

Riddelliine Expert Panel Report

Part A – Peer Review of the Background Document on Riddelliine:

The Report on Carcinogens (RoC) expert panel for riddelliine met at the Sheraton Chapel Hill Hotel on January 24 & 25, 2008, to review the draft background document on riddelliine and make a recommendation for the listing status of riddelliine in the 12th Edition of the RoC. Members of the expert panel are as follows:

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The charge to this panel was to determine whether information in the draft background document on riddelliine was presented in a clear and objective manner, to identify any information missing from the body of knowledge presented in that document, and to determine the utility of this body of knowledge for drawing conclusions regarding carcinogenicity of a candidate substance and for applying the RoC criteria for listing. Following discussion, the expert panel reached consensus concerning the critique of the draft background document, including its adequacy and proposed revisions, then voted 7 yes/0 no to accept this document (with changes specified by the expert panel). The expert panel agreed that the background document is adequate for drawing conclusions regarding the carcinogenicity of riddelliine and for applying the RoC listing criteria.

The expert panel's proposed revisions for each section of the riddelliine background document are appended.

Executive Summary

1. Human Exposure

- Page v, line 18 – change “known to contain riddelliine,” to “known to contain riddelliine as well as seneciphylline and senecionine.”

Section 1: Introduction

1. General
 - NTP should conduct a final literature review looking for additional exposure data in past 30 years.
 - Change title of Section 1 from “Introduction” to “Introduction and Chemistry.”
2. Section 1.3 (Metabolites)
 - If additional metabolites are added to section 5, NTP should add them to Section 1.3.

Section 2: Human Exposure

1. General and introduction Section 2.3.2 (Herbal products)
 - Parts of the draft background document suggest that riddelliine is the cause of many herbal and plant related toxicities and carcinogenicity. In fact riddelliine is not the major pyrrolizidine alkaloid in *Senecio jacobea* or *S. longilobus*; Seneciphylline and senecionine are much more abundant. The NTP should review the draft background document for potential biased statements towards riddelliine.
 - Page 11, line 7 – Change sentence from “Riddelliine is a PA” to “Riddelliine and riddelliine *N*-oxide are naturally occurring PAs found in plants (primarily of the genus *Senecio*) that are found . . .”
2. Section 2.3.2 Herbal products
 - Page 16, line 21 – The FDA makes a strong statement of regulation in the July 6, 2001 advisory. Change sentence from “In the United States, these products are essentially unregulated” to “In the United States, *prior to 2001*, these products were essentially unregulated (change the rest of the sentence from present tense to past tense). Add new sentences following this sentence: “In 2001, the FDA issued an advisory to dietary supplement manufacturers to remove comfrey products from the market. The advisory states that any product containing PAs is considered adulterated under the Dietary Supplement Health and Education Act (DSHEA).”
 - Page 17, line 18 – *S. longilobus* contains other PAs as well as riddelliine. Add “as well as seneciphylliine and senecionine” to text after “... *S. longilobus* has been shown to contain riddelliine.”
 - Page 19, line 21 – Children are not “disproportionately exposed;” they are uniquely susceptible. Change text to read, “Children are uniquely susceptible” [Small *et al.* J. Vet. Med A 40:213-218, 1993].
 - Page 19, line 28 – Add at the end of this paragraph (discussing human toxicity) that there are several reports in animals of neonatal and fetal toxicity with little maternal toxicity (cite Small *et al.* 1993).
3. Section 2.3.3 Food
 - *Meat*, page 21, line 29 – The potential for meat contamination is discussed. Contamination of meat is not likely. However, Americans do eat liver and a small section on this risk should be included. Add text to the end of this paragraph discussing the potential for exposure to humans eating liver.
 - *Meat*, page 22, lines 1-15 – This method [liberation of sulfur-bound pyrrolic metabolites from tissue, reaction with ethanol, and identification with GS-MS] has been problematic. It is inconsistent and no one has been able to make it truly quantitative. It is useful as a qualitative indicator of exposure. Though likely, there is no information concerning secondary poisoning and the potential toxicity of pyrrole adducts. The text on this method provides no information on levels in meat so move the information to the analytical methods (Section 2.4) and revise as needed.

- *Milk*, page 23, line 23 – “However, individuals potentially could be exposed by consuming milk from a family cow or goat grazing where *S. riddellii* ...” Add “organic milk” after “family cow or goat” based on the potential for cows supplying organic milk to graze on plants containing PAs. Then add that the potential to exposure for PAs from goat’s milk may be even greater as goats are relatively resistant to poisoning. There is a potential for relay toxicity, especially as highly susceptible infants are likely to drink goat’s milk.
4. Section 2.4 Analytical Method
 - Add information on the method for liberation of sulfur-bound pyrrolic metabolites from tissue, reaction with ethanol, and identification with GS-MS as noted above in item #3, second bullet.
 5. Section 2.6 Regulations
 - Add information on FDA advisory dated July 6, 2001. The advisory states: “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.”
 6. Section 2.7 Summary
 - Page 30, line 26 – The statement, “Two cases of fatal human exposure to riddelliine-containing plants in an herbal tea have been reported from the United States” is misleading as it implies that riddelliine is the sole cause. Add seneciphylliine and senecionine in addition to riddelliine.

Section 4: Studies of Cancer in Experimental Animals

1. Section 4.4 Metabolites

- Page 44, line 2 – Expand this section to include discussion that although riddelliine and many other hepatotoxic PAs share in common the reactive metabolite DHP (see also Sections 1 and 5), this is a simplification. Certainly they share DHR and DHH as metabolites, but we probably should not discount the effect and toxicity of PA-specific pyrroles (dehydroriddelliine). If all toxicity and carcinogenicity were attributed to DHP, the neoplasms, distribution, and lesions would be similar when the doses are adjusted for activation efficiency. This is not the case. The riddelliine data suggest that it is more likely to produce hepatic vascular neoplasms. Seneciphylline and senecionine produce more hepatocellular neoplasms. Still other PA's are likely to produce extrahepatic lesions. These alkaloid-specific changes suggest that intermediates such as dehydroseneciphylline are important nucleophiles. Add any additional information from the NTP study on riddelliine, Cook *et al.* 1950, Harris and Chen 1970, and Hooper 1978 [Hooper 1978 is a new reference identified by the expert panel].

Section 5 (Other Relevant Data)

1. General

- The addition of information in Sections 5.1.3, 5.2, 5.3.2 and creation of a new Section 5.3.3 were proposed as useful for assessing the carcinogenic potential of riddelliine in humans. The specific changes are included below for the appropriate subsections of Section 5.

2. Section 5.1.3 Metabolism

- Add information on pyrrole metabolite (dehydroriddelliine) and pulmonary toxicity from Wilson *et al.* 1992.

3. Section 5.1.3 Comparison of metabolism in humans and rodents

- Change title of subsection to '*Comparative metabolism.*'
- Page 56, line 14 – Expand discussion with information from Hooper 1978 (book chapter), Huan *et al.* 1998, and Durringer *et al.* 2004. Strengthen the current paragraph to note that human susceptibility is likely to be more similar to livestock rather than to rodents.

4. Section 5.2 Studies of DHP adduct formation

- Pages 57-61 – Add a summary of established DNA adduct structures for riddelliine (there appear to be 8); also add structures of DNA adducts derived from other pyrrolizidine alkaloids that form tumors in rats (Wickramanayake *et al.* 1985, Wiessler 1994).

5. Section 5.3.1 DNA adducts and mutations

- Add a new paragraph to discuss riddelliine crosslinking in Section 5.3.1 (Hoorn and Roth 1992, Hoorn *et al.* 1993, Kim *et al.* 1999, Wagner *et al.* 1993).

6. Section 5.3.2 DNA adducts and tumor formation

- Page 66, line 11 – Expand the explanation of half-life and persistence of DNA adducts in various species of experimental animals treated with riddelliine. [Add a new paragraph at the end of Section 5.3.2 citing Schoch *et al.* 2000, Mattocks and Jukes 1992 (ref 103), Durringer *et al.* 2004, and Stegelmeier *et al.* 1996 (includes houndstongue).]

7. New Section 5.3.3 (page 66, line 12)

- Add a paragraph discussing structure activity for toxicity and genotoxicity (Frei *et al.* 1992, Fu *et al.* 2002b, Kim *et al.* 1999).

8. Section 5.6 Toxicity

- Page 79, line 12 – Revise as follows: "*S. longilobus*, a plant known to contain PAs, including riddelliine." Parts of the background document are written such that it suggests that riddelliine is the cause of many herbal and plant-related toxicities and carcinogenicity. In fact, riddelliine is not the major pyrrolizidine alkaloid in *Senecio jacobea* or *S. longilobus*. Seneciphylline and senecionine are much more abundant.

Report Approved Redacted Date 3/24/08
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References¹

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¹ New references identified by the expert panel

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