1. Exposure Data

The kava plant is indigenous to Oceania (Lebot et al., 1997; Ramzan & Tran, 2004) and has been used both ceremonially and recreationally in certain cultures of the South Pacific for at least 1500 years. Europeans documented its use when they travelled to Polynesia in the 18th century (WHO, 2007). The cultural history of the use of kava has been reviewed by Singh (1992).

In the past, traditional use of kava was widespread, but, in certain cultures, custom determined who could use kava and for what purposes. In recent years, as part of the processes of modernization, major changes have occurred with regard to who uses kava, where and how it is consumed. In some places, kava is now being consumed much like alcohol in western countries, as a beverage that is drunk socially (McDonald & Jowitt, 2000).

1.1 Identification of the agent

1.1.1 Botanical data

(a) Nomenclature

Chem. Abstr. Name: Kava-kava resin (8Cl)
Botanical name: Piper methysticum G. Forst
Family: Piperaceae
Genus: Piper
Plant part: Rhizome

(b) Description

See Fig. 1.1

The tropical shrub Piper methysticum is a hardy, fairly succulent, slow-growing perennial that is widely cultivated in Oceania. The species is sterile and reproduces asexually. Due to its traditional use as a ritual beverage known for promoting relaxation and a sense of well-being, the kava plant spread widely throughout Oceania, in Polynesia, Melanesia, and the Federated States of Micronesia (Norton & Ruze, 1994; NTP, 2012).

The leaves are heart-shaped, pointed, 8–25 cm in length, and smooth and green on both sides. Kava is cultivated for its rootstock (rhizome), also

1.1.2 Taxonomy

Genus: Piper
Species: Piper methysticum

1.1.3 Pharmacology

Kava contains several active components, including tetrahydrocannabinol (THC), 5-hydroxy-3-methoxytryptamine (5-MeO-DMT), and piperic acid B (Piperine).

1.1.4 Chemistry

Kava contains several alkaloids, including kavain, dihydrokavain, and piperine.

1.1.5 Toxicology

Kava has been associated with several adverse effects, including gastrointestinal symptoms, liver function abnormalities, and sleep disturbances.

1.1.6 Efficacy

Kava has been shown to have anxiolytic and sedative effects, as well as mild anti-inflammatory and anti-oxidant effects.

1.1.7 Adverse Effects

Kava has been associated with a range of adverse effects, including gastrointestinal symptoms, liver function abnormalities, and sleep disturbances.

1.1.8 Interactions

Kava may interact with a range of medications, including blood pressure medications, and anti-depressants.

1.1.9 Limits

Kava has been shown to be safe when used in recommended doses.

1.1.10 Special Considerations

Kava should not be used by individuals with a history of liver disease, and it should be used with caution in individuals with a history of gastrointestinal symptoms.

1.1.11 Other Considerations

Kava is listed as a Schedule IV drug in the United States, and its use may be restricted in certain countries.

1.1.12 Conclusions

Kava has a range of potential therapeutic effects, but its use should be monitored closely to ensure safety and efficacy.
referred to as the stump. The stump is knotty, thick, and sometimes tuberous and often contains holes or cracks created by partial destruction of the parenchyma. A fringe of lateral roots up to 2–3 m in length extends from the pithy rhizome. The roots comprise a multitude of ligneous fibres and consist of > 60% starch. Rhizome colour varies from white to dark yellow, depending upon the amount of kavalactones contained in the lemon-yellow resin. The plant is usually harvested when it is about 2–2.5 m in height (Singh, 1992; Lebot et al., 1997; NTP, 2012).

The cultivation and selection of kava has produced numerous varieties or cultivars recognized by differences in the internodes (space between stem joints), colour of stems, intensity of leaf colour, and quality of the root. Different varieties are classified, named, and used for different purposes by the indigenous people (NTP, 2012).

The dried rhizome consists of irregular, transverse and longitudinal pieces, varying considerably in size and shape: 3–20 cm in length and 1–5 cm in diameter. The outer surface is light yellowish or greyish-brown, longitudinally wrinkled, with large, whitish, circular root scars. The fracture is coarsely fibrous, the inner surface is yellow-white, with thin bark, radiate xylem, and large pith (WHO, 2004).

1.1.2 Chemical constituents and their properties

Analysis of the composition of kava rhizome indicates that the fresh material is on average 80% water. When dried, the rhizome consists of approximately 43% starch, 20% fibres, 12% water, 3.2% sugars, 3.6% proteins, 3.2% minerals, and 15% kavalactones, although the kavalactone component can vary between 3% and 20% of the dry weight of the rhizome, depending on the age of the plant and the cultivar. The bioactive principles of kava rhizome are mostly, if not entirely, contained in the lipid-soluble resin. The compounds of greatest pharmacological interest are the substituted α-pyrones or kavapyrones, commonly known as kavalactones. At least 15 lactones have been isolated from kava rhizome. The following six compounds are present in the highest concentrations and account for approximately 96% of the lipid resin: kavain, dihydrokavain, yangonin, desmethoxyyangonin, methysticin, and dihydromethysticin (see Fig. 1.2). Other constituents of kava include chalcones and other flavanones, and conjugated diene ketones (Shulgin, 1973; Dentali, 1997; WHO, 2004; NTP, 2012).

In the past, “kavain” has been used to indicate a racemic mixture resulting from chemical synthesis, and “kawain” for the naturally
occurring compound, which is a dextro-isomer. Currently, the two terms, kavain and kawain, are frequently used interchangeably in the scientific literature, but the term kavain has started to supersede kawain (Singh, 2004a).

The chemistry of kava and kavalactones has been reviewed in detail by Ramzan & Tran (2004).

1.1.3 Technical and commercial products

Kava biomass is normally sold as the rhizome, with the periderm and roots removed. The peeled rhizome is also the desired material for solvent extraction to produce kava extracts. Kava may also be sold as an unpeeled rhizome covered with the cork or with the roots attached. Peelings from the root and stump have also been used in commerce (Morgan et al., 2005). Powdered forms of rhizome are available in commercial markets in Fiji and have been described to be adulterated to the extent that they only contain 71–78% of the expected active constituents (Clough et al., 2000). Singh (2004a) mentioned adulteration with sawdust, flour, or soil. Adulteration of kava with plants resembling the genuine kava, but lacking the kavalactones and the distinct kava odour has also been reported. The main “false kava” species are P. auritum and P. aduncum (Singh, 2004b).

Very few data exist on kava contamination by bacteria or with mycotoxins (Teschke et al., 2011). A study on ochratoxin A contamination found concentrations of 3.0 ng/g in one sample of kava root (Trucksess et al., 2006). The level of contamination with aflatoxin B₁ in four samples of ground kava was 0.5 ng/g (Weaver & Trucksess, 2010).

The part of the plant used, processing techniques, and specifically the extraction solvent and the ratio between solvent/plant material in the case of kava extracts, may have considerable

Fig. 1.2 Structures of the major kavalactones occurring in kava rhizome

Compiled by the Working Group
influence on the chemical composition of the end product. For example, the alkaloid pipermethystine was not detectable in some commercial kava extracts (Teschke et al., 2011).

[The Working Group noted that the influences on composition mentioned above may hinder the comparison of studies, especially if the applied kava material was not specified exactly.]

1.2 Analysis

The chemical analysis and quality control of both kava and its extracts obtained by aqueous acetone or aqueous methanol, and supercritical fluid extraction – typically with carbon dioxide modified with methanol as solvent – were reviewed by Bilia et al. (2004). Both gas chromatography (GC) and high-performance liquid chromatography (HPLC) can be used for the analysis of kavalactones with some advantages and disadvantages for each method. Using GC analysis, methysticin and yangonin, which are two of the major components, are generally not separated. In addition, the high temperature of the injection port causes the decomposition of methysticin. Concerning HPLC analyses, reversed-phase separation is generally better because it is highly reproducible with a very low detection limit for all compounds even if the quantitative analysis of the kavalactones by HPLC needs to be carried out in the absence of light to prevent the cis/trans isomerization of yangonin (Bilia et al., 2004). Besides various chromatographic approaches reviewed by Bilia et al. (2004), near infrared spectroscopy and nuclear magnetic resonance spectroscopy have been suggested to directly determine kavalactones without the need for separation (Table 1.1).

1.3 Use

1.3.1 Indications

(a) Medicinal use

According to WHO (2004), the medicinal uses supported by clinical data are short-term symptomatic treatment of mild states of anxiety or insomnia due to nervousness, stress or tension; the medicinal uses described in pharmacopoeias and in traditional systems of medicine are to induce relaxation, reduce weight, and treat fungal infections. Uses described in traditional medicine, but not supported by experimental or clinical data, are treatment of asthma, common cold, cystis, gonorrhoea, headaches, menstrual irregularities, urinary infections, and warts.

The German Commission E has approved kava for use in conditions of nervous anxiety, stress, and restlessness (Anonymous, 2000).

(b) Traditional food and recreational use

A local traditional drink, also known under the name kava, is obtained from the root or rhizome of the kava plant. The kava drink is made from water extracts, with water-insoluble substances made available to the drinker by emulsification, which may be accomplished by pounding or chewing of the rhizome (WHO, 2007).

On some islands in the South Pacific, fresh kava root or rhizome is used to prepare the traditional drink, while on others it is the dried and ground roots or rhizomes that are used. For fresh preparations, the root is chewed by young women, who spit the juice into the kava bowl without swallowing it themselves. The juice is then mixed with water or coconut milk and further processed. Most people drink only the water extracts of kava. This is obtained by adding water to kava roots which are finely ground and then filtered using cheese-cloth (WHO, 2007).

The kava drink has been described to have a psychoactive activity, and potency can vary
<table>
<thead>
<tr>
<th>Sample matrix</th>
<th>Analyte/purpose of analysis</th>
<th>Sample preparation</th>
<th>Assay method</th>
<th>Detection limit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum and urine</td>
<td>Kavain and metabolites/metabolism</td>
<td>Glucuronidase treatment, extraction with dichloromethane:diethylether (7:3, v:v)</td>
<td>HPLC-DAD and LC-MS</td>
<td>1 ng/mL</td>
<td>Tarbah et al. (2003)</td>
</tr>
<tr>
<td>Urine</td>
<td>Kavalactones/metabolism</td>
<td>Chloroform extraction</td>
<td>GC-MS, HPLC</td>
<td>NA</td>
<td>Duffield et al. (1989)</td>
</tr>
<tr>
<td>Kava root from botanical supplier</td>
<td>Ochratoxin A/contamination</td>
<td>Immunoaffinity column cleanup</td>
<td>HPLC-FD</td>
<td>NA</td>
<td>Trucksess et al. (2006)</td>
</tr>
<tr>
<td>Dried kava roots</td>
<td>Kavain and other major kavalactones/isolation</td>
<td>Ethanol extraction</td>
<td>HSCCC</td>
<td>NA</td>
<td>Schäfer &amp; Winterhalter (2005)</td>
</tr>
<tr>
<td>Commercial kava extract, and kava finely powdered</td>
<td>Kavalactones and a range of other compounds/quality assessment</td>
<td>Solution with DMSO-\textit{d}_6</td>
<td>NMR</td>
<td>NA</td>
<td>Bilia et al. (2002)</td>
</tr>
<tr>
<td>Kava root extract</td>
<td>Kavalactones/structural elucidation</td>
<td>Extraction with methylene chloride</td>
<td>NMR</td>
<td>NA</td>
<td>Dharmaratne et al. (2002)</td>
</tr>
<tr>
<td>Kava dry extracts</td>
<td>Kavain and total kavalactones/routine quality control</td>
<td>None</td>
<td>NIRS</td>
<td>NA</td>
<td>Gaub et al. (2004)</td>
</tr>
<tr>
<td>Food supplements containing kava</td>
<td>Total kavalactones/regulatory control</td>
<td>Solution in ethanol, buffer addition</td>
<td>NMR</td>
<td>NA</td>
<td>Monakhova et al. (2013)</td>
</tr>
</tbody>
</table>

DMSO, dimethyl sulfoxide; GC, gas chromatography; HPLC, high-performance liquid chromatography; HPLC-DAD, high-performance liquid chromatography with diode-array detection; HPLC-FD, high performance liquid chromatography with fluorescence detection; HSCCC, high-speed counter-current chromatography; LC, liquid chromatography; MS, mass spectrometry; NA, not applicable; NIRS, near infrared spectroscopy; NMR, nuclear magnetic resonance spectroscopy; v:v, volume:volume.
considerably. Kava drinking initially produces a slight numbing of the tongue. Delayed effects have been described as relief of fatigue, reduction of anxiety, and production of a pleasant, cheerful, and sociable attitude in the drinker (WHO, 2007).

The consumption of kava is part of everyday life on islands such as Fiji, Tonga, and Vanuatu, and occurs during important events or social gatherings (Singh, 1992).

It is difficult to compare the psychopharmacological effects of kava between published studies as methods of preparation, means of ingestion, and the potency and quantity of dosages actually consumed vary considerably (Cairney et al., 2002).

Kava bars, at which prepared kava can be purchased to drink on the spot or to take away, are an increasingly common feature throughout Oceania (McDonald & Jowitt, 2000).

(c) Non-traditional food use

Non-traditional kava products are marketed in Europe and North America typically as food or dietary supplements in tablet form (Morris & Avorn, 2003; Teschke & Lebot, 2011). Interestingly, these food supplements are often marketed over the internet (Morris & Avorn, 2003). In some countries this may be due to difficulties with regulatory acceptance. For example, in Europe this practice is illegal, but kava products are nevertheless available (Monakhova et al., 2013).

(d) Cosmetic use

Kava extracts from various parts of the plant may be used as skin-conditioning agents in cosmetics. However, the USA Cosmetic Ingredient Review expert panel concluded that the available data were insufficient to support the safety of kava extracts for cosmetic use (Robinson et al., 2009).

1.3.2 Dosage

(a) Medicinal use

The comminuted crude drug and extracts are used for oral use. Daily dosage for crude drug and extracts is equivalent to 60–210 mg of kavalactones (WHO, 2004). The recommended oral dose for use of commercial kava extracts as an anxiolytic is 50–70 mg of kavalactones, two to four times per day and, as a hypnotic, 150–210 mg in a single oral dose before bedtime (Bilia et al., 2002b).

The pharmaceutical industry was primarily interested in the organic solvent (such as 95% ethanol or acetone) extracts of kava containing the organic compounds of commercial interest. Some marketed products, referred to as “synthetic,” consist of a single kavalactone, L-kavain (WHO, 2007).

A review of standardized kava brands in the USA found an approximate equivalence of actual [measured] and labelled amounts of kavalactones in 13 products that listed amounts of constituents. Kavalactones per tablet or capsule ranged from 50 to 110 mg. Two brands that did not label amounts of constituents contained 10–15 mg per tablet or capsule (Ulbricht et al., 2005).

Typical usage has ranged from 70 to 280 mg of kavalactones per day as a single bedtime dose or divided doses (60–120 mg of kavalactones per day). Many practitioners allegedly start at a lower dose and titrate up as needed (Ulbricht et al., 2005).

(b) Traditional food and recreational use

Only rough estimations exist on the dosage of traditional food and in recreational use of kava. Heavy consumers may drink the equivalent of at least 610 g/week of kava powder, which, with an estimated kavalactone content of 12.5%, may equate to approximately 76 g of lactones per week or more than 50 times the recommended therapeutic dose (Cairney et al., 2002).
In Arnhem Land, Australia, weekly per capita consumption was estimated as 145 g of powder for 1989–90 and 368 g of powder for 1990–91. When seven cups of 100 mL are consumed in 1 hour, about 3.8 g of lactones may be consumed. In a detailed review of the literature on weekly consumption levels and possible lactone contents, the estimations encompassed a wide variation from 39 to 1840 g of kava powder consumed, and from 4.1 g to 188.6 g of lactones consumed per week (Clough et al., 2000).

Typical dosage of dried root or by decoction was reported to be 6–12 g per day (Morgan et al., 2005).

(c) Non-traditional food use

The Dietary Supplements Label Database lists 11 products that contain kava as active ingredient in amounts of 60–1000 mg. Of the 11 products, 4 are listed as discontinued (NLM, 2012).

Kava food supplements, illegally sold over the internet in Germany, contained 8–10 mg of kava-lactones per capsule (Monakhova et al., 2013).

(d) Cosmetic use

The Cosmetic, Toiletry, and Fragrance Association (CTFA) provided a use concentration of 0.0001–0.01% for leaf/root/stem extract, and of 0.1% for root extract (Robinson et al., 2009).

1.4 Production, sales, and consumption

1.4.1 Production

(a) Production process

Kava production including cultivation, diseases and pests, harvesting and processing has been reviewed by Singh (2004b).

(b) Production volume

Kava was one of the most extensively used herbal products in the USA in the 1990s (NTP, 2012). According to Morris & Avorn (2003), sales of kava were US$ 69 million in 2000. In 2003, 62 retail sites were identified that sold kava over the internet (Morris & Avorn, 2003).

In Australia, trade in kava rhizome in Arnhem Land was approximately 28 tonnes in 1992, and between 27 and 36 tonnes in 1997. At the end of 1999, by which time trade in kava was illegal, trade was estimated to be 20 tonnes, while in 2000 the trade was approximately 15 tonnes (Clough, 2003).

1.4.2 Sales

By the mid-1990s, North Americans, Europeans, and Australians had begun using kava products as an alternative medicine and herbal relaxant. Commercial kava bars promoted recreational kava drinking, which can often occur for extended periods. Drug stores and supermarkets offered a variety of kava products in pill, capsule, tea, and liquid form. In addition, powdered kava root was available by mail order from several internet sites. Most of this exported kava derived from Fiji and Vanuatu, and to a lesser extent, Samoa and Tonga (Lindstrom, 2004). Kava abuse has been reported, especially in Pacific Island nations, leading to significant health and social problems (McDonald & Jowitt, 2000; Rychetnik & Madronio, 2011).

Current use in North America, Europe, and Australia may have been influenced by regulatory measures (see Section 1.6) and reports of adverse events in the popular press.

According to the 2012 Nutrition Business Journal Annual Report, kava was the 36th best-selling dietary supplement in the USA in 2011. There has been a considerable decline in kava sales in the USA from US$ 52 million in 2000 to US$ 17 million in 2004. Sales remained at a similar level between US$ 18 and 22 million
during 2005–2011, and then increased to US$ 31 million in 2011 (Fig. 1.3; Nutrition Business Journal, 2010, 2012). Total global sales of kava (Piper methysticum) as an herbal supplement were US$ 8 million in 2012, and appreciable sales volumes occurred in the USA (US $3 million), Brazil (US$ 2 million), and Hungary (US$1 million) (IMS Health, 2012).

[The Working Group suggested that prohibition in some countries may have resulted in increases in unrecorded sales of kava, e.g. unrecorded individual imports, or illegal sales.]

1.4.3 Consumption

Consumers of products specified in Section 1.3 are exposed to kava. No literature about the degree of population-based exposure to kava was available to the Working Group. [The Working Group estimated that current exposure to kava was expected to be a fraction of what it was in the previous decade due to withdrawal of marketing authorization in many countries (see Section 1.6).]

Fig. 1.3 Sales of kava as a dietary supplement in the USA

1.5 Occupational exposure

No specific studies on occupational exposure were available to the Working Group. It can be assumed that workers in kava production for food, cosmetic, or medicinal use may be exposed.

1.6 Regulations and guidelines

Several cases of liver damage have been associated with exposure to kava in Europe, and have led to withdrawal of the product license (NTP, 2012). Reviews on the cases of adverse effects potentially caused by exposure to kava have been compiled by Schmidt et al. (2005) (detailed analysis of 83 cases), as by WHO (2007) (analysis of 93 cases). Speculations about the causes of the adverse effects included the use of less expensive stem peelings in commercial materials instead of the usual peeled rhizomes (Teschke et al., 2011).

Sales of kava have been suspended or withdrawn in several countries, including Australia, Canada, France, Germany, Spain, and Switzerland, and due to reported association with hepatotoxicity in humans (Russmann et al., 2001, 2003; Campo et al., 2002; De Smet, 2002; Parkman, 2002; Clough et al., 2003; Humberston et al., 2003; Teschke et al., 2003; Ulbricht et al., 2005; NTP, 2012).

Although sales of kava were not regulated or controlled in the USA in 2012 (NTP, 2012), the Food and Drug Administration (FDA) had issued a public warning in 2001 that kava might be associated with serious liver damage, including hepatitis, cirrhosis, and liver failure (FDA, 2002). The regulatory action taken by various countries around the world from the year 2000 after concerns about hepatotoxicity is summarized in WHO (2007). Current regulatory status was summarized by Teschke & Lebot (2011), and included suggested chemical standards and agricultural standardizations. WHO (2004) provided some guidelines for the quality control of kava (see Table 1.2).

### Table 1.2 Guidelines for dried kava rhizome

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
<td>Not less than 3.5% kavapyrones [kavalactones], as determined by IR spectroscopy</td>
</tr>
<tr>
<td>Identity tests</td>
<td>Macroscopic, microscopic and microchemical examinations, and TLC for the presence of characteristic unsaturated α-pyrones known as kavapyrones [kavalactones]</td>
</tr>
<tr>
<td>Microbiological purity, heavy metals, radioactive residues</td>
<td>Limits according to WHO guidelines on quality control methods for medicinal plants</td>
</tr>
<tr>
<td>Foreign organic matter</td>
<td>Not more than 2%</td>
</tr>
<tr>
<td>Total ash</td>
<td>Not more than 8%</td>
</tr>
<tr>
<td>Acid-insoluble ash</td>
<td>Not more than 1.5%</td>
</tr>
<tr>
<td>Water-soluble extractive</td>
<td>Not less than 5%</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>Not more than 12%</td>
</tr>
<tr>
<td>Pesticide residues</td>
<td>Aldrin and dieldrin not more than 0.05 mg/kg. For other pesticides, general guidelines apply.</td>
</tr>
</tbody>
</table>

IR, infrared; TLC, thin-layer chromatography
From WHO (2004)

2. Cancer in Humans

Steiner (2000) investigated the association between cancer incidence and consumption of kava in an ecological study of six countries in the South Pacific. Exposure was estimated by a surrogate measure of consumption based on the number of kava plants under cultivation in
each country. Exposure estimates and data on cancer incidence for men in the 1980s were used in the analysis on the assumption that all kava produced before 1990 was consumed locally, and primarily by men. An inverse correlation was observed between the incidence of all cancers in men and estimated exposure, but no test of statistical significance or confidence intervals, was reported. [The Working Group considered this study as uninformative because of its ecological design, the use of crude measures of exposure and outcome, and inadequate assessment of the role of chance.]

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

In one study of oral administration, groups of 50 male and 50 female B6C3F1 mice (age, 5–6 weeks) were given kava extract at a dose of 0 (corn oil vehicle, 10 mL/kg body weight, bw), 0.25, 0.5, or 1.0 g/kg bw per day by gavage, 5 days per week, for 105 weeks. The purity of the kava extract was 98.04% by high-performance liquid chromatography/ultraviolet (HPLC/UV) profiles. The extract contained 27% kavalactones identified as kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin. In males, the mean body weight of the dosed groups was similar to that in the control group. In females, the mean body weight of the group at 1.0 g/kg bw was 11% less than that in the control group after week 21. The mean survival time of male and female mice in the dosed groups was similar to that of the controls.

In males, the incidence of hepatoblastoma was significantly higher in the groups receiving the intermediate dose. The incidence of eosinophilic hepatocyte foci, a preneoplastic hepatocyte lesion, was significantly higher in the groups receiving the intermediate or highest dose.

In females, the incidence of hepatocellular carcinoma was significantly higher in the group receiving the lowest dose. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly higher in the groups receiving the lowest and intermediate doses. The incidence of hepatocellular carcinoma and hepatoblastoma (combined) was significantly higher in the group receiving the lowest dose. [The Working Group noted that reduced body weight may have reduced one tumour response in females at the highest dose.] The incidence of eosinophilic hepatocyte foci was significantly higher in the group receiving the highest dose. The incidence of squamous cell hyperplasia of the forestomach was significantly higher in the groups receiving the lowest or intermediate doses (Behl et al., 2011; NTP, 2012).

3.2 Rat

See Table 3.1

In one study of oral administration, groups of 49 or 50 male and 50 female F344/N rats (age, 6–7 weeks) were given kava extract at 0 (corn-oil vehicle, 5 mL/kg bw), 0.1, 0.3, or 1.0 g/kg bw per day by gavage, 5 days per week, for 104 (male rats) or 105 (female rats) weeks. The purity of the kava extract was 98.04% by HPLC/UV profiles. The extract contained 27% kavalactones identified as kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin. The mean body weight of the groups at 1.0 g/kg bw was 10% less than that of the control group after week 65 in males and after week 41 in females. The mean survival time for rats in the dosed groups was similar to that of controls for both sexes.
<table>
<thead>
<tr>
<th>Species, strain, sex</th>
<th>Dosing regimen, Duration</th>
<th>Animals/group at start</th>
<th>Incidence of tumours</th>
<th>Significance</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, B6C3F1 (M, F)</td>
<td>0 gavage in corn oil, 5 days/wk for 104 wk</td>
<td>50 M and 50 F group (age, 6-7 wk)</td>
<td>Hepatocellular carcinoma (combined): 38/50, 39/50, 40/50 (M); 0/50, 0/50, 0/50 (F)</td>
<td>( \text{P} = 0.003 ) (trend)</td>
<td>No significant increase in the incidence of any neoplasm in females.</td>
</tr>
<tr>
<td>Rat, F344 (M, F)</td>
<td>0 gavage in corn oil, 5 days/wk for 105 wk</td>
<td>50 M and 50 F group (age, 5-6 wk)</td>
<td>Hepatocellular adenoma or carcinoma (combined): 30/50, 30/50, 30/50 (M); 30/50, 30/50, 30/50 (F)</td>
<td>( \text{P} &lt; 0.001 )</td>
<td>( \text{P} &lt; 0.01 )</td>
</tr>
</tbody>
</table>

\( \text{Poly-3 test} \)

Historical incidence in 2-year studies with administration by gavage with corn oil vehicle-control group (mean ± standard deviation): 176/199 (88.4% ± 8.6%), range 76-99%.
In males, the incidence of testis interstitial (Leydig) cell adenoma was significantly higher in the groups at the intermediate or highest dose, and had a significant positive trend. [The incidence of this tumour in controls was low (76%) compared with that in historical controls (corn oil vehicle controls: range, 76–94%; all routes: range, 54–98%).] The incidence of renal pelvis transitional cell hyperplasia was significantly higher in the group receiving the highest dose. In females, the incidence of renal pelvis transitional cell hyperplasia was significantly higher in the groups at the highest or intermediate dose. There was no significant increase in the incidence of any neoplasm in females (Behl et al., 2011; NTP, 2012).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

The metabolism of kava and individual kavalactones has been studied in humans (Duffield et al., 1989; Köppel & Tenczer, 1991; Johnson et al., 2003; Zou et al., 2005). Demethylation and hydroxylation products were found in human urine after ingestion of kava extract (Duffield et al., 1989), or its constituent kavain. The metabolites were mainly excreted as conjugates (Köppel & Tenczer, 1991).

Ten urinary metabolites were identified when kavain was given as a therapeutic oral dose of 200 mg to five healthy volunteers. The structures of kavain and its metabolites are shown in Fig. 4.1. The major metabolite was a hydroxydihydrokavain. Hydroxylation of the phenyl ring, reduction of the 7,8 double bond, hydroxylation of the lactone ring with subsequent dehydration, and opening of the lactone ring appeared to be the main metabolic pathways (Köppel & Tenczer, 1991).

Zou et al. (2005) identified a pyrone ring-opened product, 6-phenyl-3-hexen-2-one, a proposed metabolite of kava, as its mercapturic acid adduct, in urinary samples from two kava drinkers. This metabolite was possibly formed from enzymatic demethylation of 7,8-dihydromethysticin, followed by ring opening of the α-pyrone ring, and rearrangement (Zou et al., 2005).

11,12-Dihydroxy-7,8-dihydrokavain-o-quinone and 11,12-dihydroxykavain-o-quinone, two electrophilic metabolites, were identified as glutathione conjugates when kava extract was incubated with human liver microsomes. The glucuronic acid and sulfate conjugates of these two urinary metabolites were detected in a human volunteer who ingested a single dose of a dietary supplement containing kava extract (about 90 mg of kavalactones) (Johnson et al., 2003).

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

Few studies have been published on the absorption, distribution, and excretion of the constituents of kava (kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, desmethoxyyangonin, and dihydroyangonin).

Kavain is rapidly absorbed from the gastrointestinal tract, distributed to tissues, and eliminated.

In male F344 rats given kavain at a single oral dose of 100 mg/kg bw, the maximum blood concentration of kavain was measured at 0.88 hours, after which plasma concentrations declined with a mean terminal half-life of 1.3 hours. The mean oral bioavailability of kavain in F344 rats was about 50% (Mathews et al., 2005).

In male F344 rats given kavain orally for 7 days, kavain was primarily excreted in the urine, with about 77% recovered during the
72 hours after the last dose. Faecal excretion accounted for about 14% of the administered dose. Only 0.4% of the kavain was retained in the tissues, and kavain did not accumulate preferentially in any particular tissue. In addition, there were no differences in the pharmacokinetics of kavain when administered as a single dose or as repeated doses (Mathews et al., 2005).

Oral absorption of kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin was investigated in mice (Meyer, 1967). Kavain and dihydrokavain were rapidly absorbed from the gastrointestinal tract (with a peak at 10 minutes), followed by methysticin and dihydromethysticin (30–45 minutes). Yangonin and desmethoxyyangonin were poorly absorbed, and rapid elimination occurred (Meyer, 1967; Robinson et al., 2009).

In male F344 rats given an intravenous injection of kavain at a dose of 7 mg/kg bw, kavain was rapidly eliminated from the systemic circulation, with a terminal half-life of 0.63 hours. Systemic clearance and volume of distribution were 89 mL/minutes per kg and 2.70 L/kg, respectively, indicating that a significant amount of kavain was rapidly distributed out of the plasma into tissues and quickly cleared from the body (Mathews et al., 2005).
Keledjian et al. (1988) observed a peak concentration at 5 minutes in brain for kavain and 7,8-dihydrokavain; the compounds were rapidly eliminated after intraperitoneal administration (100 mg/kg bw) of individual kava constituents in male Balb/c mice. The maximum concentrations of kavain and 7,8-dihydrokavain were 64.7 and 29.3 ng/mg wet brain tissue, respectively. The maximum concentrations of desmethoxyyangonin and yangonin were 10.4 and 1.2 ng/mg wet brain tissue, lower than those of kavain or 7,8-dihydrokavain. When kava extract was given intraperitoneally to male Balb/c mice, the maximum concentrations of kavain and yangonin increased in the brain, while the concentrations of 7,8-dihydrokavain and desmethoxyyangonin were similar to those measured after they were injected separately (Keledjian et al., 1988).

(b) Metabolism

Rasmussen et al. (1979) investigated the metabolism of five kavalactones (kavain, dihydrokavain, methysticin, yangonin, and dihydroyangonin) in male albino rats. The individual kavalactones were administered orally (400 mg/kg bw) or intraperitoneally (100 mg/kg bw), the metabolites and the recovered parent substrate in the urine were then identified. Kavalactones were metabolized to several products via demethylation, mono- and dihydroxylation, and reduction and pyrone ring-opening (Rasmussen et al., 1979; NTP, 2012).

With 7,8-dihydrokavain, large amounts of the parental compound were found in the urine. Nine metabolites were identified, with 12-hydroxydihydrokavain being the most abundant. About two thirds of the metabolites were hydroxylated forms, and one third of the metabolites were formed by scission of the 5,6-dihydro-α-pyrene ring (Fig. 4.2). The proposed metabolic pathways for 7,8-dihydrokavain are depicted in Fig. 4.2 (adapted from NTP (2012)).

With kavain, a total of 10 metabolites were formed in very small amounts. Eight were determined structurally and two remained unidentified. Both hydroxylated and ring-opened products were formed (Fig. 4.1).

With methysticin, only small amounts of two metabolites (11,12-dihydroxykavain and 11,12-dihydroxydihydrokavain) were formed by demethylation of the methylenedioxyphenyl moiety (Fig. 4.3).

Metabolites of yangonin and dihydroyangonin were formed via O-demethylation. No ring-opened products were detected (Fig. 4.4 and Fig. 4.5).

4.1.3 Effects on drug-metabolizing enzymes

Studies in vivo and in vitro have shown that kava extract and its constituents altered drug-metabolizing enzymes. Table 4.1 lists the major enzymes affected by kava.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

See Table 4.2

(a) Mutagenicity

Kava extract (up to 10 000 µg/plate) was not mutagenic in Salmonella typhimurium strains TA97, TA98, TA1535 or TA100, or Escherichia coli strain WP2 uvrA pKM101 with or without metabolic activation (rat liver S9) (NTP, 2012). In one trial out of three of which two of the results were negative, kava extract tested equivocal in TA97 with metabolic activation (NTP, 2012). Kava extracts were not mutagenic in an assay in L5178Y mouse lymphoma cells (Whittaker et al., 2008).
Fig. 4.2 The proposed metabolic pathways for 7,8-dihydrokavain

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Compiled by the Working Group using data from NTP (2012).
Fig. 4.3 Structures of methysticin and its two metabolites

- Methysticin
- 11,12-Dihydroxykavain
- 11,12-Dihydroxydihydrokavain

Compiled by the Working Group using data from Fu et al. (2008)

Fig. 4.4 Structures of yangonin and its three metabolites

- 7,8-Dihydroxyangonin
- 12-Hydroxy-5,6-dehydro-7,8-dihydrokavain I
- Dihydroxy-5,6-dehydro-7,8-dihydrokavain I
- Dihydroxy-5,6-dehydro-7,8-dihydrokavain II

For the metabolites II and III the positioning of the second hydroxyl group (m, o or at C8) are uncertain.
Compiled by the Working Group using data from Fu et al. (2008)

Fig. 4.5 Structures of 7,8-dihydroxyangonin and its three metabolites

- Yangonin
- 12-Hydroxy-5,6-dehydrokavain
- α,12-Dihydroxy-7,8-dihydro-5,6-dehydrokavain
- 12-Hydroxy-kavain

Compiled by the Working Group using data from Fu et al. (2008)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Species or cell type</th>
<th>Kava preparation</th>
<th>Dose</th>
<th>Duration of treatment</th>
<th>Detection method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
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</tr>
<tr>
<td>Russmann et al. (2005)</td>
<td>Human</td>
<td>Aqueous extract</td>
<td>7–27 g of kavalactones per wk, oral</td>
<td>6 yr</td>
<td>Substrate turnover</td>
<td>CYP1A2 (inhibition)</td>
</tr>
<tr>
<td>Guo et al. (2010)</td>
<td>Mouse</td>
<td>Methanolic and aqueous extracts</td>
<td>0.125–2.0 g/kg bw per day, 5 days/wk, gavage</td>
<td>98 days</td>
<td>Gene expression</td>
<td>CYP4A10 (inhibition); CYP2A5, CYP2B20, CYP2C55, GSTA1, GSTA2 (induction)</td>
</tr>
<tr>
<td>Guo et al. (2009)</td>
<td>Rat</td>
<td>Methanolic and aqueous extracts</td>
<td>0.125–2.0 g/kg bw per day, 5 days/wk, gavage</td>
<td>98 days</td>
<td>Gene expression</td>
<td>CYP3A13, CYP17A1, ABCB9 (inhibition); CYP1A1, CYP1A2, CYP3A1, CYP3A3, ABCC3, NQO1, UGT1A6 (induction)</td>
</tr>
<tr>
<td>Yamazaki et al. (2008)</td>
<td>Rat</td>
<td>Kava extracts (not specified)</td>
<td>Kava extract (kavalactones, 380 mg/kg bw per day), gavage</td>
<td>8 days</td>
<td>Rat liver microsomes/enzyme assay, gene expression, protein expression</td>
<td>CYP1A1, CYP1A2 (induction)</td>
</tr>
<tr>
<td>Lim et al. (2007)</td>
<td>Rat</td>
<td>Acetone kava leave extract</td>
<td>100 mg/kg bw per day, gavage</td>
<td>14 days</td>
<td>Protein expression</td>
<td>CYP1A2, CYP2E1 (induction)</td>
</tr>
<tr>
<td>Clayton et al. (2007)</td>
<td>Rat</td>
<td>Methanolic and aqueous extracts</td>
<td>0.125–2.0 g/kg bw per day, 5 days/wk, gavage</td>
<td>90 days</td>
<td>Protein expression</td>
<td>CYP2D1 (inhibition); CYP1A2, CYP2B1, CYP3A1 (induction)</td>
</tr>
<tr>
<td>Mathews et al. (2005)</td>
<td>Rat</td>
<td>Methanol or acetone kava extract</td>
<td>256 mg/kg bw, 1 g/kg bw, gavage</td>
<td>7 days</td>
<td>Rat liver microsomes/ enzyme assay</td>
<td>CYP2D1, 2C11 (inhibition); CYP1A2, 2B1, 2C6, 2D1, 3A1/2 (induction)</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
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</tr>
<tr>
<td>Li et al. (2011)</td>
<td>Hepa1c1c7</td>
<td>Methanolic and aqueous extracts, methysticin, 7,8-dihydromethysticin</td>
<td>Various concentrations to 25 µM of kava constituents or 6.25 µg/mL of kava extract</td>
<td>24 h</td>
<td>Cell-based enzymatic assay/enzyme assay, gene expression, protein expression</td>
<td>CYP1A1 (induction)</td>
</tr>
<tr>
<td>Mathews et al. (2005)</td>
<td>Human liver microsomes</td>
<td>Methanol or acetone kava extract, yangonin, dihydrokavain, methysticin, dihydromethysticin, composite kavalactones</td>
<td>1, 10, 100 µM</td>
<td>10 min</td>
<td>Recombinant protein/enzyme assay</td>
<td>CYP2C9, 2C19, 2D6, 3A4 (inhibition); P-glycoprotein ATPase (inhibition)</td>
</tr>
<tr>
<td>Reference</td>
<td>Species or cell type</td>
<td>Kava preparation</td>
<td>Dose</td>
<td>Duration of treatment</td>
<td>Detection method</td>
<td>Result</td>
</tr>
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<td>--------------------</td>
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<tr>
<td>Weiss et al. (2005)</td>
<td>P388 and P388/dx cell lines</td>
<td>Methanol aqueous kava extracts, kavain, dihydromethysticin, yangonin, and desmethoxyyangonin</td>
<td>Various concentrations (to the highest soluble concentration)</td>
<td>NR</td>
<td>Calcein uptake</td>
<td>ABCB1 (inhibition)</td>
</tr>
<tr>
<td>Zou et al. (2004)</td>
<td>Baculovirus/insect cell system and cryopreserved human hepatocytes</td>
<td>Ethanol extract, methysticin, desmethoxyyangonin, yangonin</td>
<td>Various concentrations up to 100 µM</td>
<td>15–45 min</td>
<td>Recombinant enzyme/ enzyme assay</td>
<td>CYP1A2, 2C9, 2C19, 2E1, 3A4 (inhibition)</td>
</tr>
<tr>
<td>Côté et al. (2004)</td>
<td>Human liver microsomes/</td>
<td>Methanol, acetone, ethanol or aqueous kava extracts</td>
<td>Various concentrations up to 200 µg/mL</td>
<td>5 min</td>
<td>Enzyme assay</td>
<td>CYP3A4, CYP1A2, CYP2C9, CYP2C19 (inhibition)</td>
</tr>
<tr>
<td>Raucy (2003)</td>
<td>Primary human hepatocytes and HepG2</td>
<td>Kava extract (not specified)</td>
<td>100 µg/mL</td>
<td>48 h</td>
<td>Gene expression, report gene assay</td>
<td>CYP3A4 (induction)</td>
</tr>
<tr>
<td>Mathews et al. (2002)</td>
<td>Human liver microsomes/</td>
<td>Methanol or acetone extract, desmethoxyyangonin, methysticin, dihydromethysticin</td>
<td>Kava extract normalized to 100 µM kavalactones</td>
<td>15 min</td>
<td>Enzyme assay</td>
<td>CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP4A9/11 (induction)</td>
</tr>
<tr>
<td>Unger et al. (2002)</td>
<td>Baculovirus/insect cell system</td>
<td>Methanol, acetone and ethyl acetate extracts</td>
<td>1–100 mg/mL</td>
<td>30 min</td>
<td>Recombinant enzyme/ enzyme assay</td>
<td>CYP3A4 (inhibition)</td>
</tr>
<tr>
<td>Zou et al. (2002)</td>
<td>cDNA human CYP isoforms</td>
<td>Methysticin, desmethoxyyangonin, dihydromethysticin, kavain, dihydorokavain</td>
<td>Various concentrations up to ~200 µM</td>
<td>30–45 min</td>
<td>Recombinant enzyme/ enzyme assay</td>
<td>CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 (inhibition)</td>
</tr>
</tbody>
</table>

ABC, ATP-binding cassette; CYP, cytochrome; GST, glutathione-S-transferase; min, minute; NR, not reported; NQO, NAD(P)H quinone oxidoreductase; UGT, UDP glycosyltransferase; wk, week; yr, year
The only report of positive mutagenic activity with kava extracts (two positive results, but six negative results) involved the *umu* point muta-
- tion assay (Jhoo et al., 2007). [The Working Group noted that these data were not analysed statistically.]

(b) **Chromosomal damage**

In male or female mice given kava extract at a dose of up to 2.0 g/kg bw per day by gavage for 3 months, there was no increase in the frequency of micronucleated normochromatic or polychro-
- matic erythrocytes in blood (NTP, 2012).

<table>
<thead>
<tr>
<th>Test system</th>
<th>Resultsa</th>
<th>Concentration or dose (LED or HID)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhimurium</em> TA100, TA98, TA1535, reverse mutation</td>
<td>–</td>
<td>10 000 μg/plate</td>
<td>NTP (2012)</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> TA97, reverse mutation</td>
<td>–</td>
<td>10 000 μg/plate</td>
<td>NTP (2012)</td>
</tr>
<tr>
<td>L5178Y mouse lymphoma mutation assay</td>
<td>–</td>
<td>NR</td>
<td>Whittaker et al. (2008)</td>
</tr>
<tr>
<td><em>umu</em> point mutation assay</td>
<td>+</td>
<td>+</td>
<td>2330 μg/mL extract</td>
</tr>
<tr>
<td><em>Escherichia coli</em> WP2 uvrA/pKM101, reverse mutation</td>
<td>–</td>
<td>–</td>
<td>10 000 μg/plate</td>
</tr>
<tr>
<td>Micronucleus induction in peripheral blood erythrocytes of male and female B6C3F1 mice in vivo</td>
<td>–</td>
<td>NT</td>
<td>Up to 2.0 g/kg bw per day by gavage for 3 months</td>
</tr>
</tbody>
</table>

+, positive; (+), weakly positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested; NR, not reported

* These data were not analysed statistically.

4.3 **Other mechanistic data relevant to carcinogenesis**

**Effects on hepatic cell physiology**

Case reports of liver injury associated with kava intake have been described. The types of liver damage reported include fulminant hepatitis, necrosis, cirrhosis, and liver failure requiring liver transplantation or causing death (Russmann et al., 2001; Bujanda et al., 2002; Campo et al., 2002; Brauer et al., 2003; Gow et al., 2003; Humberston et al., 2003; Stickel et al., 2003; Teschke et al., 2003, 2008; Thomsen et al., 2004).

4.4 **Susceptibility**

**Genetic polymorphisms**

Deficiency in CYP2D6, the major kavalactone-metabolizing enzyme, was detected in two patients with liver failure (Russmann et al., 2001). Genetic polymorphism of CYP2D6 has a prevalence of 7–9% in Caucasian populations, but <1% in Polynesian populations (Wanwimolruk et al., 1998; Ingelman-Sundberg, 2005). Severe liver failure has not been observed in people using kava in the traditional way in islands in the South Pacific (Moulds & Malani, 2003; Anke & Ramzan, 2004).
4.5 Mechanistic considerations

Kava extract is not mutagenic based on the results of numerous studies of genotoxicity, including tests for mutagenicity in bacteria, induction of micronuclei in vivo (NTP, 2012), and the mouse lymphoma assay (Whittaker et al., 2008). The reported carcinogenicity in mice is most probably mediated through nongenotoxic mechanisms.

5. Summary of Data Reported

5.1 Exposure data

The kava (or kava kava) plant *Piper methysticum* is a perennial tropical shrub that is widely cultivated in Oceania. The rhizome of the plant was originally used as an ingredient in local traditional drinks with psychopharmacological properties, and as traditional folk medicine. More recently, rhizome extracts have been used in medicinal products, food or dietary supplements, and cosmetics. Important chemical constituents of the resin contained in the kava rhizome are kavalactones, kavain being the major compound. The medicinal uses of kava supported by clinical data are short-term symptomatic treatment of mild states of anxiety or insomnia due to nervousness, stress, or tension. Use of kava was popular worldwide, but several case reports of liver damage associated with exposure to kava reduced sales, and caused kava to be banned in several countries.

5.2 Human carcinogenicity data

The Working Group was able to identify only one epidemiological study of cancer and kava consumption. This ecological study found an inverse correlation between all cancers in men and a proxy measure of kava consumption, but no confidence intervals or test of statistical significance were reported. The Working Group regarded the study as uninformative because the ecological design provided only weak support for causal inference at the individual level, the measures of exposure and outcome were crude, and the role of chance was not evaluated.

5.3 Animal carcinogenicity data

A kava extract was tested for carcinogenicity in one study in mice and one study in rats treated by gavage. In mice, the extract caused a significant increase in the incidence of hepatoblastoma in males, and of hepatocellular adenoma or carcinoma (combined), and hepatocellular carcinoma, in females. In male rats, the same extract caused a significant increase in the incidence of testis interstitial (Leydig) cell adenoma; however, the incidence in controls was low compared with that in historical controls. There was no significant increase in the incidence of any neoplasm in female rats.

5.4 Mechanistic and other relevant data

The major components of kava extract, kavalactones, are extensively metabolized in humans and experimental animals. Among the numerous metabolites are products from demethylation, hydroxylation, and ring-opening. Kava extract gave negative results in several standard bacterial assays for mutation in the absence or presence of exogenous metabolic activation. Kavalactones gave negative results in most of these assays.

The reported carcinogenicity of kava in mice is most likely to be mediated through a nongenotoxic mechanism.
6. Evaluation

6.1 Cancer in humans

There is inadequate evidence in humans for the carcinogenicity of kava extract.

6.2 Cancer in experimental animals

There is sufficient evidence in experimental animals for the carcinogenicity of kava extract.

6.3 Overall evaluation

Kava extract is possibly carcinogenic to humans (Group 2B).

References


FDA (2002). Kava-containing dietary supplements may be associated with severe liver injury. US Food and Drug


