	x		x
Lemon verbena	*Lippia* sp.	x		
Nettle leaves	*Urtica* sp.			x
Parsley leaves	*Petroselinum* sp.	x	x	
Raspberry leaves	*Rubus* sp.			x
Red clover	*Trifolium* sp.	x		
Rosehips	*Rosa* sp.			x
Spearmint leaves	*Mentha* sp.	x		x
Stevia leaves	*Stevia* sp.	x		
Squawvine	*Mitchella* sp.			x
Strawberry leaves	*Fragaria* sp.			x
Uva ursi leaves[b]	*Arctostaphylos uva-ursi*	x	x	

[a] = PMS tea, active ingredient (500 mg/tea bag) (PMS, premenstrual syndrome).
[b] = PMS tea and Weightless Tea, active ingredient (80 mg and 45 mg/tea bag, respectively).

Extraction of alkaloids and sample preparation techniques have been reported to be the major event in the detection aad identification processes (Chizzola 1994; Porter 1994). Because pyrrolizidine alkaloids occur as minor constituents in medicinal plants, determination of these alkaloids even at low concentration is imperative. Rapid sample preparation using solid-phase columns avoids the use of separating funnels and allows enrichment of the alkaloids, which is countered by the lengthy sample cleanup and

concentration steps for gas chromatography (GC), high performance liquid chromatography (HPLC), or thin-layer chromatography (TLC). The procedure includes the reduction of N-oxides with the oxygen-absorbing resin Serdoxit and cleanup with strong cation-exchange solid-phase columns (Chizzola 1994).

B. Chromatographic Techniques

Molyneux and Roitman (1980) described a TLC technique for detecting PAs. The extracts obtained were applied to silica gel 60, 0.25-mm aluminum-backed precoated TLC plates and developed with chloroform:methanol:17% NH_4OH (82.5:15.5:2). Silica gel HL plates have been evaluated for their ability to separate biologically active compounds in water extracts (Manteiga 1991).

The TLC plates were dried and sprayed with a 1% solution of orthochloranil (tetrachloro-obenoquinone)/benzene, dried on a steam bath for approximately 1 min, resprayed with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde), and again heated on the steam bath for 1 min. Presence of PAs was confirmed by the development of pyrrole-characteristic, stable purple spots. While screening crude extracts, possible n-oxides can be converted to pyrroles by treatment with acetic anhydride spray, followed by the application of Ehrlich's reagent. The presence of PAs (N-oxides or parent alkaloids) should be confirmed by the development of pyrrole characteristics. For comparison, standard reference pyrrolizidine alkaloids (senecionine, lycopsamine, echimidine, intermedine, riddelliine, and acetylcopsamine) should be concurrently chromatographed with the chloroform extract.

PAs have been separated by column chromatography using silica gel 60, 70–230 mesh. Before packing, the silica was activated by baking at 120 °C for 1 hr. Columns were packed with enough silica gel to obtain a column height of at least 13 cm. The silica gel was allowed to settle in each column by passing chloroform. The chloroform is rinsed from the columns with absolute methanol. Sample extracts or infusions are applied after lowering the level of the solvent so that they are near the top of the silica gel. The sample was allowed to penetrate the silica by slow elution (<0.8 mL/min).

Further characterization of the alkaloidal extracts using GC and mass spectrometry (MS) is required to conclusively establish the true identity of the pyrrolizidine alkaloids present, since several other PAs have Rf values equal to or close to those detected. Stelljes et al. (1991), who previously reported the presence of PAs in *Senecio mikanioides* Otto, analyzed four *Senecio* species by designing a more sensitive GC-MS system using a medium-low polarity DB-17 column; this provided a tentative identification of complex mixtures of PAs that are difficult and time consuming to assay when using nuclear magnetic resonance (NMR) analysis (Stelljes et al. 1991). Most of the species, including *S. dimorphophyllus, S. serra, S. hydrophyllus*, and *S. mikanioides*, showed the same profile of PAs. The presence of senecionine and integerrine were reported in *S. dimorphophyllus*, and complex mono- and diesters of PAs were detected by GC-MS analysis (Stelljes et al. 1991). Betz et al. (1994) recently developed a method of extraction, solid-phase concentration, and capillary GC determination of alkaloids. The same method was adapted to determine PAs in comfrey (*Symphytum* sp.), which is rich in unsaturated PAs (Mossoba et al. 1994). Since comfrey has been consumed in the form of herbal tea, a green drink, and in capsule form, it is a potential route of exposure to toxicity and carcinogenecity. The comfrey root extracts were identified by capillary gas chromatography/matrix isolation/Fourier transform infrared (GC/MI/FTIR) spectroscopy and electron ionization and positive ion chemical ionization GC/MS techniques, resulting in greater utility of GC/MI/FTIR spectroscopy to PA isomer identification (Mosroba et al. 1994).

HPLC techniques have been adopted for food analysis over the past two decades as a novel analytical technique for the determination and quantification of different food components. Hertog et al. (1993) developed an analytical method for the determination of flavonoids in freeze-dried foods. Later, the same method was adapted for aglycon determination in different beverages, e.g., tea infusions, wines, and fruit juices, by reverse-phase HPLC on a Nova-Pak C_{18} column using acetonitrile:phosphate buffer (25:75 v/v, pH 2.4) as a mobile phase and UV detection (370 nm) (Hertog et al. 1993). Although the literature regarding the use of HPLC for alkaloid determination is scarce, this technique has the potential to improve the determination and quantification of alkaloids in herbal tea infusions. The use of cochromatography (TLC, HPLC, and/or MS with UV or a fluorescence detector) has been reported for better identification and quantification of ergot alkaloids (Porter 1994).

C. Bioassay Techniques for Toxicity Testing

With the development of short-term bioassay techniques, it is possible to determine toxic and mutagenic potentials *in vivo* and *in vitro*. Crude or fractionated extracts of herbal tea mixtures can be screened after the chromatographic and other seperation techniques.

In addition to the effects described, PAs have been reported to induce dose-related fetal abnormalities in rats (Green and Christie 1961; Peterson and Jago 1980). The mutagenic potential of a number of PAs and PA-containing plant extracts has been demonstrated in *Drosophila melanogaster* (Candrian et al. 1984; Clark 1959), *Salmonella typhimurium* TA100 (White et al. 1983; Yamanaka et al. 1979), *Vicia faba* (Furmanowa et al. 1983), cultured mammalian cells (Bruggeman and Van der Hoeven 1985), and in other test systems (Bruggeman and Van der Hoeven 1985; Green and Muriel 1975). The carcinogenic potential of a number of purified PAs, PA-containing plants, and crude extracts of such plants has been demon-

strated in the rat by different investigators (Hirono et al. 1976, 1977, 1978, 1979, 1983; Manteiga 1991; Schoental et al. 1954). Tumors are produced over a wide range of tissues and organs, but the liver is the organ most commonly involved.

The brine shrimp assay (*Artemia* sp.) has been used to rapidly screen crude extracts from commercially available herbal teas (see Table 2) for toxicity potential as well as to monitor the separation of toxic fractions (Manteiga 1991) (Figs. 4 and 5). When testing different sample concentrations, crude extracts or isolates were dispensed into 13 × 100 mm glass test tubes and the solvent evaporated to dryness by heating under a current of nitrogen gas. Solvent and negative controls were included to determine the natural background mortality of the organism. Mortality readings are taken at 16, 24, and 48 hr during incubation. After 48 hr, the larvae remaining alive are killed by heating the test tubes, and the total number of larvae per tube are determined. Based on the percentage mortality, a dose-response curve was tabulated.

Acute toxicity in the mouse was determined by intraperitoneal injection of crude plants extracts into CD-1 inbred female mice. A minimum of three dose levels per extract were tested. After dosing, mice were observed for any signs of toxicity or death at 16, 24, 48, and 72 hr. The chicken embryo

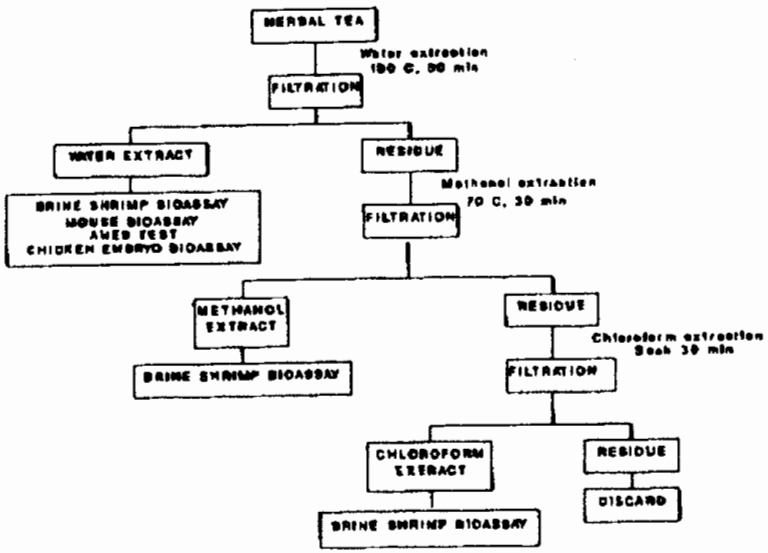

Fig. 4. Extraction and preliminary toxicity testing outline for commercially available herbal teas.

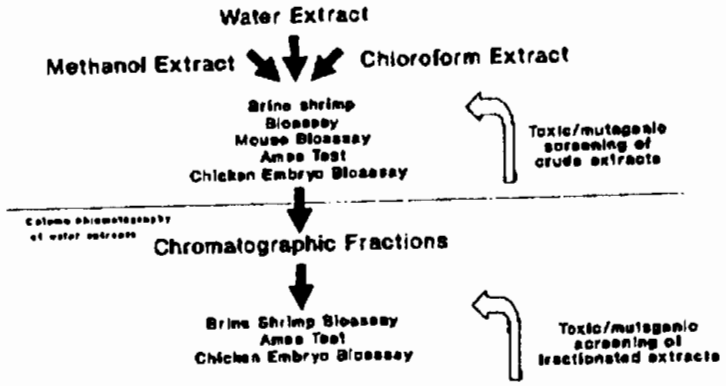

Fig. 5. Herbal tea toxic/mutagenic screening outline.

bioassay, useful in determining acute toxicity and teratogenic potentials, is described in the Official Methods of Analysis, Sections 26.084-26.087 (AOAC 1984; Williams and Weisburg 1986). The *Salmonella*/microsomal mutagenicity assay (Maron and Ames 1983), using *Salmonella typhimurium* tester strain TA 100, has been useful for determining mutagenic potential of crude plant extracts and selected column chromatographic fractions. Both the plate incorporation and the preincubation test procedures are recommended.

Manteiga (1991) concluded that water extracts were more toxic than methanol extracts except for the chaparral tea. Water extracts were found to be nonmutagenic in the *Salmonella* assay, and chloroform extracts were nontoxic in the brine shrimp assay, while no hepatotoxicity of PAs was detected in water or methanol extracts from all herbal teas. The greatest toxic and cathartic effects in mice were observed when exposed to the weightless tea extract.The PAs present in the alkaloidal extract from weightless tea were identified as senecionine and riddelline by TLC, and their concentrations were estimated to be 6.7 and 10 mg/kg dry wt, respectively. The lack of toxicity observed in the crude chloroform extracts could be attributed to the lack of toxic principles in the extracts, to insensitivity of brine shrimp to the extracts, or, more likely, to insolubility of the extracted compounds in the brine shrimp media.

V. Public Health Significance

Many commercial preparations contain substantial amounts of psychoactive substances, and their use has resulted in a number of intoxications requiring clinical attention. Of 396 distinct herbs and spices commercially available and used either singly or in blended mixtures as herbal teas, Siegel

(1976) identified 43 that contained psychoactive agents. Herbs such as yohimbe, catnip, damiana, wormwood, Mormon tea (*Ephedra* sp.), mandrake, Jamestown weed, nutmeg, and *Lobelia*, among others, contain biologically active principles with hallucinogenic, stimulatory, or narcotic properties (Siegel 1976). Jamestown weed (*Datura stramonium*), when taken in the form of an infusion or tea, produces skin flushing, tachycardia, dryness of mouth and skin, excitement, delirium, irritability, fever, convulsions, depression, and bilateral mydriasis (Weintraub 1960). The ingestion of about 4–5 g of the leaves or seeds could be fatal.

Pyrrolizidine alkaloids were among the first naturally occurring carcinogens found in products of plant origin. Their wide distribution, both geographical and botanical, makes their probable presence in herbal tea preparations a matter of public health significance. Other compounds related to toxic and carcinogenic potential are tannins and safrole. Although there are no reports on the induction of tumors in humans by the dietary intake of tannins, some studies have linked the consumption of tannin-rich plants with high incidence of esophageal cancer in Caracao (West Indies), South Carolina (U.S.), and other geographical regions of the world (Morton 1970, 1972, 1980; Segi 1975).

A. Human Exposure and Symptoms

The two main sources of PA poisoning reported in humans are the consumption of cereal grains contaminated with weeds containing the alkaloids and consumption of PA-containing plants as medicinal or dietary items. A third source of exposure is represented by the presence of low levels of alkaloids in honey and milk.

PA toxicosis in humans is manifested as hepatic veno-occlusive disease (VOD), a condition characterized by obstruction of the central and some lobular veins of the liver (Bras and Hill 1956). This acute disease is associated with a subacute or chronic onset that may lead to cirrhosis and high mortality. Its clinical course, as outlined by Stuart and Bras (1957), is represented in Fig. 6. Onset of the disease may be acute or insidious (subacute or chronic). The acute form may result in either complete recovery or rapid death. About 20% of acute-phase patients show clinical recovery, after which some patients may go into the subacute phase of VOD or, after a latent period of years, develop cirrhosis of the liver. In the acute phase, 13% of patients will progress to subacute VOD and either recover completely, progress rapidly to the chronic phase, or, after clinical improvement, develop cirrhosis. In humans, the characteristic effect of PA intoxication is hepatic VOD. This condition, associated with high mortality, has an acute onset and is manifested by rapid swelling of the upper portion of the abdomen and development of rapidly filling ascites and oliguria. Edema of the feet, nausea, and vomiting may be present. A subacute or chronic onset of the disease has been associated with the development of nonportal cirrhosis (WHO 1988).

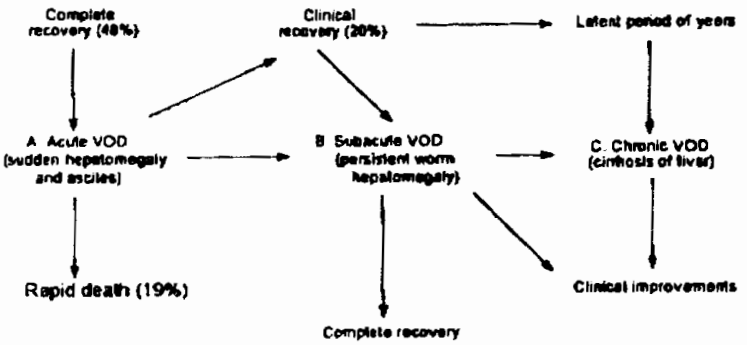

Fig. 6. Clinical history of hepatic veno-occlusive disease (VOD) (from Stuart and Bras 1957).

Liver disease caused by the contamination of cereal grains has been reported in rural areas of Afghanistan (Mohabbat et al. 1976), India (Tandon et al. 1976), South Africa (Selzer and Parker 1951), and the former U.S.S.R. (Mirochnik 1938). Outbreaks resulting from this source of exposure are characterized by high mortality and are associated with periods of abnormally dry weather. In the largest reported outbreak in northwestern Afghanistan, an estimated 8000 people from a population of 35,000 were affected. Approximately 1600–2000 deaths (25% mortality rate) were reported (Mohabbat et al. 1976). The source of the alkaloids was traced to *Heliotropium* plants that were found to be growing extensively in wheat fields and whose seeds contaminated the crop during harvesting. Examination of wheat samples from several villages showed contamination with 300 mg *Heliotropium* seeds per kilogram of wheat. In an outbreak in Central India, *Crotalaria* species growing in cereal fields were responsible for 67 cases of hepatic VOD and a mortality rate of 42% (Tandon et al. 1976). Other cases of human intoxication caused by consumption of contaminated cereal grains and hot infusions are summarized in Tables 3 and 4.

Human poisoning through the medicinal or dietary use of PA-containing plants has been reported worldwide. Consumption of *Crotalaria* sp., in the form of "bush teas," was identified in 1957 as the etiological factor in the high incidence of hepatic VOD disease in Jamaican children (Bras et al. 1957). Schoental (1968) was the first to suggest a link between the high incidence of primary liver carcinoma and the use of *Senecio* plants in the Bantu population of South Africa. Laboratory experiments using South African *Senecio*, retrorcine, and its N-oxide, isatidine, proved effective in inducing liver tumors, including malignant hepatocarcinomas and lung lesions, when administered to female rats.

Another case of PA intoxication was reported in Switzerland, where a

Table 3. Reported cases of human poisoning associated with the ingestion of pyrrolizidine alkaloid-containing plants.

Country/number of cases	Suspected vehicle of intoxication	Name of Plant	Nature of lesion	Outcome
Ecuador (1)	Herbal infusion	*Crotalaria Juncea*	Acute	Recovered
Hong Kong (4)	Herbal infusion	*Heliotropium Lasiocarpium*	VOD	1 died
India (8)	Herbal decoction and pills	*Heliotropium eichwaldii*	Acute cirrhosis	3 died
United Kingdom (1)	Herbal infusion	*Symphytum officinale*	Thrombotic VOD	
United States (3)	Herbal infusion	*Senecio longilobus/ Symphytum* sp.	Acute lesions	1 died 1 cirrhosis
West Indies (95)	Herbal infusion	Bush tea; *Crotalaria* and *Senecio*	Acute to cirrhosis	11% cirrhosis 27% died

VOD, Veno-occlusive disease.
Source: WHO Task Group on Pyrrolizidine Alkaloids (1988).

Table 4. Reported cases of human toxicity caused by pyrrolizidine alkaloid-contaminated cereal grain.

Country/number of cases	Name of plant	Nature of lesion	Outcome (cirrhosis/died)
Afghanistan (8000)	*Heliotropium Popovii Gillianum*	All stages; mostly acute to subacute	1600–2000 died
India (108)	*Crotalaria Nana*	Various stages of disease	Up to 63% died
Iraq (9)	Possibly *Senecio*	Acute	1 died
South Africa (11)	*Senecio flicifolius; S. Burchelli*	Centrilobular hemorrhages;	"Majority died"
(Former) U.S.S.R. (1000-1500)	*Heliotropium Lasiocarpum*	Acute lesions	13%–15% died; cirrhosis in many

Modified from WHO Task Group on Pyrrolizidine Alkaloids (1988).

newborn infant died of hepatic VOD (Roulet et al. 1988). The mother confirmed the daily consumption of an herbal infusion during pregnancy as an expectorant. Chemical analysis of the incriminated tea showed a concentration of 0.60 mg/kg senecionine (PA) (dry wt).

Reported cases of VOD of liver resulting from the consumption of herbal tea preparations has increased in the U.S. A 49-yr-old woman, who for 6 mon had consumed 1 qt/d of herbal tea known as MU-16 with comfrey pepsin pills, was diagnosed with VOD (Ridker et al. 1985). Analysis of tea and comfrey pepsin pills for PA content revealed a consumption of a minimum of 85 mg of PAs (15 μg/kg per day).

Other fatal cases of PA poisoning in children have been documented in Arizona (Huxtable 1980a,b). The plant identified with the herbal tea preparation was *Sencero longilobus*, a known source of hepatic PAs (Centers for Disease Control 1977; Huxtable 1980a,b). It is believed that many more cases of PA poisoning take place, but the condition passes unreported due to the deciduous nature of the disease.

Since 1970, a strong correlation between a high incidence of esophageal cancer in various geographic areas of the world and the consumption of tannin-rich teas or medicines has been revealed by many investigators (Dunham 1968; Morton 1972; Schoenberg et al. 1971).

O'gara et al. (1971), after surveillance of the use of herbal teas by 50 esophageal cancer patients in Curacao (West Indies), assayed the carcinogenic activity of decoctions made from 14 of the most commonly used plants. Of the plants assayed, reduced extracts of *Krameria ixina* (Krameriaceae), *Acacia villosa* (Leguminosae), and *Melochia tomentosa* (Sterculisceae) were shown to produce fibrosarcomas at the site of injection in 100% of NIH black rats. These plants were found to be alike in their richness of condensed tannins and related anthrocyanins. The tumorigenic properties of the tannin fractions were shown when tannin-free extracts produced no tumors in the rats (O'Gara et al. 1971, 1974).

Deliberate consumption of herbal teas containing PA is a public health problem common to many areas of Africa, South and Central America, and, in particular, to Jamaica, where hepatic VOD is endemic. Aside from *Senecio* and *Crotalaria* species, known to be consumed by the Jamaican population as "bush teas," other sources of hepatotoxic PAs consumed worldwide in the form of herbal teas or tonics include comfrey (*Symphytum officinale*), Russian comfrey (*Symphytum xuplandicum*), coltsfoot (*Tussilago farfara*), *Farfugium japonicum*, *Petasites japonicus*, borage (*Borago officinalis*), and *Eupatorium stoechadosmum*.

The low country of South Carolina (U.S.) is an area where the rate of esophageal cancer has attracted considerable scientific attention (Morton 1986). During plantation times, the poor inhabitants of this region habitually drank infusions of plants rich in tannins in an attempt to fight profuse diarrhea and dysentery, which were the leading causes of death at the beginning of the 1900s, and, this consumption of tannin-rich plants has contin-

ued. Plants such as wax myrtle (*Myrica cerifera*), blackberry (*Rubus trivialis*), unripe persimmon (*Diospyrus americana*), and longleaf pine (*Pinus palustris*) are consumed daily by the African-American community of the low country in the form of tea or wine or are used in cookery (Morton 1986). Further, tea drinking begins at an unusually early stage; in low-income families, a child of 6 mon is taken off milk and placed on tea. Green peanuts, including the bitter, tannin-rich testa, are consumed after being boiled unshelled in heavily salted water. Also, the deep red-brown decoction of the bark of the cherrybark oak (*Quercus falcata* var. *pagodaefolia*) is consumed as a beverage and used to color locally distilled "moonshine" whiskey. Extracts from this plant have been shown to produce fibrosarcomas at the site of injection in 100% of experimental rats. The tannin fraction extracted from this plant produced fibrosarcomas in 93% of experimental rats (Kapadia et al. 1976).

Aside from their carcinogenic potential in tannin-rich infusions, tannins have been shown to have antinutritive properties. The presence of tannins in high-tannin sorghums, as determined by animal feeding trials, has been shown to diminish their nutritional value (Cousins et al. 1981; Rostagno et al. 1973). This phenomenon is explained by their propensity to bind with proteins by means of both hydrogen bonding and hydrophobic interactions, with proteins thus limiting their digestibility (Butler et al. 1984). Tannins have also been reported to influence the absorption of Vitamin B_{12} in rats (Disler et al. 1975).

Hot water infusions prepared from the root bark of the sassafras tree (*Sassafras albidum*) have long been employed for tonics as well as for a variety of unsubstantiated therapeutic purposes. In South Carolina, where sassafras trees are common in open woods, many inhabitants also consume the fresh roots (Morton 1986). Safrole (4-allyl-1,2-methylenedioxybenzene), the major chemical constituent of the aromatic oil present in sassafras root bark, was shown to be a hepatocarcinogen in rats (Hagan et al. 1965; Long et al. 1963), and dihydrosafrole, one of its metabolic products, was shown to produce esophageal cancer in rats (Taylor et al. 1964). Even though the FDA in 1960 banned the use of safrole or any of its derivatives in foods, sassafras continues to be freely available in health food stores and similar outlets in the U.S. Consumers ingesting one cup of sassafras tea brewed from one tea bag containing 2.5 g of the bark could be ingesting as much as 200 mg of safrole, or the equivalent of 3.0 mg/kg body wt (Segelman et al. 1976). Exposure to this amount of safrole could represent a considerable health hazard, considering that a small total dose of 0.66 mg of safrole (approximately 66 mg/kg) administered subcutaneously over a period of 21 d to infant male mice produced hepatomas (Epstein et al. 1970). Exposed animals surviving for 1 yr had lymphomas, pulmonary adenomas, and adenocarcinomas. In addition, safrole is also a potent inhibitor of certain liver microsomal hydroxylating systems (Jaffe et al. 1968), a property that could lead to toxicity problems if drugs metabolized by these enzymes are administered together with sassafras tea.

Another important effect of herbal teas is their allergenic potential. Most of these teas are crude, complex mixtures that are neither uniformly prepared nor assayed for purity. The compositions of three commercially available herbal tea mixtures are shown in Table 2. Many contain a variety of allergens (as yet unidentified) that possess potentially adverse effects. Cases of anaphylactic shock or allergic rhinitis in atopic persons known to be sensitive to ragweed pollen have been reported after consumption of chamomile tea (*Matricaria chamomilla*) (Benner and Howard 1973).

Misidentification of wild herbal plants has also resulted in numerous fatalities, as in the case of an elderly couple in Washington who drank a home-prepared tea in which foxglove had been used instead of comfrey (a known source of hepatotoxic PAs) (Centers for Disease Control 1977). One hour after ingestion of the tea, the wife experienced nausea, vomiting, dizziness, and sweating, with death occurring before admission to the hospital. The husband was admitted to the hospital, where after treatment he remained stable for 17 hr; he then suddenly developed an episode of ventricular tachycardia that caused his death from refractory ventricular fibrillation.

B. Prevention

The presence of naturally occurring toxic and carcinogenic compounds in teas raises important concerns about product safety. Cases of human intoxication following consumption of herbal teas have been widely reported. Although none of the reported ingredients of tea mixtures belong to known plant sources of PAs and other biologically active compounds, the individual ingredients should be analyzed for the presence of toxins. The possibility that the PAs detected are present as contaminants or adulterants in the herbal preparation should not be neglected. It is also a possibility that intrinsic components of plants can be metabolized in the liver to mutagenic compounds. Identification and quantification of the compounds responsible for the observed mutagenic potentials would be necessary to better assess consumer risk when ingesting infusions from herbal preparations.

Plants contain compounds that have both beneficial (nutrition, disease prevention) and hazardous (toxic and carcinogenic) potentials. It is crucial that health scientists and consumers become informed of the relative risks associated with the consumption of food products in particular herbal teas.

C. Beneficial Effects of Teas

Tea, originating from China, is one of the world's oldest prepared beverages. It is extensively consumed throughout the world in the form of different tea extracts, including hot and cold infusions. The natural presence of flavonoids in plant foods has demonstrated a wide range of biochemical and pharmacological effects including antiinflammatory and antiallergic effects (Middleton and Kandaswami 1992). It was also reported that quercithin, kaempherol, and myricetin inhibited carcinogen-induced tumors in

rats and mice (Deschner et al. 1991; Wei et al. 1990). Quercithin, a major flavonoid in teas (*Camelia sinensis*), including black, green, and oolong teas, has been demonstrated to inhibit oxidation and cytotoxicity of low-density lipoproteins *in vitro* (De Whalley et al. 1990; Nagre Salvagyre and Salvagyre 1992) and to decrease cancer and cardiovascular diseases in humans.

Different teas have been analyzed for their antimutagenic and anticarcinogenic effects (Hertog et al. 1993; Yen and Chen 1994). Tea extracts, including black (fermented), oolong and pouchong (semifermented), and green (nonfermented) teas, were compared for their toxicity and mutagenicity in *Salmonella*/microsomal assay in the presence of different toxic and mutagenic chemicals (Yen and Chen 1994). All teas were found to inhibit mutagenicity, especially the semifermented teas (oolong and pouchong) in which up to 90% reduction in mutagenicity was observed. Thus, semifermented teas may be beneficial to humans for chemoprevention of mutations.

Summary

Plants have been used for medicinal purposes for centuries. Health-oriented individuals are turning to herbal teas as alternatives to caffeinated beverages such as coffee, tea, and cocoa and for low-caloric supplements. The popularity of herbal tea consumption has increased significantly during the past two decades in the U.S. Hundreds of different teas made up of varied mixtures of roots, leaves, seeds, barks, or other parts of shrubs, vines, or trees are sold in health food stores. Although chemists have been characterizing toxic plant constituents for over 100 years, toxicological studies of herbal teas have been limited and, therefore, the safety of many of these products is unknown.

Plants synthesize secondary metabolites that are not essential in the production of energy and whose role may be in the defense mechanisms as plant toxins to their interactions with other plants, herbivores, and parasites. Pyrrolizidine alkaloids (PAs) were among the first naturally occurring carcinogens identified in plant products, and their presence in herbal teas is a matter of public health significance. Some herbal tea mixtures and single-ingredient herbal teas have been analyzed for toxic/mutagenic potential by bioassay and chromatographic techniques. Numerous human and animal intoxications have been associated with naturally occurring components, including pyrrolizidine alkaloids, tannins, and safrole. Thus, the prevention of human exposure to carcinogens or mutagens present in herbal tea mixture extracts is crucial. Preparation of infusion drinks prepared from plants appears to concentrate biologically active compounds and is a major source of PA poisoning. The quantity and consumption over a long period of time is of major concern. It is recommended that widespread consumption of herbal infusions should be minimizaed until data on the levels and varieties of carcinogens, mutagens, and toxicants are made available.

References

Ames NB (1983) Dietary carcinogens and anticarcinogens. Science 221:1256–1264.

AOAC (1984) Official methods of analysis of the Association of Analytical Chemists. Association of Analytical Chemists, Arlington, VA, p 489.

Benner MH, Howard JL (1973) Anaphylactic reaction to chamomile tea. J Allergy Clin Immunol 52(5):307–308.

Betz JM, Eppley RM, Taylor WC, and Andrzejewski D (1994) Determination of pyrrolizidine alkaloids in commercial comfrey products (*Symphytum* sp.). J Pharm Sci 83:649–653.

Borchert P, Miller EC, Miller JA (1971) 1′-Hydroxysafrole, a new metabolite and 1′-acetoxysafrole, a reactive intermediate. Proc Am Assoc Cancer Res 12:34.

Borchert P, Wislocki PG, Miller JA, Miller EC (1973) The metabolism of the naturally occurring hepatocarcinogen safrole to 1′-hydroxysafrole and the electrophilic reactivity of 1′-acetoxysafrole. Cancer Res 33:575–530.

Bras G, Jelliffe DB, Stuart KL (1954) Veno-occlusive disease of the liver with nonportal type of cirrhosis occurring in Jamaica. Arch Pathol 57:285–300.

Bras G, Hill KR (1956) Veno-occlusive disease of the liver—essential pathology. Lancet 2:161–163.

Bras G, Berry DM, Gyorgy P (1957) Plants as etiological factor in veno-occlusive disease of the liver. Lancet 2:960–962.

Brown KS (1984) Chemical ecology of dehydropyrrolizidine alkaloids in adult Ithomiinae (Lepidoptera: Nymphalidae) Rev Bras Biol 44:435–440.

Bruggeman IM, Van der Hoeven JCM (1985) Induction of SCEs by some pyrrolizidine alkaloids in V79 Chinese hamster cells co-cultured with chick embryo hepatocytes. Mutat Res 142:209–212.

Butler GL, Riedl DJ, Lebryk DG, Blytt HJ (1984) Interaction of proteins with sorghum tannin: mechanism, specificity and significance. J Am Oil Chem Soc 61:916–920.

Candrian U, Luthy J, Graf U, Schlatter C (1984) Mutagenic activity of the pyrrolozidine alkaloids seneciphylline and senkirkine in *Drosophila* and their transfer into rat milk. Food Chem Toxicol 22:223–225.

Centers for Disease Control (1977) Poisoning associated with herbal teas: Arizona, Washington. Morbid Mortal Wkly Rep 267:10–12.

Chizzola R (1994) Rapid sample preparation technique for the determination of pyrrolizidine alkaloids in plant extracts. J Chromatogr 668:427–433.

Clark AM (1959) Mutagenic activity of the alkaloid heliotrine in *Drosophila*. Nature (London) 183:731–732.

Cousins BW, Tanksley TD, Knabe DA, Zebrowsila T (1981) Nutrient digestibility and performance of pigs fed sorghums varying in tannin concentration. J Anim Sci 53:1524.

Culvenor CCJ (1985) Pyrrolizidine alkaloids: some aspects of the Australian involvement. Trends Pharmacol Sci 6:18–24.

Culvenor CCJ, Edgar JA, Smith LW (1986) *Heliotropium lasiocarpum* Fisch and Mey identified as cause of veno-occlusive disease due to a herbal tea. Lancet 325: 978.

Deschner EE, Ruperto J, Wong G, Newmark HL (1991) Quercithin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis (Oxford) 7:1193–1196.

De Whalley CV, Rankin SM, Hoult JRS, Jessup W, Leake DS (1990) Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem Pharmacol 42:1743–1750.

Disler PB, Lynch SR, Charlton RW, Torrance JD, Bothwell TH, Walker RB, Mayet F (1975) The effects of tea on iron absorption. Gut 16:193–200.

Dorange JL, Delaforge M, Janiaud P, Padieu P (1977) Pouvoir mutagene de metabolites de la voie epoxydediol du safrol et d'analogues. Etude sur *Salmonella typhimurium*. C R Seanc Soc Biol Fil 171:1041.

Dunham LJ (1968) A geographic study of the relationship between oral cancer and plants. Cancer Res 28:2369–2371.

Epstein SS, Fujii K, Andrea J, Mantel N (1970) Carcinogenicity testing of selected food additives by parenteral administration to infant Swiss mice. Toxicol Appl Pharmacol 16:321–334.

Federal Register (1960) Refusal to extend effective date of statute for certain specified food additives. Fed Reg 25:12412 (Dec. 3).

Federal Register (1974) Substances prohibited from use in food. Fed Reg 39:26748–26749, 34172–34173.

Fox DW, Hart MC, Begerson PS, Jarret PB, Stillman AE, Huxtable RJ (1978) Pyrrolizidine (*Senecio*) intoxication mimicking Reye syndrome. J Pediatr 93: 980–982.

Furmanowa M, Guzewska J, Beldowska B (1983) Mutagenic effects of aqueous extracts of *Symphytom officinale* L. and its alkaloid fractions. J Appl Toxicol 3: 127–130.

Green RC, Christie CS (1961) Malformations in fetal rats induced by pyrrolizidine alkaloid, heliotrine. Br J Exp Pathol 42:369–378.

Green MH, Muriel WJ (1975) Use of repair-deficient strains of *Escherichia coli* and liver microsomes to detect and characterize DNA damage caused by the pyrrolizidine alkaloids heliotrine and monocrotaline. Mutat Res 28:331–336.

Hagan EC, Jenner PM, Jones WI, Fitzhugh OG, Long EL, Brouwer JG, Webb WK (1965) Toxic properties of compounds related to safrole. Toxicol Appl Pharmacol 7:18.

Hartmann T, and Zimmer M (1985) Organ-specific distribution and accumulation of pyrrolizidine alkaloids during the life history of two annual *Senecio* species. J Plant Physiol 122:67–80.

Hartmann T, Witte J (1994) In: Pollitier SW (ed) Alkaloids: Chemical and Biological Perspectives. Pergamon Press, New York, p 155.

Hertog MGL, Hollman PCH, Van de Putte B (1993) Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. J Agric Food Chem 41:1242–1246.

Hirono I, Mori H, Culvenor CCJ (1976) Carcinogenic activity of coltsfoot, *Tussilago farfara* L. Gann Monogr Cancer Res 67:125–129.

Hirono I, Mori H, Yamada K, Hirata Y, Haga M, Tatematsu H, Kanie S (1977) Carcinogenic activity of petasitenine, a new pyrrolizidine alkaloid isolated from *Petasites japonicus Maxim*. J Natl Cancer Inst 58:1155–1156.

Hirono I, Mori H, Haga M (1978) Carcinogenic activity of *Symphytum officinale*. J Natl Cancer Inst 61:865–869.

Hirono I, Mori H, Haga M, Fujii M, Yamada K, Takanashi H, Uchida E, Hosaka S, Ueno I, Matsushima I, Umezava K, Shirai A (1979) Edible plant containing carcinogenic pyrrolizidine alkaloids in Japan. In: Miller BC, et al. (eds) Naturally Occurring Carcinogens, Mutagens, and Modulators of Carcinogenesis. University Park Press, Baltimore, MD, pp 79–87.

Hirono I (1981) Natural carcinogenic products of plant origin. CRC Crit Rev Toxicol 9:235–277.

Hirono I, Ueno I, Aiso S, Yamaji T, Haga M (1983) Carcinogenic activity of *Farfagium japonicum* and *Senecio cannabifolius*. Cancer Lett 20:191–198.

Hodgson E, Philpot RM (1974) Interaction of methylenedioxyphenyl (1,3-benzidioxole) compounds with enzymes and their effect on mammals. Drug Metab Rev 3:231.

Hoveland CS (1993) Importance and economic significance of the *Acremonium endocytes* to performance of animals and grass plant. Agric Ecosyst Environ 44:3.

Huxtable RJ (1980a) Herbal teas and toxins: novel aspects of pyrrolizidine poisoning in the United States. Perspect Biol Med 24:2–14.

Huxtable RJ (1980b) Problems with pyrrolizidines. Trends Pharmacol Sci 1:299–303.

Huxtable RJ, Cooper R, Yan CC (1996) Pyrrolizidine alkaloids: relationships between structure, metabolism and toxicity. Presented at 13th Rocky Mountain regional meeting of the American Chemical Society, 9–12 June, Denver, CO (abstr. 77).

Ioannides C, Delaforge M, Park DV (1981) Safrole: its metabolism, carcinogenicity and interactions with cytochrome P-450. Food Cosmet Toxicol 19:657–666.

Jaffe H, Fujii K, Sengupta M (1968) *In vivo* inhibition of mouse liver microsomal hydroxylating systems by methylene dioxyphenyl insecticidal synergists and related compounds. Life Sci 7(1):1051–1062.

Janiaud P, Delaforge M, Levi P, Maume BF, Padieu P (1977) Metabolisme d'un hepatocancerogene naturel, le safrol. Etude chez le rat et dans des cultures de cellules hepatiques de rat, de l'action d'effecteurs sur plusieurs voies metaboliques et des formes de transport. Coll Int CNRS 286:431.

Johnson A, Molyneux RJ, Merrill GB (1985) Chemistry of toxic range plants: variation in pyrrolizidine alkaloid content of *Senecio, Amsinckia*, and *Crotalaria* species. J Agric Food Chem 33:50–55.

Kapadia GJ, Paul BD, Chung EB, Ghosh B, Pradham SN (1976) Carcinogenicity of *Camellia sinensis* (tea) and some tannin-containing folk medicinal herbs administered subcutaneously in rats. J Natl Cancer Inst 57(1):207–209.

Kingsbury JM (1983) The evolutionary and ecological significance of plant toxins. In: Keeler RF, Tu TA (eds) Handbook of Natural Toxins. Plant and Fungal Toxins, Vol. 1. Marcel Dekker, New York, pp 675–706.

Kirby KS (1960) Induction of tumors by tannin extracts. Br J Cancer 14:147.

Korpassy B, Mosonyi M (1950) Liver tumors induced in rats by prolonged subcutaneous administration of tannic acid solutions. Br J Cancer 4:411–420.

Korpassy B (1961) Tannins as hepatic carcinogens. Prog Exp Tumor Res 2:245–290.

Kumana CR, Lin HJ, Wu PC, Todd D (1983) Hepatic veno-occlusive disease due to toxic alkaloid in herbal tea. Lancet 2:1360–1361.

Larkin T (1983) Herbs are more toxic than magical. FDA Consumer 17:5–10.

Liddell RJ, Logie CG (1993) 7-Angelyl-1-methylenepyrrolizidines from *Senecio chrysocoma*. Phytochemistry 34(4):1198–1199.

Long EL, Nelson AA, Fitzhugh OG, Heasen WH (1963) Liver tumors produced in rats by feeding safrole. Arch Pathol 75:595.

Manteiga R (1991) Toxic and mutagenic potentials of herbal teas. M.S. thesis, University of Arizona, Tucson.

Maron DM, Ames BN (1983) Revised methods for the *Salmonella* mutagenicity test. Mutat Res 113:173–215.

Mattocks AR (1968) Toxicity of pyrrolizidine alkaloids. Nature 217:723–728.

Mattocks AR (1969) Dihydropyrrolizidine derivatives from unsaturated pyrrolizidine alkaloids. J Chem Soc Sect C Org Chem 19:2698–2700.

Mattocks AR (1970) Role of acid moieties in the toxic actions of pyrrolizidine alkaloids on liver and lung. Nature 228:174–175.

Mattocks AR, Bird I (1983) Pyrrolic and N-oxide metabolites formed from pyrrolizidine alkaloids by hepatic microsomes *in vitro*: relevance to *in vivo* hepatotoxicity. Chem Biol Int 43:209–222.

Mattocks AR, Driver HE (1987) Toxic actions of senaetnine, a new pyrrolizidine-type alkaloid, in rats. Toxicol Lett 38:315–319.

McGee JO, Patrick RS, Wood CB, Blumgart LH (1976) A case of veno-occlusive disease of the liver in Britain associated with herbal tea consumption. J Clin Pathol 29:788–794.

McLean EK (1970) The toxic actions of pyrrolizidine (*Senecio*) alkaloids. Pharmacol Rev 22:429–482.

Merino FC, Amesty C, Grand R (1979) Immunological studies in a Venezuelan high gastric cancer risk population. Cancer Detect Prev 2(3):373–389.

Middleton E, Kandaswami C (1992) Effects of flavonoids on immune and inflammatory cell functions. Biochem Pharmacol 43:1167–1179.

Mirochnick MF (1938) Clinical course and etiopathogenesis of toxic hepatites with ascites. In: Medical Institute, Scientific Papers of the Second Therapeutic Clinic, Vol. I. Medical Literature Publishers, Tashkent.

Mohabbat O, Srivastava RN, Younos MS, Sediq GG, Menzad AA, Aram GN (1976) An outbreak of hepatic veno-occlusive disease in Northwestern Afghanistan. Lancet 2:269–271.

Molyneux RJ, Roitman JN (1980) Specific detection of pyrrolizidine alkaloids on thin-layer chromatograms. J Chromatogr 195:412–415.

Morton JF (1970) Tentative correlation of plant usage and esophageal cancer zones. Econ Bot: 217–226.

Morton JF (1972) Further associations of plant tannins and human cancer. Q J Crude Drug Res 12:1829–1840.

Morton JF (1973) Plant products and occupational materials ingested by esophageal cancer victims in South Carolina. Q J Crude Drug Res 13(1):2005–2022.

Morton JF (1980) Search for carcinogenic principles. In: Swain T, Kleiman R (eds) Recent Advances in Phytochemistry, Vol. 43. The Resource Potential in Phytochemistry. Plenum Press, New York, pp 53–73.

Morton JF (1986) The potential carcinogenicity of herbal tea. Environmental carcinogens review. J Environ Sci Health 2:203–223.

Mossoba MM, Lin HS, Anderzejeweski D, Sphon JA (1994) Application of gas chromatography/matrix isolation/Fourier transform infrared spectroscopy to the identification of pyrrolizidine alkaloids from comfrey root (*Symphytum officinale* L). J Assoc Anal Chem Int 77(5):1167–1174.

Nagre-Salvagyre A, Salvagyre R (1992) Quercithin prevents the cytotoxicity of oxidized low density lipoproteins by macrophages. Free Radical Biol Med 112:101–106.

O'Gara RW, Lee C, Morton JF (1971) Carcinogenicity of extracts of selected plants from Curacao after oral and subcutaneous administration to rodents. J Natl Cancer Inst 46:1131–1137.

O'Gara RW, Lee C, Morton JF, Kapadia GJ, Dunham LJ (1974) Sarcoma induced in rats by extract of plant and by fractionated extracts of *Krameria ixina*. J Natl Cancer Inst 52(2):443–448.

Peele JD, Oswald EO (1978) Metabolism of the proximate carcinogen 1'-hydroxysafrole and the isomer 3'-hydroxyisosafrole. Bull Environ Contam Toxicol 19:396.

Peterson JE, Jago MV (1980) Comparison of the toxic effects of dehydroheliotridine and heliotrine in pregnant rats and their embryos. J Pathol 131:339–355.

Peterson JE, Culvenor CCJ (1983) Plant and fungal toxins. In: Keeler RF, Tu AT (eds) Handbook of Natural Toxins, Vol. 1. Marcel Dekker, New York, pp 637–681.

Porter JK (1994) Analysis of endophyte toxins: fescue and other grasses toxic to livestock. J Anim Sci 73(3):871–880.

Pradhan SN, Chung EB, Ghosh B, Paul BD, Kapadia GJ (1974) Potential carcinogens I. Carcinogenicity of some plant extracts and their tannin-containing fractions in rats. J Natl Cancer Inst 52(5):1579–1582.

Ridker PM, Ohkuma S, Mcdermott WV, Trey C, Huxtable R (1985) Hepatic venoocclusive disease associated with the consumption of pyrrolizidine-containing daily supplements. Gastroenterology 88:1050–1054.

Rostagno HW, Featherson WR, Rogler JC (1973) Studies on the nutritional value of sorghum grains with varying tannin contents for chicks. Poult Sci 52:765–772.

Roulet M, Laurini M, Calame A (1988) Hepatic veno-occlusive disease in newborn infant of a woman drinking herbal tea. J Pediatr 112:433–436.

Schneider D, Boppre M, Zweig J, Horsley SB, Bell TW, Meinwald J, Hansen K, Diehl EW (1982) Scent organ development in *Creatonotos* moths: regulation by pyrrolizidine alkaloids. Science 215:1264–1265.

Schoenberg BS, Bailar JC, Fraumeni JF (1971) Certain mortality patterns of esophageal cancer in the United States. J Natl Cancer Inst 46:63–73.

Schoental R, Head MA, Peacock PR (1954) *Senecio* alkaloids: primary liver tumors in rats as a result of treatment with (1) mixtures of alkaloids from *S. jacobaea* Lin., (2) retrorsine, (3) isatidine. Br J Cancer 8:458–465.

Schoental R (1968) Toxicology and carcinogenic action of pyrrolizidine alkaloids. Cancer Res 28:2237–2246.

Segelman AB, Segelman FP, Kerliner J, Duane RF (1976) Sassafras and herb tea: potential health hazards. J Am Med Assoc 236:477.

Segi M (1975) Tea-gruel as a possible factor for cancer of the esophagus. Gann 66: 199–202.

Selzer G, Parker RGF (1951) *Senecio* poisoning exhibiting as Chiari's syndrome: a report of 12 cases. Am J Pathol 27:885–907.

Siegel RK (1976) Herbal intoxication. J Am Med Assoc 236:473–476.

Singleton VL (1981) Naturally occurring food toxicants: phenolic substances of plant origin common in foods. Adv Food Res 27:149–242.

Smith LW, Culvenor CCJ (1981) Plant sources of hepatotoxic pyrrolizidine alkaloids. J Nat Prod (Lloydia) 44:129-152.

Stelljes ME, Kelly RB, Molyneux RJ, Seiber JN (1991) GC-MS determination of pyrrolizidine alkaloids in four *Senecio* species. J Nat Prod (Lloydia) 54(3):759-773.

Street HE, Cockburn W (1972) Secondary plant products. In: Plant Metabolism. Pergamon Press, London, pp 186-211.

Stuart KL, Bras G (1957) Veno-occlusive disease of the liver. Q J Med 26:291-315.

Swanson AB, Chambliss DD, Bolinquist JC, Miller EC, Miller JA (1979) The mutagenicities of safrole, estragole, eugenol, trans-anethole and some of their known or possible metabolites for *Salmonella typhimurium* mutants. Mutat Res 60:143.

Swick RA (1984) Hepatic metabolism and bioactivation of mycotoxins and plant toxins. J Anim Sci 58(4):1017-1027.

Tandon BN, Tandon HD, Tandon RK, Narendranathan M, Joshi YK (1976) Epidemic of veno-occlusive disease in Central India. Lancet 2:271-272.

Taylor JM, Jenner PM, Jones WI (1964) A comparison of the toxicity of some allyl, propenyl and propyl compounds in the rat. Toxicol Appl Pharmacol 6:378.

Tyler VE (1987) Comfrey. In: The Honest Herbal. George F. Stickley, Philadelphia, pp 77-80.

Van Dam NM, Witte L, Theuring C, Hartmann T (1995) Distribution, biosynthesis and turnover of pyrrolizidine alkaloids in *Cynoglossum officinale*. Phytochemistry (Oxford) 39(2):287-292.

Volimer JJ, Steiner NC, Larsen GY, Muirhead KM (1987) Pyrrolizidine alkaloids: testing for toxic constituents of comfrey. J Chem Educ 64:1027-1030.

Wei H, Tye L, Bresmik E, Birt DF (1990) Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. Cancer Res 50:499-502.

Weinstraub S (1960) Stramonium poisoning. Postgrad Med 27:364-371.

Were OA, Benn M (1991) Pyrrolizidine alkaloids from *Senecio hadiensis*. J Nat Prod (Lloydia) 54(2):491-499.

White RD, Krumperman PH, Cheeke PR, Buhler DR (1983) An evaluation of acetone extracts from six plants in the Ames mutagenicity test. Toxicol Lett 15: 25-31.

WHO Task Group on Pyrrolizidine Alkaloids (1988) Pyrrolizidine alkaloids. In: Environmental Health Criteria 80. World Health Organization, Geneva.

Williams GM, Weisburg JH (1986) Chemical carcinogens. In:Klaassen CD, Amdur MO, Doull J (eds) Casarett and Doull's Toxicology. The Basic Science of Poisons, 3rd ed. McMillan, New York, p 128.

Wislocki PG, Borchert P, Miller JA, Miller EC (1976) The metabolic activation of the carcinogen 1'-hydroxysafrole *in vivo* and *in vitro* and the electrophilic reactivities of possible ultimate carcinogens. Cancer Res 36:1686.

Yamanaka H, Nagao M, Sugimura T, Fureya T, Shirai A, Matsushima T (1979) Mutagenicity of pyrrolizidine alkaloids in the *Salmonella*/mammalian microsome test. Mutat Res 68:211-216.

Yen GC, Chen HY (1994) Comparison of antimutagenic effect of various tea extracts (green, oolong, pouchong, and black tea). J Food Prot 57(1):54-58.

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Effects of Pesticides on Amphibians and Reptiles in Sub-Saharan Africa

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Contents

I. Introduction

General concern about the effects of insecticides and herbicides on nontarget amphibians and reptiles has been expressed in the world's developed countries, where pesticides have been widely used, especially for agricultural pest control. Impact of usage in the United Kingdom on amphibians has been addressed in a series of papers by Cooke (1972a,b, 1977, 1981), and the toxicological literature covering this group has been reviewed by Power et al. (1989). Effects of pesticides and other environmental contaminants on reptiles, based mainly on work in the United States, was reviewed by Hall (1980), who showed that reptiles are susceptible to such organochlorine (OC) insecticides as DDT, dieldrin, heptachlor, and toxaphene. He later concluded that toxicity testing of amphibians and reptiles for purposes of chemical registration was difficult because of intraspecific variation (Hall and Henry 1992).

Most previous studies have been carried out in temperate regions, with

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