D. *SENECIO* SPECIES AND RIDDELLIINE

1. Exposure Data

1.1 Origin, type and botanical data (Molyneux *et al.*, 1991)

Senecio riddellii (Asteraceae) (Riddell groundsel) is a grey-white half-shrub, 30–90 cm tall with pinnatifid and relatively hairless leaves, revealing its bright green leaf colour. It has bright yellow flowers on the stems at about the same height above the ground. This gives the plant a flat-topped appearance when in bloom. It produces flowers in late summer to early autumn and dies back to ground level after the first frost. It grows in dry, sandy soils and its roots are long and about as thick as a lead pencil.

Senecio longilobus (also known as woolly groundsel and thread-leaf groundsel) is a shrubby, erect, branched, leafy plant, 30–60 cm tall. It has narrowly linear leaves which are thick, white, and occasionally pinnately lobed, up to 10 cm long. The composite yellow flower heads contain numerous clusters. In North America, this is a common plant with a range extending from Colorado to Utah, south to Texas and Mexico (Kingsbury, 1964).

1.2 Use

The ‘bush tea’ used in Jamaica to treat children for a cold an a herbal tea that is popular in the south-west USA, gordolobo yerba, may contain riddelliine (Stillman *et al.*, 1977a; Huxtable, 1980; National Toxicology Program, 2002).

1.3 Chemical constituents

The plants (ragworts) from which riddelliine and other pyrrolizidine alkaloids are isolated are found in the rangelands of the western USA. Cattle, horses and, less commonly, sheep that ingest these plants can succumb to their toxic effects (called pyrrolizidine alkaloidosis). Riddelliine residues have been found in meat, milk and honey. The plants may contaminate human food sources as intact plants, and their seeds may contaminate commercial grains such as wheat (Fu *et al.*, 2001; National Toxicology Program, 2002).

The main chemical constituents of importance in *S. riddellii* are pyrrolizidine alkaloids.

Four pyrrolizidine alkaloids have been identified as constituents of *S. longilobus*, namely riddelliine, retrorsine, senecionine and seneciphylline (Segall & Molyneux, 1978).

1.4 Sales and consumption

No information was available to the Working Group.

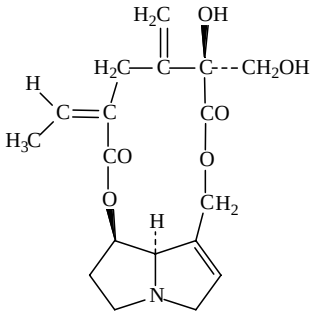
1.5 Component with potential cancer hazard (riddelliine)

Riddelliine was evaluated previously (IARC, 1976).

1.5.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 23246-96-0
Chem. Abstr. Serv. Name: 13,19-Didehydro-12,18-dihydroxysenecionan-11,16-dione
Synonym: Riddelline

1.5.2 *Structural and molecular formulae and relative molecular mass*



$C_{18}H_{23}NO_6$ Relative molecular mass: 349.38

1.5.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Crystalline solid (National Toxicology Program, 2002)
(*b*) *Melting-point*: 197–198 °C (decomposes) (Buckingham, 2001)
(*c*) *Solubility*: Sparingly soluble in water; soluble in chloroform; slightly soluble in acetone and ethanol (National Toxicology Program, 2002)
(*d*) *Optical rotation*: $[\alpha]_D^{25}$ –109.5 (chloroform) (Buckingham, 2001)

1.5.4 *Analysis*

Underivatized pyrrolizidine alkaloids, including riddelliine, from natural sources (plants and insects) have been analysed by capillary gas chromatography–mass spectro-

metry (Witte *et al.*, 1992). Thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR) spectroscopy have also been used to detect pyrrolizidine alkaloids in plant extracts (Molyneux *et al*., 1979; Molyneux & Roitman, 1980).

Riddelliine, retrorsine, senkirkine, retronecine, integerrimine, seneciphylline and senecionine (0.007, 0.008, 0.012, 0.005, 0.008, 0.042 and 0.036%, respectively) were determined in methanol extracts from dry *Senecio vernalis* using reversed-phase high-performance liquid chromatography (HPLC) with spectrometric detection at 225 nm (Sener *et al*., 1986).

Pyrrolic metabolites from pyrrolizidine alkaloids were detected in liver and dried blood samples from pigs fed varying amounts of riddelline using gas chromatography/tandem mass spectrometry (Schoch *et al*., 2000).

1.5.5 *Production*

Riddelliine is produced commercially only as a reference standard and as a research chemical (National Toxicology Program, 2002).

1.5.6 *Use*

Riddelliine has no known commercial use.

1.5.7 *Occurrence*

Riddelliine is found in *S. riddellii*, *S. longiflorus*, *S. eremophilus*, *S. vernalis*, *S. cruentus*, *S. longilobus*, *S. aegyptus*, *S. desfontainei* (*S. coronopifolius*) and *S. jacobaea* (Klásek *et al*., 1968; Segall & Molyneux, 1978; Asada *et al*., 1982; Sener *et al*., 1986; Mirsalis *et al*., 1993; Fu *et al*., 2001; Röder, 2002).

Structurally, riddelliine belongs to a class of toxic pyrrolizidine alkaloids that are esters of unsaturated basic alcohols (necines) and of a necic acid produced by plants growing in climates ranging from temperate to tropical. The pyrrolizidine alkaloid-producing plants are unrelated taxonomically. The alkaloids occur in different parts of the plants, with the highest content in the seeds and flowering tops. The quantity of the alkaloids varies, depending on the season, climate and soil constitution (Fu *et al*., 2001; National Toxicology Program, 2002).

2. Studies of Cancer in Humans

Riddelliine

No report of cancer related to the intake of riddelliine or of *Senecio* spp. in humans was available to the Working Group.

3. Studies of Cancer in Experimental Animals

Riddelliine

Oral administration

Mouse: Groups of 50 male B6C3F$_1$ mice, 5–6 weeks of age, were administered 0, 0.1, 0.3, 1.0 or 3 mg/kg bw riddelliine by gavage in sodium phosphate buffer on five days per week for 105 weeks. Groups of 50 female B6C3F$_1$ mice, 5–6 weeks of age, were administered 0 or 3 mg/kg riddelliine by gavage in sodium phosphate buffer on five days per week for 105 weeks. Mean survival (days) among these groups was 696 (0.0 mg/kg), 705 (0.1 mg/kg), 716 (0.3 mg/kg), 701 (1 mg/kg) and 667 (3 mg/kg) for males and 670 (0.0 mg) and 678 (3 mg/kg) for females. The incidence of hepatic haemangiosarcomas in males that received 3 mg/kg was significantly greater than that of the controls (2/50, 1/50, 0/50, 2/50, 31/50 ($p < 0.001$) at doses of 0, 0.1, 0.3, 1.0 and 3 mg/kg, respectively). The incidence of hepatocellular tumours was negatively correlated with dose in male mice and was significantly decreased in females given the 3 mg/kg dose. The incidences of bronchiolo-alveolar adenoma (1/50 control versus 9/50 treated) and of adenoma and carcinoma combined (2/50 control versus 13/50 treated) were significantly increased in the highest-dose females compared with control females. The incidence of bronchiolo-alveolar tumours in this group exceeded the historical control range for this neoplasm (National Toxicology Program, 2002).

Rat: Groups of 50 female Fischer 344 rats, 5–6 weeks of age, were administered riddelliine in sodium phosphate buffer by gavage at doses of 0, 0.01, 0.03, 0.1, 0.3 and 1 mg/kg bw on five days per week for 105 weeks. Groups of 50 males were administered riddelliine similarly at doses of 0 or 1 mg/kg bw for only 72 weeks, due to mortality in high-dose males. All high-dose (1 mg/kg bw) females died before week 97. Survival of females in the other groups was not affected. In females, there was increased incidence of haemangiosarcoma at the highest dose (0/50, 0/50, 0/50, 0/50, 3/50 and 38/50 ($p < 0.001$) at doses of 0, 0.01, 0.03, 0.1, 0.3 and 1 mg/kg, respectively). Hepatocellular adenoma/carcinoma combined were also increased (1/50, 0/50, 0/50, 0/50, 2/50 and 8/50 ($p < 0.001$) at doses of 0, 0.01, 0.03, 0.1, 0.3 and 1 mg/kg, respectively). Mononuclear-cell leukaemia was found in 12/50, 8/50, 13/50, 18/50, 18/50 and 14/50 at doses of 0, 0.01, 0.03, 0.1, 0.3 and 1 mg/kg, respectively (trend test positive, $p = 0.009$). In treated males, 43/50 ($p < 0.001$) developed haemangiosarcomas of the liver, 4/50 ($p = 0.03$) developed hepatocellular adenomas and 9/50 versus 2/50 controls ($p = 0.004$) had mononuclear-cell leukaemia (National Toxicology Program, 2002).

Groups of 20 male and 20 female Fischer 344 rats, 6–8 weeks old, were given 0, 0.1, 0.33, 1.0, 3.3 or 10 mg/kg bw riddelliine by gavage in phosphate buffer five times per week. Ten rats per sex per dose were sacrificed after 13 weeks of treatment. Five rats per

sex were killed after a seven-week recovery period and the remaining five rats per sex were killed after 14 weeks' recovery. No liver lesions were observed in control animals. Two of 10 female rats in the 10-mg/kg group examined at 13 weeks had hepatocellular adenomas. Three other females in this group had hepatocellular foci or focal nodular hyperplasia. One female in this group that died during the 14-week recovery period had multiple adenomas and multi-focal nodular hyperplasia and cholangiocellular hyperplasia (Chan *et al*., 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

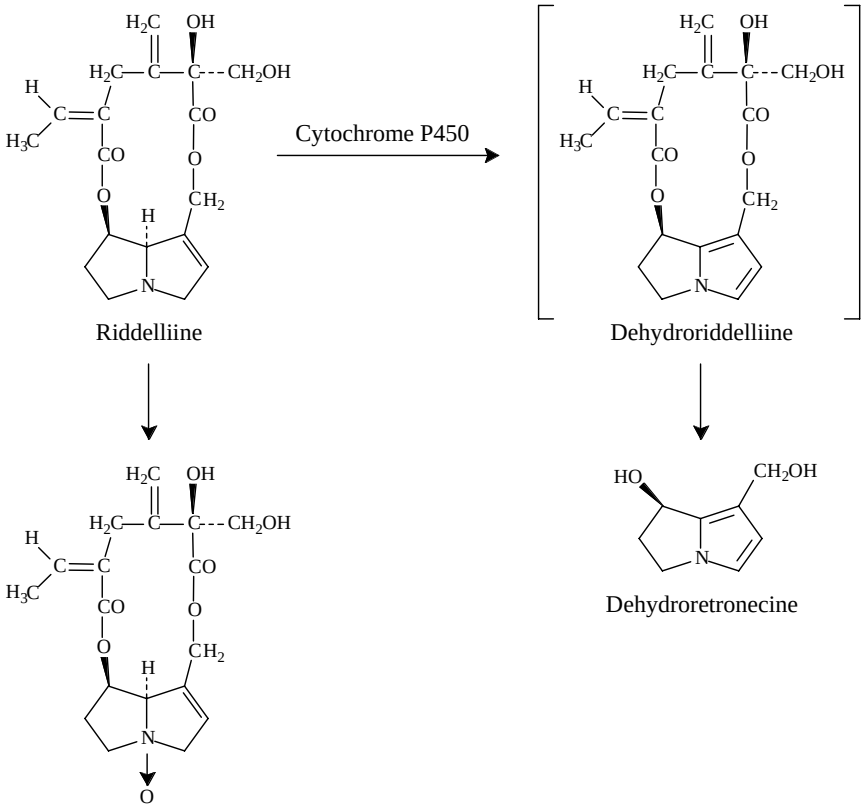
No data were available to the Working Group.

4.1.2 *Experimental systems*

Several feeding experiments have indicated that riddelliine and its *N*-oxide are absorbed in the gastrointestinal tract of domestic farm animals. Calves treated for 20 days with either riddelliine *N*-oxide (40.5 mg/kg/day) or a mixture of riddelliine *N*-oxide (40.5 mg/kg/day) and riddelliine (4.5 mg/kg/day) showed 100% morbidity, implying absorption in each case (Molyneux *et al.*, 1991). Tissue samples from pigs fed various amounts (3, 10 or 15 mg/kg bw in gelatin capsules) of riddelliine for 40 days also showed absorption, as determined by liquid chromatography and tandem mass spectrometric (LC/MS) analysis of pyrrolic metabolites in blood and liver samples collected one day after the end of treatment (Schoch *et al.*, 2000).

Pyrrolizidine alkaloids exist in plants in two forms, the free-base alkaloids and their *N*-oxides, neither of which is toxic *per se*. In numerous experimental studies, especially in rats, free riddelliine base has been found to be absorbed and transported to the liver, where it is converted to hepatotoxic pyrroles by microsomal enzymes (Mattocks & White, 1971; Molyneux *et al.*, 1991). In liver microsomes of Fischer 344 rats pretreated with phenobarbital, the major metabolites of riddelliine obtained after aerobic incubation for 30 min were riddelliine *N*-oxide and dehydroretronecine, as determined by HPLC and LC/MS (Figure 1). The latter metabolite interacts with DNA to form various adducts (Yang *et al.*, 2001a).

Figure 1. Metabolism of riddelliine by rat liver microsomes



From Yang *et al.* (2001a)

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group on the toxicity of riddelliine itself to humans.

Riddelliine is one constituent of the plant *Senecio longilobus*, which has been used as a folk remedy called gordolobo yerba by Mexican–Americans in the south-western USA (Segall & Molyneux, 1978). The consumption of gordolobo yerba has been linked with the incidence of acute hepatic veno-occlusive disease (Stillman *et al.*, 1977a,b). Two infants were diagnosed as exhibiting hepatic veno-occlusive disease due to pyrrolizidine alkaloid intoxication, as a result of ingesting *S. longilobus* as a herbal tea used as a cough medicine. One of these infants subsequently died (Stillman *et al.*, 1977a).

4.2.2 *Experimental systems*

Cheeke (1988) reviewed the general toxicity of pyrrolizidine alkaloids in *Senecio*, *Crotalaria* and other plant species in large animals, small herbivores and other laboratory animals. Cattle and horses are highly susceptible to pyrrolizidine poisoning, whereas sheep, goats, rabbits and guinea-pigs are much more resistant. A few studies have been published specifically on the toxic signs and symptoms of riddelliine. Thus, preflowering *Senecio riddellii* whole plants (Riddell's groundsel), a species that contains mainly (> 90%) riddelliine and its *N*-oxide as alkaloidal constituents, caused seneciosis when given to calves for 20 days either by gavage or in gelatin capsules at an equivalent dose of 15–20 mg/kg bw pyrrolizidine alkaloid per day. The main clinical signs were malaise, depression, erratic or unpredictable behaviour, aimless walking and ataxia. Diarrhoea with tecnesmus and rectal prolapse, and abdominal distension were frequently observed. Gross pathological findings included ascites, abomasal oedema, mesenteric lymph node enlargement and oedema, and oedema of the mesentery between loops of the ansa spiralis. Hepatobiliary lesions were present in all cattle necropsied. Portal biliary hyperplasia, formation of new bile ductules and periportal fibrosis were also seen. In more severe cases, vacuolated and enlarged hepatocytes were seen throughout the liver lobule. Central nervous system lesions were present in all cattle with seneciosis, such as spongy degeneration along the axonal tracts of the white matter. Other microscopic lesions included abomasal oedema, mucosal haemorrhage, lymph node oedema and occasional pulmonary haemorrhage (Johnson *et al.*, 1985).

In a follow-up study, under the experimental conditions described by Johnson *et al.* (1985), a group of calves fed 4.5 mg/kg bw riddelliine free base per day showed no sign of toxicosis or serum enzyme changes. However, two further groups fed pure riddelliine *N*-oxide (40.5 mg/kg/day) and a mixture of pure riddelliine (4.5 mg/kg/day) and pure riddelliine *N*-oxide (40.5 mg/kg/day) showed 100% morbidity, with the latter group showing fewer liver lesions. It was established from this study that the *N*-oxide of riddelliine alone is capable of inducing seneciosis in cattle (Molyneux *et al.*, 1991).

The toxicity of riddelliine was studied in male and female Fischer rats and in B6C3F$_1$ mice. The compound was given by gavage in 0.1 M phosphate buffer five times per week at daily doses of 0, 0.1, 0.33, 1, 3.3 and 10 mg/kg bw (rats) and of 0, 0.33, 1, 3.3, 10 and 25 mg/kg bw (mice). The animals were necropsied after 13 weeks of treatment or after an additional 7 or 14 weeks of recovery. Body weight gain was inversely related to dose in both rats and mice. The initial group sacrificed after 13 weeks showed dose-related hepatopathy and intravascular macrophage accumulation in rats (at doses ≥ 0.33 mg/kg bw) and hepatocytomegaly in mice (only at 25 mg/kg bw). Some of these lesions persisted throughout the 14-week recovery period, with hepatic foci and cellular alterations observed in male rats, and increasingly severe bile duct proliferation in female rats and in male and female mice (Chan *et al.*, 1994).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The developmental toxicity of pyrrolizidine alkaloids has been evaluated (IARC, 1976; WHO, 1988). Several pyrrolizidine alkaloids, pyrrolizidine alkaloid derivatives and related compounds have been shown to produce teratogenic and fetotoxic effects in experimental animals (WHO, 1988). However, none of the studies reviewed was on riddelliine. According to Chan *et al.* (1994), pyrrolizidine alkaloids have been detected in milk of lactating rats and cows.

In 13-week gavage studies combined with mating trials, groups of male and female Fischer 344 rats and B6C3F_1 mice were administered riddelliine at doses of up to 10 and 25 mg/kg bw, respectively. Body weight gain was inversely related to dose in both rats and mice. In rats, decreased epididymal and testis weights were observed in males given 1.0 mg/kg bw, but spermatozoal measurements were not affected. At 10 mg/kg bw (a dose lethal to the males), riddelliine caused persistent estrus in females. In mating trials with 1.0 mg/kg bw as the highest dose, no effect was seen on fertility, weight gain of dams during gestation, litter size or percentage of live pups. At 14 and 21 days of age, female pups had lower body weight than control pups. In mice, females in the 25-mg/kg bw group showed marked prolongation of length of the estrus cycle. Despite this, dosed dams were able to conceive and continue with pregnancy. There was no effect on maternal body weight gain during pregnancy at the 25-mg/kg bw dose, but live litter size was reduced and pups of treated dams had lower body weight at birth and during the lactation period than control pups. There was no effect on litter size or pup body weight at 3.3 mg/kg bw (National Toxicology Program, 1993; Chan *et al*., 1994).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Riddelliine was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of a metabolic activation system, but not in strains TA1535, TA97 and TA98. It caused DNA–protein cross-links in bovine kidney CCL 22 cells *in vitro*. Sister chromatid exchange and chromosomal aberrations were induced in Chinese hamster ovary cells *in vitro*. Transformation of BALB/c3T3 cells was observed in a single study.

Table 1. Genetic and related effects of riddelliine and its metabolite dehydroxyretronecine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Riddelliine				
Salmonella typhimurium TA1535, TA97, TA98, reverse mutation	–	–	5000 µg/plate	Zeiger *et al.* (1988)
Salmonella typhimurium TA100, reverse mutation	–	+	1000 µg/plate	Zeiger *et al.* (1988)
DNA-protein cross-links, bovine kidney (MDBK) CCL 22 cells *in vitro*	+	+	17.5	Hincks *et al*. (1991)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	3	Galloway *et al*. (1987)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	+	300	Galloway *et al*. (1987)
Cell transformation, BALB/c3T3 mouse cells, *in vitro*	+	NT	225	Matthews *et al*. (1993)
Unscheduled DNA synthesis, male and female Fischer 344 rat hepatocytes *in vivo*	+		50 po × 1	Mirsalis (1987)
Unscheduled DNA synthesis, male and female Fischer 344 rat hepatocytes *in vivo*	–		25 po 30 d[c]	Mirsalis *et al*. (1993)
Unscheduled DNA synthesis, male and female $B6C3F_1$ mouse hepatocytes *in vivo*	?[d]		25 po 30 d[c]	Mirsalis *et al*. (1993)
Unscheduled DNA synthesis, male and female $B6C3F_1$ mouse hepatocytes *in vivo*	+		25 po × 5	Chan *et al.* (1994)
Unscheduled DNA synthesis, male and female Fischer 344 rat hepatocytes *in vivo*	+		1 po × 5	Chan *et al.* (1994)
Micronucleus induction, male and female $B6C3F_1$ mouse bone marrow, *in vivo*	–		25 po 5 or 30 d[c]	Mirsalis *et al*. (1993)
Micronucleus induction, male $B6C3F_1$ mouse peripheral blood, *in vivo*	+		150 po × 1	Chan *et al.* (1994)
Micronucleus induction, male $B6C3F_1$ mouse bone marrow, *in vivo*	+		270 po × 1	Chan *et al.* (1994)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus induction, male Swiss mouse peripheral blood erythrocytes, *in vivo*	+		150 ip × 1	National Toxicology Program (2002)
Micronucleus induction, male Swiss mouse bone-marrow erythrocytes, *in vivo*	+		270 ip × 1	National Toxicology Program (2002)
Micronucleus induction, male and female B6C3F$_1$ mouse peripheral blood, *in vivo*	–		25 po 13 wk	Witt *et al*. (2000)
Micronucleus induction, male and female Fischer 344 rat bone marrow, *in vivo*	–		3.3 po 30 d[c]	Mirsalis *et al*. (1993)
DNA adducts, female Fischer 344 rat liver, *in vivo*, ^{32}P-postlabelling	+		0.01 po 3 mo[c]	Yang *et al*. (2001a)
Dehydroretronecine				
Salmonella typhimurium TA92, reverse mutation	+	NT	500 µg/plate	Ord *et al*. (1985)
DNA cross-links, pBR322 plasmid and M13 viral DNA	+	NT	29	Reed *et al*. (1988)
DNA–protein cross-links, bovine kidney (MDBK) CCL22 cells *in vitro*	+	NT	46	Kim *et al*. (1995)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.12	Ord *et al*. (1985)
DNA adducts, calf thymus DNA *in vitro*	+	NT	3.9	Yang *et al*. (2001b)
Alkylation of N^2 of deoxyguanosine *in vitro*	+	NT	1530	Robertson (1982)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; d, day; po, oral; ip, intraperitoneal; mo, month; wk, week
[c] Treated 5 days/week
[d] Equivocal response in males; modest response in females at highest dose only

Riddelliine induced unscheduled DNA synthesis in rat and mouse hepatocytes *in vivo* in some studies, but not in others. It induced micronuclei in peripheral blood erythrocytes and bone marrow of mice after a single high dose (intraperitoneal or oral) but not after repeated treatment of rats or mice at low dose. Riddelliine did form DNA adducts in rat liver following oral exposure.

A major metabolite of riddelliine, dehydroretronecine, was mutagenic in *S. typhimurium* strain TA92 and induced sister chromatid exchange in human lymphocytes in the absence of exogenous metabolic activation. Dehydroretronecine also induced DNA–DNA cross-links in pBR322 plasmid and M13 viral DNA and DNA–protein cross-links in bovine kidney CCL 22 cells *in vitro*. Exposure of calf thymus DNA or deoxyguanosine to dehydroretronecine *in vitro* caused DNA-adduct formation and alkylation of the exocyclic amino group, respectively.

Eight different dehydroretronecine-derived DNA adducts were detected in all liver samples from female Fischer 344 rats fed riddelliine at five different doses (0.01, 0.033, 0.1, 0.33, 1.0 mg/kg bw per day). Two of these were characterized as dehydroretronecine-3*N*-deoxyguanosin-N^2-yl epimers (Figure 2) by a ^{32}P-postlabelling/HPLC technique, supported by spectroscopic and synthetic procedures (Yang *et al.*, 2001b). The other six adducts detected were not identified (Yang *et al.*, 2001a).

4.5 Mechanistic considerations

Orally administered riddelliine forms dehydroretronecine–DNA adducts and induces unscheduled DNA synthesis in rat liver, indicating that liver is a target for its genotoxic activity. Dehydroretronecine forms DNA adducts and cross-links *in vitro* without further metabolic activation. This metabolite is also mutagenic to *S. typhimurium*. This activity, as well as the hepatotoxicity with consequent hyperplasia, could be significant events in riddelliine-induced hepatocarcinogenicity. It is not known, however, whether the basic metabolic step occurs in humans.

5. Summary of Data Reported and Evaluation

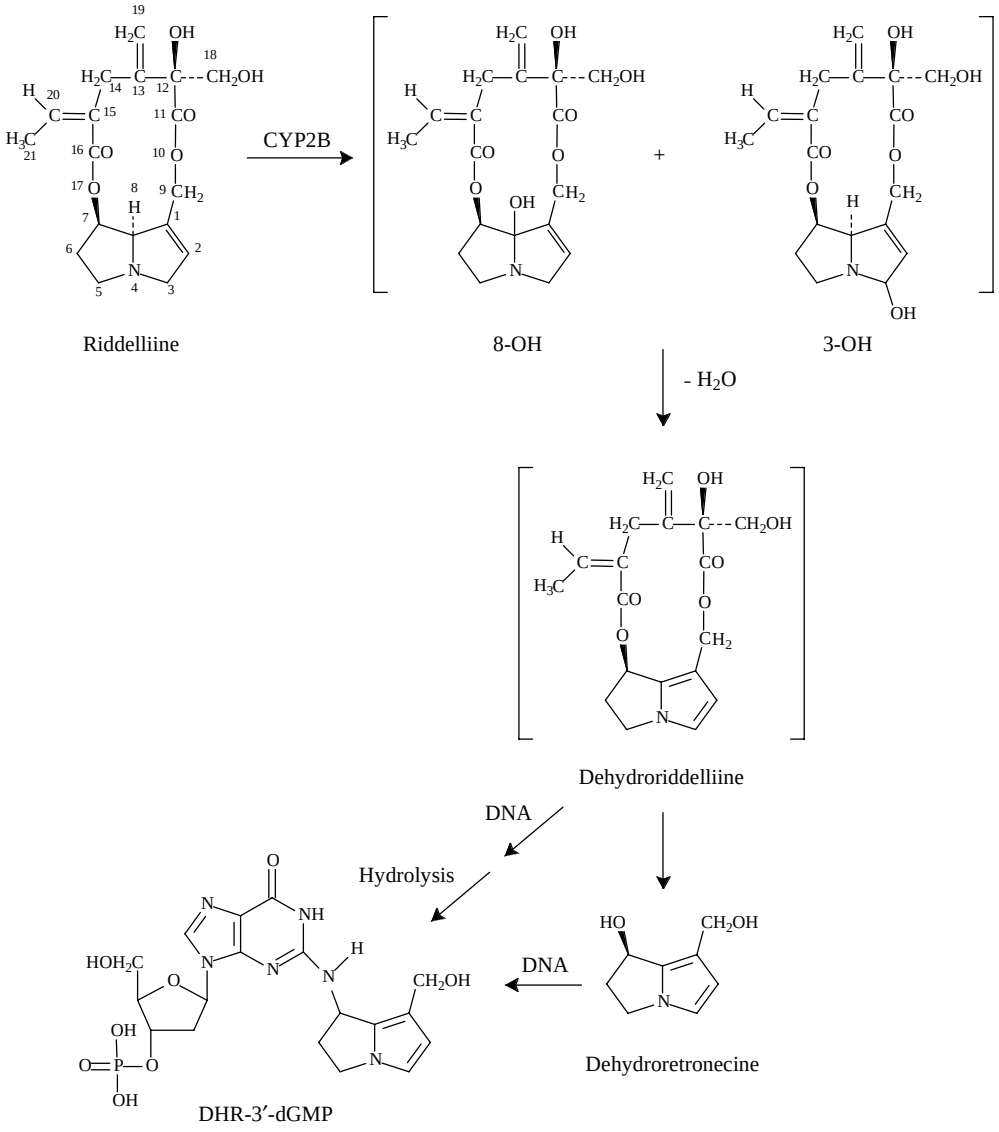
5.1 Exposure data

Riddelliine is a pyrrolizidine alkaloid that is found in *Senecio riddellii* and other *Senecio* species, including *S. longilobus*, which is used as a herbal remedy in the southwestern USA.

5.2 Human carcinogenicity data

No data on the carcinogenicity of riddelliine to humans were available to the Working Group.

Figure 2. Proposed metabolic activation of riddelliine leading to dehydroretronecine–DNA adducts in female Fischer 344 rats fed riddelliine



From Yang *et al.* (2001a)

5.3 Animal carcinogenicity data

In mice, oral administration of riddelliine induced hepatic haemangiosarcomas in males and bronchiolo-alveolar adenomas and carcinomas in females. In rats, oral administration of riddelliine increased the incidence of hepatic haemangiosarcomas, hepatocellular carcinomas and/or adenomas and mononuclear cell leukaemia in both males and females. In a short-term study, a few rats developed hepatocellular adenomas after 13 weeks of oral administration.

5.4 Other relevant data

Riddelliine and its *N*-oxide are absorbed from the gastrointestinal tract. Riddelliine is metabolized to riddelliine *N*-oxide and dehydroretronecine in rat liver microsomes.

A herbal preparation made from *Senecia longilobus*, of which riddelliine is a constituent, causes hepatic veno-occlusive disease in humans. Intoxication of calves with either *S. riddellii* or one of its two main pyrrolizidine alkaloid constituents, riddelliine *N*-oxide, led to the typical signs and symptoms of seneciosis. A similar toxicity profile was induced by riddelliine in rodents.

Riddelliine disturbs the estrus cycle in rodents. It causes developmental toxicity in the absence of marked toxicity in rodents.

DNA adducts of dehydroretronecine are found in rat liver following oral administration of riddelliine. Dehydroretronecine is genotoxic in a number of in-vitro systems. It induced sister chromatid exchange in human lymphocytes, DNA–protein cross-links in bovine kidney epithelial cells *in vitro* and gene mutations in bacteria.

5.5 Evaluation

There are no data on the carcinogenicity of riddelliine to humans.

There is *sufficient evidence* in experimental animals for the carcinogenicity of riddelliine.

Overall evaluation

Riddelliine is *possibly carcinogenic to humans (Group 2B)*.

6. References

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