SYMPHYTI RADIX

SUMMARY REPORT

1. *Symphyti radix* is the fresh or dried root section of *Symphytum officinale* (synonym: comfrey) which is a plant of the *Boraginaceae* family.

   *Symphyti radix* contains allantoin (0.6 to 0.8%), triterpene saponins (including symphytoxide A), isobauerolen, the phytoestrogens 8-sitosterol and stigmasterol as well as pyrrolizidine alkaloids (up to 0.7% in dried root material) most of which are unsaturated in the 1:2 position of the necine base and are esterified at the hydroxyl groups in the C7 and/or C9-position (e.g. echinatine, lycopsamine, 7-acetyllycopsamine, echimidine, lasiocarpine, symphytine, symlandine, intermedine, acetyllintermedine, synveridine, heliocarpine and heliosupine, viridiflorine and echumine). The content of individual pyrrolizidine alkaloids vary considerably dependent on the plant variety, species hybridisation, and place and time of growth. Additionally, the alkaloids symphytocynoglossin, consolicin and its derivative consolidin are found as well as lithospermic acid, rosmarinic acid and several other substances. Furthermore, mucilaginous substances (up to 30% of the dry weight), tannins (to 6%) and silicic acid (about 4%) are contained in the plant.

2. *Symphyti radix* and preparations thereof are contained in 2 veterinary medicinal products used in phytotherapy. One preparation is a solution containing 5 active ingredients, of which 1 is a tincture prepared with 60% isopropanol from 4 different plants, including 1 g *Symphyti radix*. This tincture constitutes 53.8% (w/w) of the finished product and additionally 22% (w/w) of a decoct of *Symphyti radix* (in total extracts from 2.1 g *Symphyti radix* in 100 g of the finished product). It is used for topical administration to all food producing species, particularly cows, horses, sheep and goats, to treat strains and ruptures of muscles and tendons, and swollen joints. The usual dose is up to 10 ml of the product, depending on the size of the lesion. The product is only intended for use on intact skin and duration of treatment is 2 to 3 days. The pulverised dried *Symphytum* roots (17.5% (w/w) and 3 other ingredients are part of an anti-diarrhoeic powder for cattle, horses, sheep and goats, to treat strains and ruptures of muscles and tendons, and swollen joints. The usual dose is 30 to 90 g of the powder for cattle and horses, and 15 to 30 g for calves, foals, pigs and sheep, given for 3 days.

In homeopathy, preparations of *Symphytum officinale* according to homeopathic pharmacopoeias are alcoholic extracts from the roots collected before the time of blossoming. In veterinary homeopathy 1:10 and 1:1000 dilutions of *Symphytum officinale* are intended for parenteral or oral (drops, tablets) use in all food-producing species. The maximum recommended parenteral dose for large animals is 10 ml/animal. Treatment may be repeated but a fixed dose schedule is not common in homeopathy.

In humans, the use of medicinal (phytotherapeutic) products containing *Symphyti radix* and preparations thereof has been restricted to external application on undamaged skin only in some Member States. Ointments and other preparations for external application may contain 5 to 20% dried drug or equivalent preparations. The duration of treatment is limited to a maximum of 4 to 6 weeks and the daily dose of pyrrolizidine alkaloids with 1,2-unsaturated necine structure should not exceed 100 µg per person per day (not to be used by pregnant and breast-feeding women without special consultation).
In human homeopathy, the oral and parenteral use of *Symphytum officinale* is still allowed but limited to doses containing a maximum of 1 µg pyrrolizidine alkaloids with unsaturated necine configuration per person and day, including the N-oxide-derivatives. For topical uses this limit is 100 µg/person and day. Contraindicated in pregnant and breast-feeding women are pyrrolizidine alkaloid doses greater than 0.1 µg/person and day for oral or parenteral administration and 10 µg after topical use. In some countries the use of these drugs is discouraged in infants. Duration of medication should not exceed 6 weeks per year.

In some countries, e.g. Japan, comfrey leaves are used as food.

3. In rats wound-healing, anti-inflammatory and analgesic effects were observed, possibly due to allantoin and rosmarinic acid contained in *Symphytum officinale* (no details available). Consolicina, consolindine and symphytocynoglossine are reported to inhibit central nervous system activity. Aqueous extracts of *Symphytum officinale* roots containing oxidated lithospermic acid have been shown to exert significant antigonadotrophic effects *in vitro*. *In vivo*, cessation of cyclus was observed after several injections to female rats. Threshold doses for these effects have not been determined. Pyrrolizidine alkaloids are not considered necessary for the therapeutic activity.

3. After oral administration, pyrrolizidine alkaloid bases generally appear to be well absorbed. Pyrrolizidine alkaloids are mainly excreted via urine (50 to 80%). Pyrrolizidine alkaloid-N-oxides, which represent the main fraction of pyrrolizidine alkaloids in *Symphyti radix*, have to undergo reduction in the gut to be absorbed in appreciable amounts.

Nearly no absorption of pyrrolizidine alkaloids takes place when used externally on intact skin. In rats after dermal application of a crude alcoholic extract of *Symphyti radix* at an approximate dose of 194 mg pyrrolizidine alkaloid-N-oxides/kg bw for 2 days, 0.1 to 0.4% of the administered dose were excreted via urine as pyrrolizidine alkaloids. After oral administration, 20 to 50 times higher levels of N-oxides and free alkaloids were detected in urine (no further details provided) in the above experiment in rats. N-oxides are reduced by reductases of the gut flora to the more toxic free alkaloid.

No further pharmacokinetic data are available for pyrrolozidine alkaloids contained in *Symphyti radix*. The following information relates to other pyrrolizidine alkaloids. After intraperitoneal administration of $^{14}$C-senecionine and $^{14}$C-seneciphylline to lactating mice, pyrrolizidine alkaloids were excreted rapidly via urine (66 to 75% of the dose) and faeces (14 to 18%) within 16 hours. In the exhaled air 0.2 to 0.5% of the dose was detected and 0.04% was excreted in milk. In liver tissue, which contained 1.5% of the dose, the radioactivity was mainly bound covalently to proteins, DNA or RNA. Elevated concentrations were also found in lungs and kidneys. Excretion of pyrrolizidine alkaloids into milk has also been observed in rats at a rate of 0.08% of the dose within the first 3 hours after administration of $^{3}$H-senecionine or $^{3}$H-seneciphylline. Parent compound accounted for 20% of the residues found in rat milk. After administration of high dietary amounts of tansy ragwort (another alkaloid-containing plant) to cows and goats, (approximate daily intakes of up to 16 mg pyrrolizidine alkaloid/kg bw) excretion in milk amounted to 0.84 and 0.53 mg pyrrolizidine alkaloid/kg milk, respectively, which corresponds to 0.1 to 0.2% of the dose.

In general, pyrrolizidine alkaloids with unsaturated 1,2-necine configuration such as those found in *Symphyti radix* are reported to be metabolised to dehydropyrrolizidines (pyrrolizidine alkaloid pyroles), which are able to alkylate macromolecules. They undergo rapid hydrolysis to form the alkyllating necine pyroles (e.g. dehydroretronecine). Both pyrole species are supposed to exert toxicity by covalent binding to DNA or proteins. A second pathway, leading to detoxification, is the formation of pyrrolizidine alkaloid-N-oxides in the liver followed by hydrolysis to form more polar metabolites (necine base and acid moiety) which are eliminated mainly via urine. Conjugates of the pyroles, such as 7-gluthathionyl-dehydroretronecine, have been found in the bile of rats treated with pyrrolizidine alkaloids containing an ester-bond at the C7 site.

No data on the pharmacokinetics and metabolism of other constituents of *Symphyti radix* were available.
4. Most pyrrolizidine alkaloids tested are of moderate acute toxicity. The intraperitoneal LD$_{50}$ in rats and mice for symphytine was given with 130 mg/kg bw and 300 to 350 mg/kg bw. The oral, intraperitoneal, and intravenous LD$_{50}$ values of lasiocarpine were 110, 78, and 88 mg/kg bw, respectively. A mixture of the monoesters lycopsamine and intermedine was reported to lead to deaths in rats after oral doses of 1500 mg/kg bw and to acute central nervous system effects at 1000 mg/kg bw. Chronic liver damage was seen after single oral doses of 500 mg/kg bw. Pyrrolizidine alkaloids with an unsaturated 1,2-necine-configuration and one or two ester groups at carbon C7 and/or C9 induce hepatotoxic effects after high acute doses, characterised by acute necrosis and liver failure. Megalocytosis and veno-occlusive disease, sometimes followed by fatal liver cirrhosis have been observed in animals and humans. Lithospermic acid was reported to be of low toxicity: At intravenous doses up to 200 mg/kg bw in mice, no pathological signs were encountered. No information on other constituents of Symphytum officinale was available.

5. No adequate repeated oral dose toxicity studies were available for Symphyti radix or any of its constituents. For several different pyrrolizidine alkaloids, hepatic tissue damage as well as lesions in other tissues have been demonstrated after repeated administration of low doses. In rats, single gavage doses of 200 mg/kg bw or repeated doses of 50 to 100 mg/kg bw (3 times/week for 3 weeks) of pyrrolizidine alkaloids of Russian comfrey (slightly different pattern of pyrrolizidine alkaloids to Symphytum officinale) led to histologically identifiable liver damage, with swelling of liver cells, haemorrhagic necrosis and hepatic vessel changes indicative of the first stages of veno-occlusive disease. In rats, fed dietary amounts of 5000 to 80 000 mg Symphyti radix/kg food, corresponding to 250 to 4000 mg of crude dried root material/kg bw, hepatotoxicity and early deaths were encountered at doses of 10 000 mg/kg feed or higher, which corresponds to approximately 1.5 mg of Symphyti radix derived pyrrolizidine alkaloids/kg bw/day.

6. The susceptibility of animals species to the toxic effects of pyrrolizidine alkaloids varies widely, exhibiting differences of more than 2 orders of magnitude. Swine are reported to be exceptionally sensitive, followed by chicken (less sensitive by a factor 5), cattle and horses (factor 14), rats and mice (factor 50 and 150) and sheep (factor 200). Ruminal microflora is assumed to detoxify pyrrolizidine alkaloids to a significant extent. However, numerous outbreaks of poisonings partly connected to extensive morbidity and mortality have been recorded in animals grazing pastures contaminated with pyrrolizidine alkaloids containing plants.

7. No adequate information on developmental toxicity of Symphyti radix or any of its constituents was available. Data exist for other pyrrolizidine alkaloids showing embryotoxic and teratogenic potential.

8. The mutagenic activity of Symphytum radix and its main pyrrolizidine alkaloids has been tested in several prokaryotic and eukaryotic cell systems in vitro. Salmonella-microsomal assays performed with the drug did not always reveal positive effects. However, chromosomal aberrations and antimitotic activity of the aqueous extracts and individual fractions of Symphytum officinale root and one of its ingredients, lasiocarpine, were described in lateral root cells of Vicia faba. A crude pyrrolizidine extract of Symphytum officinale roots induced sister chromatid exchanges (SCEs) and structural chromosomal aberrations in human lymphocytes in vitro, at a concentration of 140 µg/ml with metabolic activation and at 1400 µg/ml without metabolic activation. Symphytine was reported to induce mutations in the Salmonella typhimurium strain TA 100 with metabolic activation. It also induced forward mutations in V79 Chinese hamster lung cells, but did not transform cryopreserved hamster embryo cells. The main individual pyrrolizidine alkaloids occurring in comfrey, 7-acetylintermedine, 7-acetylycopsamine, symphytine, symlandine, intermedine and lycopsamine, gave positive results in the wing spot test in Drosophila melanogaster when fed at concentrations of $10^{-5}$ to $10^{-3}$ mol to 72 h-old third-instar larvae during 48 hours, i.e. until pupation. Dehydroretronecine, a possible metabolite of several pyrrolozidine alkaloids contained in Symphyti radix, was mutagenic in the Salmonella-microsomal assay in strain TA 92 at concentrations of approximately 500 µg/plate, and induced sister chromatid exchanges in human lymphocytes at concentrations of about $10^{-6}$ mol. No detailed reports of these studies were available.
In general, pyrrolizidine alkaloids with an unsaturated necine configuration and a mono- or diester-bond at the C7 and/or C9 sites exert mutagenic effects in bacterial test systems, as well as in eukaryotic systems using test organisms such as *Drosophila melanogaster* and mammalian cells *in vitro* and *in vivo*. Many of these compounds have also shown antimitotic activity, sometimes interfering with the expression of their mutagenicity in mammalian test systems.

9. Available studies on possible tumour induction by *Symphytum officinale* or its constituents were not carried out according to current standards of carcinogenicity testing: Liver tumours were seen in rats receiving *Symphytum officinale* at dietary concentrations of 1 to 8% for several months. Due to toxicity the doses were reduced to 1, 0.5 or 0% after distinct intervals. The liver tumour incidence was not clearly dose-correlated, possibly due to shortened life spans in the higher dose groups. Additionally, 30 rats of both sexes were exposed to 0.5% comfrey root via diet for 754 days (65 male and 65 female controls). Of these animals 8 out of 30 developed hepatocellular adenomas and 9 out of 30 had hemangioendothelial sarcomas of the liver. Of animals surviving longer than 590 days, 69% (9 of 13) had hemangioendothelial sarcoma of the liver. Most of the tumour bearing animals developed both kinds of liver tumours. No liver tumours were observed in the controls. The pyrrolizidine alkaloid doses in the experiment roughly correspond to 0.75 mg/kg bw at the low dose level of 0.5%. Twenty male rats were treated with intraperitoneal injections of 0 or 13 mg/kg bw symphytine twice weekly for 4 weeks, followed by 1 treatment/week for further 52 weeks. The experiment was terminated after 650 days. Hemangioendothelial sarcoma of the liver was seen in 3 of 20 rats of the symphytine-group and 1 had a liver cell adenoma while none of the control rats showed any liver tumours. Lasiodaricine, at low doses (15 mg/kg in the feed, approximately 0.75 mg/kg bw) induced liver tumours in about one third of 24 female rats, mainly in the second year on trial. Synergistic effects (100% hepatomas and death within 1 year) were observed by concomitant administration of cycasin, another hepatocarcinogen, which in some parts of the world also occurs in the human food chain (cycad flour). Lasiodaricine was also found to induce neoplasias of the skin, lung, ileum and hematopoietic system.

Single gavage doses of 500 to 1500 mg/kg bw of a mixture of lycopsamine and intermedine, pyrrolizidine alkaloid monoesters, which both are present in comfrey root, to weanling rats caused pancreatic islet tumours in 3 of 15 animals after 1 to 2 years.

In conclusion, several pyrrolizidine alkaloids of comfrey have shown tumorigenic activity in rodents. It is further known that all pyrrolizidine alkaloid diesters with unsaturated necine configuration tested to date for mutagenicity and carcinogenicity have proved to be genotoxic carcinogens in rodents. Monoesters less consistently reveal positive results in the respective genotoxicity studies, but may generally be considered active at the same endpoints as the diesters. No information on human carcinogenicity from epidemiological studies is available at present.

10. In humans cirrhosis has been reported as a frequent sequela to early life (foetal, early postnatal, childhood) liver lesions by pyrrolizidine alkaloids. Fatal veno-occlusive disease was diagnosed in a young man, who had eaten 4 to 5 leaves of fresh comfrey every day, for 1 to 2 weeks only. In another incidence veno-occlusive disease was observed in a woman after daily intake of average doses of comfrey-pyrolizidines of 0.015 mg/kg bw.

However, *Symphytum officinale* is permitted in European countries as flavouring substance. Comfrey leaves are sometimes eaten as vegetable or salad. In some cases the varieties used as vegetables contain lower amounts of the toxic pyrrolizidine alkaloids. Though the use of comfrey leaves as vegetable and of comfrey root preparations as medicinal drug can be assumed to be frequent, sometimes resulting in a relatively high intake of pyrrolizidine alkaloids (8.5 to 26 mg/cup of tea), reports of adverse effects are relatively rare. However, minor evidence of liver damage may normally not be noticed and cirrhosis after chronic intake may not be easily differentiated from liver damage due to other causes.

11. No information on residues of *Symphytum officinale* and their depletion following treatment of food producing animals was provided.
Conclusions and recommendation

Having considered the criteria laid down by the Committee for Veterinary Medicinal Products for the inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- after long term oral administration of *Symphytii radix* in laboratory animals hepatotoxicity leading to tumours have been observed, which, however, is not expected after application to intact skin as no significant absorption takes place,
- preparations for topical use containing *Symphytii radix* are used in a small number of individual animals and for non-regular treatment only,
- the animals are unlikely to be sent to slaughter immediately after treatment;

the Committee for Veterinary Medicinal Products recommends the inclusion of *Symphytii radix* in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

<table>
<thead>
<tr>
<th>Pharmacologically active substance(s)</th>
<th>Animal species</th>
<th>Other provisions</th>
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<tbody>
<tr>
<td><em>Symphytii radix</em></td>
<td>All food producing species</td>
<td>For topical use on intact skin only</td>
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