Riddelliine
CAS No. 23246-96-0
Reasonably anticipated to be a human carcinogen
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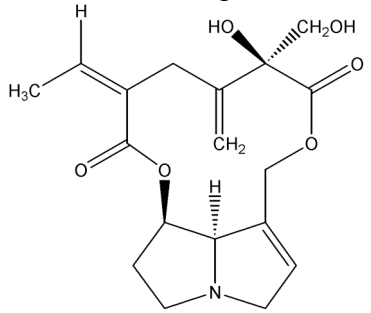

Riddelliine

Carcinogenicity

Riddelliine is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic data. When repeatedly administered by gavage (stomach tube) throughout a two-year chronic study, riddelliine caused malignant and benign tumors at multiple tissue sites in mice and rats and early onset of tumors in female rats (Chan *et al.* 2003, NTP 2003). In male and female mice, riddelliine exposure significantly increased the incidences of hemangiosarcoma (blood vessel cancer) in the liver in males and alveolar/bronchiolar (lung) tumors in females. In female and male rats, hepatocellular adenoma, hemangiosarcoma in the liver, and mononuclear-cell leukemia (cancer of white blood cells) were observed (NTP 2003). Hepatocellular adenoma (benign liver tumor) was observed in some female rats in a 13-week study.

No studies on the relationship between human cancer and exposure specifically to riddelliine were identified.

Additional Information Relevant to Carcinogenicity

Riddelliine and other pyrrolizidine alkaloids are absorbed primarily via ingestion (although dermal absorption can occur), distributed to the liver, and excreted in the urine and feces (NTP 2008). Riddelliine is metabolized in the liver to two reactive metabolites *R*- and *S*-dihydropyrrolizine (DHP) [also called dehydroretronecine and dehydroheliotridine or (±)-6,7-dihydro-7-hydroxy-1-hydroxymethyl-5*H*-pyrrolizine] by the cytochrome P450 isozymes CYP3A and CYP2B6. Both *R*- and *S*-DHP have been shown to cause tumors in rodents (NTP 2008).

DHP can bind DNA, which may be a key step leading to its genotoxicity and tumorigenicity. A set of eight DHP-derived adduct peaks has been detected in DNA reacted with riddelliine in the presence of rat or human microsomes (NTP 2008, Xia *et al.* 2003). Dose-dependent DHP adduct formation also has been detected in livers of rats exposed to riddelliine (NTP 2008, Yang *et al.* 2001). Adduct levels were higher in DNA in endothelial cells than in parenchymal cells in rats and were more persistent in endothelial cells than in parenchymal cells in both rats and mice suggesting that tumor

specificity was due to higher levels of DNA damage in the endothelial cells that form liver hemangiosarcomas (Chou *et al.* 2003, 2004, NTP 2008). The kinetic parameters (V_{max} and K_m) for formation of DHP are comparable in human and rat microsomes, and the same profile of DHP-adduct peaks is also detected (Xia *et al.* 2003). In addition, other pyrrolizidine alkaloids have been shown to be metabolized to DHP, and it has been proposed that any pyrrolizidine alkaloid that is metabolized to DHP will be carcinogenic in rodents (Fu *et al.* 2002). Available studies in rats have shown that pyrrolizidine alkaloids cause liver tumors and, to a lesser extent, tumors of other organs, including the central nervous system, lung, pancreas, urinary bladder, skin, testes, pituitary gland, and adrenal gland (NTP 2008).

There are sufficient data to conclude that the metabolites of riddelliine are genotoxic, both *in vitro* and *in vivo*, and the data suggest that genotoxicity contributes to riddelliine's carcinogenic activity. Riddelliine induced a higher frequency of mutations in non-neoplastic endothelial cells (but not in parenchymal cells) in the *cII* gene mutation assay in transgenic Big Blue rats (Mei *et al.* 2004b). The predominant mutations observed were G·C → T·A transversions, which are consistent with riddelliine-induced formation of DNA adducts involving G·C base pairs (Mei *et al.* 2004a). These changes were consistent with mutations in the *K-ras* oncogene identified in riddelliine-induced hemangiosarcomas in the mouse (Hong *et al.* 2003). The DHP metabolites clearly form a number of different DNA adducts in cultured cells as well as in treated animals (NTP 2008).

Riddelliine also induced base-pair substitutions in *S. typhimurium*. In mammalian cells *in vitro*, it increased the frequency of sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells, cell transformation in BALB/c-3T3 fibroblast cells, and DNA cross-linking, but not DNA strand breaks, in bovine kidney epithelial cells. In rats exposed *in vivo*, riddelliine induced S-phase synthesis in hepatocytes and endothelial cells and increased p53 protein expression in endothelial cells but did not induce micronucleus formation in polychromatic erythrocytes (immature red blood cells). In mice, riddelliine caused unscheduled hepatocyte DNA synthesis (in females only), but did not induce micronucleus formation (NTP 2008).

Riddelliine metabolites appear to cause damage to endothelial cells, as shown by karyomegaly (abnormally large cell nuclei) and cytomegaly (enlarged cells) and accumulation of intravascular macrophages in many organs (NTP 2008). A mechanism for the pathogenesis of hemangiosarcomas in the liver of animals exposed to riddelliine has been proposed by Nyska *et al.* (2002) and Moyer *et al.* (2004). Short-term exposure to riddelliine in rats increased apoptosis and S-phase nuclei in endothelial cells and hepatocytes, and increased levels of p53 protein were detected in endothelial cells. The nuclear and cytoplasmic enlargement of endothelial cells causes sinusoidal obstruction and local hypoxia, which stimulates the production of vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen, by hepatocytes. Development of hemangiosarcoma in the liver may have resulted from endothelial cell DNA-adduct formation, apoptosis, proliferation of endothelial cells, and mutations (Nyska *et al.* 2002, Moyer *et al.* 2004).

Riddelliine also exhibits significant non-cancer toxicity and pathology. It is acutely and chronically toxic in animals, and human toxicity has been demonstrated when foods or herbal products containing riddelliine or other pyrrolizidine alkaloids were consumed. The primary toxic effect of riddelliine, venous occlusion, occurs in the same target tissue (i.e., liver) as the primary tumor. The non-cancer effects are likely to involve the same reactive intermediate(s). However, given the strong evidence for a genotoxic mode of action, there is no reason to suspect that tumorigenicity is due solely to compensatory cell proliferation (NTP 2008).

Properties

Riddelliine is a pyrrolizidine alkaloid of the macrocyclic diester class and exists in plants as the free-base alkaloid and as an *N*-oxide, which can be converted back to riddelliine after ingestion. Both riddelliine and riddelliine *N*-oxide are white crystalline solids. Alcohol and aqueous solutions of riddelliine are stable at room temperature when protected from light; the solid form is stable at room temperature in diffuse light for several years (R.J. Molyneux, personal communication 2006). Riddelliine reacts readily with oxidizing agents to form DHP and other derivatives; however, it reacts slowly with atmospheric oxygen. It hydrolyzes readily in aqueous alkali (IARC 1976). Riddelliine *N*-oxide in solid form is stable at freezer temperature, but not at room temperature. Available information on chemical properties of riddelliine and riddelliine *N*-oxide are summarized in the following table.

Property	Information	
	Riddelliine	Riddelliine N-oxide
Molecular Weight	349.4	365.4
Melting Point	197°C–198°C dec	156°C–158°C dec
HCl salt	225°C–226°C dec	NA
MeI salt	260°C–262°C dec	NA
Water Solubility	sparingly soluble	soluble

Sources: Buckingham 2000, Mattocks 1986, Molyneux *et al.* 1991; R.J. Molyneux, personal communication 2006.
dec = decomposes at or below its melting point; NA = not applicable.

Use

Riddelliine-containing plants are not used for food in the United States, and riddelliine and riddelliine *N*-oxide have no known commercial uses. However, the riddelliine-containing plant *Senecio longilobus* has been used in medicinal herb preparations in the United States, and *S. jacobaea* and *S. vulgaris*, both of which have been shown to contain riddelliine, have been reported to be used in medicinal preparations in other parts of the world (Mattocks 1986).

Production and occurrence

The only known production of riddelliine has been for experimental purposes by purification from *S. riddellii*. Riddelliine *N*-oxide has been synthesized from riddelliine by oxidation with hydrogen peroxide in ethanol (Molyneux *et al.* 1991). No vendors for these products were identified. However, riddelliine occurs naturally in plants (primarily of the genus *Senecio*) found in the western United States and other parts of the world. At least 13 *Senecio* species have been identified that are used in herbal medicines or possibly as food worldwide.

The prototypical riddelliine-containing *Senecio*, Riddell's groundsel (*S. riddellii*), generally grows in desert areas of western North America, especially in sandy soils. It is a low, shrubby plant with bright green, thread-like leaves and intensely yellow composite flowers. The plant sprouts in the early spring and dies back to a woody crown in the early fall, although sufficient moisture from summer rains may initiate re-growth on the stems. The early-season growth and re-growth at periods when little other green leafy material is available may make it attractive to grazing animals. This plant was one of the earliest *Senecio* species to be identified as poisonous to animals, causing "walking disease" in horses in Nebraska and adjacent areas of Colorado and Wyoming. The syndrome was characterized by aimless wandering and cirrhosis of the liver (Johnson *et al.* 1985b).

Riddelliine and riddelliine *N*-oxide are the predominant alkaloids in *S. riddellii*, occurring in yields of up to 18% of the dry weight of the plant (Molyneux and Johnson 1984); however, alkaloid content may be highly variable, depending on growth stage, environmental conditions, and location (Johnson *et al.* 1985a). It has been calculated that at 18% total pyrrolizidine alkaloid, as little as 33 g of dry or 176 g of fresh *S. riddellii* consumed per day would be toxic to a 300-kg cow.

The environmental fate of riddelliine and other pyrrolizidine alkaloids is not well established. In *Senecio* species, the alkaloids are biosynthesized in the roots and, as the *N*-oxides, translocated in the phloem to the flower structure, where they are preferentially stored (Hartmann *et al.* 1989). After flowering, the pyrrolizidine alkaloid content of the remaining plant is drastically reduced, presumably because the majority of the alkaloid is dispersed in seeds and flower fragments. Nevertheless, the alkaloid content in the remaining leaves can be appreciable. For example, in *S. riddellii* collected in Oklahoma over a five-year period, the total alkaloid content in the leaves immediately before senescence ranged from 3% to 6% on a dry-weight basis (Johnson *et al.* 1985a).

No data on U.S. production volume, sales, or imports of riddelliine or riddelliine-containing plants were identified.

Exposure

Herbal products containing pyrrolizidine alkaloids, some from plants of the genus *Senecio*, have been extensively documented as causing toxicity in humans (Huxtable 1989). Two cases of accidental poisoning of infants were reported from the southwestern United States in which *S. longilobus*, a species known to contain riddelliine as well as

seneciphylline, senecionine, and retrorsine, was accidentally used to prepare an herbal tea known as gordolobo yerba (Stillman *et al.* 1977). The distribution of *S. longilobus* was traced to a major U.S. importer, who also was a major supplier of herbs in the western United States (Huxtable 1980). *Senecio*-containing products have been inadvertently distributed by pharmacies and herb stores and also could be consumed by people who gather herbs for private use (Fox *et al.* 1978). Riddelliine has been identified in at least 13 species of the genus *Senecio*, including 5 North American species (*S. riddellii*, *S. longilobus*, *S. jacobaea*, *S. spartioides*, and *S. vulgaris*) (Hartmann and Witte 1995, Mattocks 1986).

Senecio species containing riddelliine are not generally used as food plants in the United States, but human exposure could result from direct contamination of foodstuffs by parts of *Senecio* plants or from indirect introduction of the alkaloid through products derived from animals that have fed on the plants. Evidence for ingestion of these products comes from reports of toxicity in animals and humans. Cases have been reported from outside the United States of accidental human poisoning from grains and flours contaminated with *Senecio* plant parts. Experimental studies of cows fed *Senecio* plants have demonstrated that pyrrolizidine alkaloids can be transmitted in milk, with riddelliine *N*-oxide concentrations estimated as high as 5 mg/L (Molyneux and James 1990). Pyrrolizidine alkaloids other than riddelliine have been detected in eggs, and honey has been shown to contain either pyrrolizidine alkaloids or pollen from pyrrolizidine alkaloid-containing plants.

Regulations

No regulations or guidelines were identified for riddelliine.

Guidelines

In an advisory dated July 6, 2001, the FDA stated: "The agency [FDA] strongly recommends that firms marketing a product containing comfrey or another source of pyrrolizidine alkaloids remove the product from the market and alert its customers to immediately stop using the product. The agency advises that it is prepared to use its authority and resources to remove products from the market that appear to violate the Act."

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