Riddelliine

CAS No. 23246-96-0

Reasonably anticipated to be a human carcinogen

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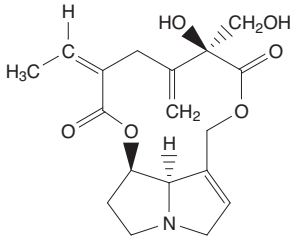

Carcinogenicity

Riddelliine is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting data from studies on mechanisms of carcinogenesis.

Cancer Studies in Experimental Animals

Oral exposure to riddelliine caused tumors at several different tissue sites in mice and rats and early onset of tumors in rats. Administration of riddelliine by stomach tube throughout the course of two-year studies caused blood-vessel cancer (hemangiosarcoma) of the liver in male mice and in rats of both sexes. It also caused benign liver tumors (hepatocellular adenoma) and mononuclear-cell leukemia in rats of both sexes and increased the combined incidence of benign and malignant lung tumors (alveolar/bronchiolar adenoma and carcinoma) in female mice. Benign liver tumors (hepatocellular adenoma) were observed in some female rats in a 13-week study (Chan *et al.* 2003, NTP 2003).

Studies on Mechanisms of Carcinogenesis

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. Riddelliine is metabolized in the liver to two reactive metabolites, *R*- and *S*-dihydropyrrolizine (DHP) (also known as 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 was detected in DNA reacted with riddelliine in the presence of rat or human microsomes (Xia *et al.* 2003, NTP 2008). Dose-dependent DHP adduct formation also was detected in livers of rats exposed to riddelliine (Yang *et al.* 2001, NTP 2008). 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, from which liver hemangiosarcomas are formed (Chou *et al.* 2004, NTP 2008). The kinetic parameters for formation of DHP are comparable in human and rat microsomes, and the same profile of DHP-adduct peaks was detected in humans and rats (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). Studies in rats have shown that pyrrolizidine alkaloids cause liver tumors and, to a lesser extent, tumors at other tissue sites, including the central nervous system, lung, pancreas, urinary bladder, skin, testes, pituitary gland, and adrenal gland (NTP 2008). It has been proposed that tumor specificity and relative species susceptibility to riddelliine carcinogenicity may be due to variability in the balance between the formation of toxic metabolites, such as DHP, and the availability of glutathione or other detoxification pathways (Fu *et al.* 2002b).

The evidence is sufficient 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. In the *cII* gene mutation assay in transgenic Big Blue rats, riddelliine increased the frequency of mutations in nonneoplastic endothelial cells (but not in parenchymal cells). The predominant mutations were G:C to T:A transversions, which is consistent with riddelliine-induced formation of DNA adducts involving G:C base pairs (Mei *et al.* 2004a,b). These changes were consistent with mutations in the K-*ras* oncogene identified in riddelliine-induced hemangiosarcomas from mice (Hong *et al.* 2003). The DHP metabolites clearly form several different DNA adducts in cultured cells as well as in exposed animals (NTP 2008). Riddelliine also caused base-pair substitutions in *Salmonella typhimurium*. In mammalian cells *in vitro,* it caused 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 production in endothelial cells but did not induce micronucleus formation in polychromatic erythrocytes. 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 and local inflammation (arteritis) in endothelial cells, as evidenced by abnormally large cell nuclei and enlarged cells, resulting in complete or partial occlusion of the vessel lumina and accumulation of intravascular macrophages in many organs (NTP 2008). Reactive oxygen species produced by macrophages and other mediators of the inflammatory response may have a role in the carcinogenicity of riddelliine through the depletion of cellular detoxification pathways. However, a specific biochemical pathway linking inflammation to riddelliine carcinogenicity has not been determined. 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, an endothelial cell-specific mitogen, by hepatocytes. Development of hemangiosarcoma in the liver may result 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 for consumption of foods or herbal products containing riddelliine or other pyrrolizidine alkaloids. The primary toxic effect of riddelliine, venous occlusion, occurs in the same target tissue (i.e., liver) as the primary tumor, and 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).

Cancer Studies in Humans

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

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. In water, riddelliine is sparingly soluble, and riddelliine *N*-oxide is soluble. 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. Riddelliine reacts readily with oxidizing agents to form DHP and other derivatives; however, it reacts slowly with atmospheric oxygen. It hydrolyzes readily in aqueous alkalis. Riddelliine *N*-oxide in solid form is stable at freezer temperature but not at room temperature (IARC 1976, NTP 2008). Physical and chemical properties of riddelliine and riddelliine *N*-oxide are listed in the following table.

Property	Riddelliine	Riddelliine *N*-oxide
Molecular weight	349.4	365.4
Melting point (decomposes)	197°C to 198°C	156°C to 158°C
Hydrochloride	225°C to 226°C	N/A
Methiodide	260°C to 262°C	N/A

Source: NTP 2008. N/A = 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, are 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 worldwide have been identified that are used in herbal medicines or possibly as food. The following plant species have been identified as containing riddelliine (Mattocks 1986, Hartmann and Witte 1995, NTP 2008) (*indicates North American species):

- *Senecio aegypticus*
- *Senecio ambrosioides (S. brasiliensis)*
- *Senecio cruentus*
- *Senecio cymbalarioides*
- *Senecio desfontanei (S. coronopifolius)*
- *Senecio douglasii var. longilobus* (S. longilobus)* (woody or threadleaf groundsel)
- *Senecio eremophilus*
- *Senecio jacobaea** (erucifoline chemotype) *(*tansy ragwort, stinking willie)
- *Senecio riddellii** (Riddell's ragwort, Riddell's groundsel)
- *Senecio spartioides** (broom groundsel)
- *Senecio vulgaris** (common groundsel)
- *Senecio pseudo-orientalis*
- *Senecio vernalis*
- *Crotalaria juncea*

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 regrowth on the stems. The early-season growth and regrowth 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 is 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 found.

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 the alkaloids 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).

Although *Senecio* species containing riddelliine are not used as food plants in the United States, 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. There is thus the potential for cumulative effects from low-level exposures. Evidence for ingestion of contaminated products comes from reports of toxicity in animals and humans. Outside of the United States, accidental human poisoning from grains and flour contaminated with *Senecio* plant parts

has been reported. Studies of cows fed *Senecio* plants demonstrated that pyrrolizidine alkaloids could 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 (NTP 2008).

Regulations

No regulations or guidelines relevant to reduction of exposure to riddelliine were identified.

Warnings and Alerts

Food and Drug Administration (FDA, an HHS agency)

In a 2001 alert (FDA 2001), the agency strongly recommended 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 advised that it was prepared to use its authority and resources to remove products from the market that appeared to violate the Federal Food, Drug, and Cosmetic Act.

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