European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

PICEAE TURIONES RECENTES EXTRACTUM

SUMMARY REPORT

1. *Piceae turiones recentes extractum* (spruce-tip extract) is manufactured from the crude drug, which is defined as the fresh 10 to 15 cm long shoots, collected in spring, of *Picea abies* (L.) Karsten (*Pinaceae*) [synonym *Picea excelsa* (Lam.) Link.] or *Abies alba* Miller [Synonym *Abies pectinata* (L.) DC]. The raw material is extracted by hot water (90°C). The resulting extract is dried to a dry matter content of 35 to 55% to form a black/brown viscous paste. This extract is mixed with starch and included in a herbal powder product which also contains *foliae melissae*, *flores chamomilae* and *herba absinthii*. The herbal product is also formulated as aqueous preparation. One hundred g of final herbal powder product and 650 ml of solution contain about 3.06 g dry extract. Details concerning the manufacturing process suggested an extraction yield of about 10%.

   In veterinary medicine, the product containing the herbal extract is intended for oral use (via feed, drinking water or directly per os) in the treatment of diarrhoea in cattle, horses, pigs, sheep and poultry. The recommended dose is about 0.1 g to 1 g powder/kg bw or 0.6 to 6.4 ml solution/kg bw respectively, which is equivalent to 3.1 mg to 30. 6 mg spruce-tips extract/kg bw. The product is also used added to the feed in cases of unthriftiness and poor feeding.

2. Characteristic constituents of spruce foliage are the terpenes (mono-, sesqui-, di- and triterpenes) of which, according to published literature, the monoterpenes (bornyl acetate, α-pinene, camphene, limonene) and the diterpenes (manool and dehydroabietate) are the most abundant subclasses. Another complex group of compounds of quantitative importance are phenolic compounds of different classes encompassing tannins (mostly of condensed type) and flavonoids (e.g. glycosideically bound kaempferol, taxifolin, isorhamnetin, laricitrin, quercetin, myricetin, syringetin), furthermore stilbene glycosidees/stilbenes (piceatannol/astringin, isorhapontin and piceid) and lignans (e.g. conidendrin, pinoresinol, lariciresinol, hydroxymatairesinol). Other components reported in literature are alkaloids of the piperidine type.

   Analysis of a preparation of the dry hot-water extract of spruce-tip by pharmacopoeia methods showed the following composition: tannins (24.8%), total phenolic compounds other than tannins (23.4%), essential oils (2.1%), triterpene saponins/glycosidees (2.8%), basic substances (1.0%) and flavone glycosidees (0.05 %). An investigation applying GC and/or HPLC methods roughly confirmed the result with respect to the tannins (about 20%) but found substantially less essential oils (about 0.25%). The reason for this difference was not provided.

   The analysis of the extract by HPLC and GC/MS methods displayed that, compared to the contents of fresh needles, tannins were largely recovered, while the fraction of essential oils was depleted or practically absent. In hot water, phenolic compounds can get condensed oxidatively/enzymatically to higher molecular derivatives. Other components of possible relevance for the assessment such as piperidine alkaloids (125.43 µg/g extract dry weight), stilbenes (10.7 µg/g extract dry weight) and diterpenes (1.88 mg/g extract dry weight) were also considerably decreased, up to 100-fold. Additionally limonene (0.15 µg/g extract dry weight), borneol (1.42 µg/g), caryophyllene (4.73 µg/g), and sesquiterpene related substances (10.2 mg/g), kaempferol-3-rutenoside (9.7 µg/g), isorhamnetin-3-glucoside (3.3 µg/g), quercetin-3-rutenoside (22.9 µg/g), and syringetin (4.4 µg/g), as well as 1.2-dehydropinedinone, camphene, longipinene, farnesene, cedrene, and neoisolongifolene

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(not quantified) were found in the extract. The following substances: pinidine, 6-epi-dihydropinidine, 1.2-dehydropinidinol, pinidinol, 6-epi-9-epi-pinidinol, 2-methyl-6-propyl-1.6-piperidine, pinidinone, 6-epi-pinidinone, 1-methyl-3-granatone, daidzin, glycitin, genistin, daidzein, glycitein, genistein, bisabolol, α-pinene, β-pinene and bornyl acetate were not detected.

3. No specific pharmacokinetic studies have been performed with spruce-tip extract in laboratory or target animals. In literature, a considerable amount of data is available on pharmacokinetic parameters of individual substances or classes of compounds observed in *Piceae turiones recentes extractum* (spruce-tip). The overall results allowed the following observations to be made: essential oils as relatively lipophilic compounds can be expected to be absorbed almost completely in the gastro-intestinal tract and distributed extensively. These compounds are mainly metabolised in the liver and excreted in conjugated form in bile or urine.

   Phenolic compounds show a considerable structural diversity, which can range from simple molecules such as phenolic acids to highly complex polymerised compounds, such as tannins. Absorption and metabolism of orally administered phenolics are primarily dependent on their chemical structure, which is determined by the degree or type of glycosylation, conjugation with other phenolics, as well as molecular size and degree of polymerisation. Oral absorption of condensed tannins is generally considered to be poor. The gastrointestinal mucosal membrane is believed to be impermeable to tannic acid, the best known hydrolysable tannin. Aglycons and free simple phenolic compounds, like flavonoids, stilbenes, lignans, phenolic acids and acetophenones can be absorbed directly while glycosidees may need to be hydrolysed before absorption can occur. Bioavailability of these compounds can vary largely from one compound to another. While literature data suggested relatively low bioavailability of ^{14}C-quercetin in pigs (17%), higher bioavailability was observed in humans (35 to 50%). A high amount of radioactivity in exhaled air suggested that a considerable portion of the compound was totally metabolised. Data for trans-resveratrol suggested relatively good oral bioavailabilty of the stilbene. In general, absorbed phenols/polyphenols are rapidly conjugated (to form glucuronides, sulphate esters and methyl ether) and excreted via urine and bile. Elimination half-lives for most absorbed phenolic compounds of plant origin are relatively short.

4. An oral acute single dose toxicity study (gavage) was carried out with spruce-tip extract in rats (3 female, 3 male) using the upper limit dose of 2000 mg/kg bw. The study demonstrated very low acute toxicity of the extract. Mortality or any clinical signs were not observed in the 14 day observation period. At necropsy, no macroscopic pathological findings were recorded. The oral LD_{50}, thus, in rat was more than 2000 mg/kg bw.

   The result confirmed data from open literature where acute oral toxicity for some mono-terpenes and their derivatives (α-pinene, β-pinene and turpentine-oil and limonene) were in the range of 1800 to 6800 mg/kg bw. With respect to tannins an acute oral LD_{50} value for gallic acid of more than 5000 mg/kg bw in mice has been reported.

5. No repeated dose toxicity study with *Piceae turiones recentes extractum* (spruce-tip extract) has been provided. For some terpene-constituents of spruce, like d-limonene, camphene and polyterpene, limited repeated dose toxicity data were available in summarised form, and a NOEL of 5 mg/kg bw was reported for d-limonene (oral administration of 2 to 75 mg d-limonene/kg bw to rats for 13 weeks). For gallic acid, a basic component of hydrolyzable tannins, a NOEL of 1000 mg/kg bw was reported from a study in mice (oral administration for 28 days). Some indirect information was available from use of spruce and/or pine needles in animal nutrition. Feeding pine-needle pellets (20% of feed intake) to cattle under experimental conditions (3 animals, pine-needle pellets for 4 months) or in fattening cattle under normal husbandry conditions (81 animals, for 4 months) did not reveal significant clinical or pathological/histological changes. In Lithuania, from 1962 to 1990 meal produced from spruce and pine needles was used as an admixture to cattle feed.

6. No studies on developmental toxicity/teratogenicity were conducted using *Piceae turiones recentes extractum* (spruce-tip extract). Limited published literature data on individual compounds was provided in summarised form. Data on developmental toxicity of terpenes suggested that essential oils (composed of 20 to 25% α-pinene, 15 to 18% β-pinene and 38 to 42 % sabinene) had no significant effects on maternal or foetal parameters in mice, rats and

hamsters with apparent oral NOELs of 260 to 600 mg/kg bw. Camphene had no teratogenic effects in rats up to the highest tested dose of 1000 mg/kg.

The stilbene compound trans-resveratrol (3 mg/l drinking water, corresponding to about 0.6 mg/kg bw) was tested in a preliminary two-generation study in mice. The substance exerted some effect on selected reproductive organs of both male and female mice of the parenteral generation. Also, spleen and liver weights of parenteral generation males, as well as kidney weight of F1-generation males were affected. There was no impact on spermatogenesis and sperm quality. No NOEL could be retrieved from this study.

7. *Piceae turiones recentes extractum* (spruce-tip extract) was tested in the reverse mutation test using strains of *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and *E. coli* strain WP2*uvr*A in the presence and absence of metabolic activation at 0.05, 0.1, 0.5, 1.0 and 5.0 mg/plate. No biologically relevant increase in revertant numbers was seen at any treatment level for any strain of both prokaryotes. Studies on mutagenicity in mammalian cell systems were not conducted for the extract. In addition to the test for the extract, a review of published literature on genotoxicity of single phenolic compounds and terpenes (α-pinene, camphene, limonene) in bacteria and mammalian test systems has been provided. Tested mono-terpenes did not provide evidence for relevant mutagenic potential. Some data on polyphenols indicated a mutagenic potential of quercetin and the stilbene resveratrol. Quercetin and resveratrol are known to give positive results in most *in vitro* tests but negative in most *in vivo* tests. From the available information it was considered that overall the risk of genotoxicity from residues of *Piceae turiones recentes extractum* is not of concern.

8. No carcinogenicity studies for *Piceae turiones recentes extractum* (spruce-tip extract) were provided. In published literature there are numerous citations concerning carcinogenicity and anti-carcinogenicity of secondary plant metabolites also present in spruce. A weight of evidence evaluation of data for several terpenes suggested very low probability for carcinogenicity. Some *in vitro* and *in vivo* experiments suggested that tannins and several phenolic compounds can exhibit tumourigenic as well as anticarcinogenic properties. In a study reported by the International Agency for Research on Cancer (IARC), tannic acid is reported to be carcinogenic in rats and mice following subcutaneous injection (liver tumours). However, in long-term oral toxicity studies in rats and dogs, there was no evidence for carcinogenicity. Catechin was tested for carcinogenicity by oral administration in a study in mice and in two studies in rats. No increase in the incidence of malignant tumours was found in mice. In rats, it induced adenocarcinomas in the glandular stomach in several strains. In several experiments in rats involving co-administration with known carcinogens, catechin enhanced the incidence of papillomas of the tongue, carcinomas of the oesophagus, squamous-cell carcinomas of the forestomach and adenocarcinomas of the glandular stomach (IARC). Catechin is a phenolic compound and is a common constituent in animal and human foodstuffs (e.g. in fruits like berries, cocoa beans, apple, beaches and plums).

   From the available information and considering particularly that most of the constituents of *Piceae turiones recentes extractum* belong to classes which are also components of human and/or animal diet and/or pharmacokinetic studies indicate low absorption it was considered that the risk of carcinogenicity from residues of *Piceae turiones recentes extractum* is not of concern.

9. Though there is some evidence of certain antimicrobial properties of spruce-tip constituents as terpenes or tannins, a specific assessment of microbiological consumer hazards of potential residues was not considered necessary.

10. Spruce leaf oil and preparations from spruce shoots (e.g. lozenges, teas) are traditionally used to alleviate cold, bronchitis and fever symptoms. In human phytotherapy *Piceae turiones recentes* is traditionally used for treatment of respiratory diseases and in case of rheumatic complaints. For instance, the median dose of an oral formulation is 5 to 6 g corresponding to 83 to 100 mg crude product per day. The crude product also is used as food and in confectionery. Fresh shoots of *Picea abies* (L.) Karsten are used for various food stuffs prepared from fresh spruce tip, for example spruce tip as snacks, spruce tip honey, jam of spruce tip, sweets containing spruce tip etc. In the USA, the extract of fresh spruce-tip is used to prepare the so-called “spruce beer”.

11. A NOEL from formal repeated dose and developmental toxicology studies using *Piceae turiones recentes extractum* (spruce-tip extract) was not available and an ADI for the extract could not be established. Acute oral toxicity studies with *Piceae turiones recentes extractum* (spruce-tip extract) showed very low acute toxicity (LD_{50} greater than 2000 mg/kg). Most constituents of spruce foliage identified/characterised so far represent common classes of secondary plant components. The review of safety documentation data as well as the search of published literature did not indicate a significant hazard potential for the majority of chemical constituents in spruce foliage at the concentration levels present. Particular attention was paid to identifying any compounds potentially exhibiting relevant non-threshold effects as mutagenicity/genotoxicity. An assessment of information on diterpenes, piperidine alkaloids and stilbenes and the results of the available refined chemical analysis did not indicate specific pharmacological or toxicological concern for any members of these substance classes at the levels present in the extract. Most substances of potential concern were considerably depleted or absent in the extract.

12. Residue depletion studies for Piceae turiones recentes extractum (spruce-tip extract) were not performed. The phenolic compounds (flavonoids/polyphenols such as tannins), which form the largest fraction of active ingredients (about 50%), are generally of limited/low bioavailability and residue formation potential. Phenolic constituents, as those present in spruce, are present in many botanical families including those used as animal feed or as regular human food (and medicinal plants). Human dietary exposure from plant sources can be expected to far exceed the potential intake via residues from use of Piceae turiones recentes extractum (spruce-tip extract): the average daily intake of flavonoids in human diets derived from plants was estimated to be about 20 mg to 70 mg/day (quercetin being one of the most abundant flavonoids). The average consumption of total polyphenols with the human diet was estimated to be 1 g/day. Relatively high daily exposure of food producing animals to phenolic compounds via normal forage/feed may be considered as well. Other phenolic compounds as stilbenes present in spruce (as for instance E-piceid) can be found as secondary metabolites in a variety of dietary plants as grapes (wine), nuts, fruits and berries and also in normal animal diet of plant origin or certain forage grasses. Also for these compounds considerable dietary background exposure of animals and humans may be assumed. Specific residue studies for the extract or individual constituents of Piceae turiones recentes extractum were not considered necessary.

Conclusions and recommendations

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:

- constituents of *Piceae turiones recentes extractum* (spruce-tips extract) did not indicate specific pharmacological or toxicological concern at the concentrations present,
- most constituents of spruce foliage identified/characterised represent common plant component of natural forage,
- the crude drug as well as many of the components in *Piceae turiones recentes extractum* (spruce-tip extract) are common constituents in human food stuffs;

the Committee for Medicines for Veterinary Use (CVMP) concludes that there is no need to establish an MRL for *Piceae turiones recentes extractum* (spruce-tip extract) and recommends its inclusion in Annex II of Council Regulation (EEC) 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Piceae turiones recentes extractum	All food producing species	For oral use only