The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ARNICA MONTANA

SUMMARY REPORT

1. *Arnica montana* is a plant of the *Asteraceae* family. In medicinal products the dried flower heads (*Arnicae flos*), the whole plant (*Arnicae planta tota*), and the roots (*Arnicae radix*) are used.

   The main constituents of the crude drug obtained from the dried flower heads and the aerial parts of the plant are sesquiterpene lactones, predominantly helenalin, 11a,13-dihydrohelenalin and their short-chain carbonic acid esters (0.3 to 1% of dry weight in the flower heads, 0.1 to 0.5% in leaves) and flavonoids (0.4 to 0.6%) such as apigenin, luteolin, hispidulin, kaempferol and quercetin. In the aerial parts, the essential oil constitutes 0.2 to 0.35% (flower heads) or 0.2 to 0.5% (leaves) and contains mainly fatty acids, and n-alkanes as well as thymol derivatives and mono- or sesquiterpenes. The flower heads further contain phenolcarbonic acids (chlorogenic acid, caffeic acid, cynarin), umbelliferon, scopoletin and traces (ca. 0.0005 mg/kg) of pyrrolizidine alkaloids (tussilagine and isotussilagine).

   Main constituents of *Arnica montana* roots and rhizomes are the essential oil (2 to 4% in roots, 3 to 6% in rhizomes), which mainly (about 90%) contains thymol and derivatives (thymolester and -ether). Polyines, chlorogenic acid, cynarin, and oligosaccharides are also present. Sesquiterpene lactones of the helenalin type have not been detected in the roots and rhizomes of *Arnica montana.*

2. In veterinary phytotherapy *Arnicae flos* is used topically for the treatment of acute inflammations of tendons, joints and udder, but also for cleaning and treatment of wounds of skin and mucous membranes, eczema and skin inflammations, mostly in combination with other active principles, in several liquid preparations (tinctures, fluids) and ointments. The products contain between 2 and 100% (w/w) of a 10% (w/w) alcoholic or isopropanolic extract of *Arnicae flos*. One product contains 54% of an isopropanolic extract prepared with an unspecified amount of isopropanol from 4 different plants, including 1 g *Arnicae flos*; another product contains 2% of the fluid extract of *Arnicae flos.* Target species are cattle, sheep, horses, swine and goats. The products are administered in doses between 0.03 g and 1.0 g *Arnicae flos* per day, either as single applications (0.03 g/day to 0.1 g/day) or 2 to 3 times per day for 2 to 5 days.

   In veterinary homeopathy, a dilution of 1:100 of the mother tincture, which is prepared by ethanolic extraction of the whole fresh flowering plant, and alcoholic extracts of the underground parts of *Arnica montana* in the homeopathic dilution of 1:10 are used. In addition the pure dried flowers prepared with olive oil are used for dermal application only. The preparations are intended for use in all food-producing animals. The dermal dose is not specified. The recommended maximum dose for large animals is 10 ml/animal for parenteral administration or 5 tablets for oral use. Doses of *Arnica montana* root preparations in form of tablets, globules or drops are reported to contain lower amounts of *Arnica montana* than the corresponding injectable form. The use follows the principles of homeopathic therapy and animals are diagnosed on basis of the individual pattern of clinical signs. Treatment may be repeated but a fixed dose schedule is not common in homeopathy.

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Arnica montana is also used in human medicine. In phytotherapy the use is recommended for topical administration only for treatment of distortions, rheumatic pain, and to promote wound healing. *Arnica montana* tincture (0.15 to 0.3 ml per day) was previously used orally in humans; however, nowadays the oral intake is obsolete except in homeopathic dilutions. Homeopathic products for use in humans are prepared from whole plants or roots but not from flower heads of *Arnica montana*. Topical and oral use of the mother tincture of homeopathic *Arnicae radix* preparations is contraindicated for patients with epistaxis or retinal bleeding as well as for patients in apoplectic state, while parenteral use is restricted to concentrations of *Arnicae radix* not exceeding 1 part in 1000 due to possible allergic reactions.

Arnica montana is part of the European vegetation of meadows particularly in mountain areas. Sheep and goats will eat the fresh plant, but cattle reject it. However, as part of the hay cattle would also consumed it. However, the plant is protected as nowadays it occurs rarely.

3. The main pharmacodynamic effects of *Arnicae flos* and *Arnicae planta tota*, such as increased respiration, inotropic effects on the heart muscle (*in vitro*), primary decrease of blood pressure followed by secondary increase, uterotonic and anti-inflammatory activity, and inhibition of thrombocyte aggregation, are related to the sesquiterpene lactones and their esters. Cholagogue effects were noted after administration of 2.5 ml of an extract of *Arnica montana* flower heads in dogs. Uterotonic and contraction-enhancing activity was caused in the isolated uterus of rabbits and rats by extracts of flower heads or single sesquiterpene lactones, and in the uteri of cats *in situ* by intravenous administration of 0.3 ml of an extract of *Arnica montana* flower heads.

   For sesquiterpene lactone constituents of *Arnicae flos* and *Arnicae planta tota* further data are available. Intravenous administration of 0.25 mg/kg bw of 6-O-acetyl-11,13-dihydrohelenalin increases respiration frequency and volume by 35% and 43%, respectively, in rats. Similar effects are observed in rabbits for other sesquiterpene lactones contained in *Arnica montana*. Cardiac effects include a biphasic positive inotropic effect of helenalin on the myocardium of guinea pigs has been demonstrated at concentrations of 10^{-5} to 10^{-3} mol. Concentrations of and above 10^{-3} mol cause an irreversible negative inotropic action leading to a block of muscle contraction. High intravenous doses of 6-O-acetyl-11,13-dihydrohelenalin (0.6 mg/kg bw or more) evoke clonic convulsions and lead to myocardial tissue damage in narcotised rabbits. Furthermore blood parameters are affected. Helenalin at a concentration of 0.1 µmol alters the shape of thrombocytes and at 100 µmol significantly inhibits thrombocyte aggregation. Helenalin and 11,13-dihydrohelenalin inhibit collagen-induced platelet aggregation, thromboxane formation and 5-hydroxytryptamine secretion at concentrations of 3 to 300 µmol in a concentration-dependent manner. Effects on metabolism include effective *in vitro* inhibition of several hepatic microsomal mixed-function oxidases of mice. In male BDF1 mouse hepatic microsomal cytochrome P450 contents *in vitro* and *in vivo* are decreased by a single intraperitoneal dose of 25 mg helenalin/kg bw, which is further increased in the presence of NADPH. Sesquiterpene lactones of the helenalin type with α-methylene-β-lactone structure exert anti-inflammatory, analgesic, antiarthritic activity and induce delayed hypersensitivity and mitogenic effects *in vitro* and *in vivo*. Intraperitoneal effective doses are reported as 2.5 mg/kg bw in mice and 5 mg/kg bw in rats. The anti-inflammatory action of substances of the helenalin type has partly been explained by their ability to inhibit transcription factor NF-κB by selectively alkylating the p65 subunit at micromolar concentrations. Sesquiterpene lactones of the helenalin type are strong cytotoxic agents. In primary rat hepatocyte cultures, helenalin at micromolar concentrations cause rapid lethal injury. Co-treatment of cultures with N-acetylcysteine afforded significant protection. Sesquiterpene lactones, especially of the helenalin type have been studied for antitumour activity. Mice inoculated with P388 lymphocytic leukaemia cells receiving repeated intraperitoneal doses of helenalin of 8 mg/kg bw, survived longer (by approximately 30%). DNA and protein synthesis in tumour cells was considerably inhibited. Helenalin further produced degranulation of mast cells isolated from the peritoneal cavity of rats.

   Immunostimulating properties (increase of phagocytosis by granulocytes) of a polysaccharide fraction of *Arnicae flos* were noted.

   The main pharmacodynamic effects of *Arnicae radix* have been connected with the thymol derivatives contained in the drug. These thymol derivatives have been reported to have bactericidal and fungicidal activity. The essential oil of the roots also was reported to show antiphlogistic action.

4. Pharmacokinetic information on *Arniace flos* and *Arnicae planta tota* is only available for 1 constituent. After intravenous, intraperitoneal and oral administration of radiolabelled 11,13-dihydrohelenalin to BDF_1 mice maximum serum levels were observed after approximately 15 minutes. Urinary excretion (48 hours) accounted for 64.4%, 47.2% and 38.0% after intravenous, intraperitoneal, and oral administration, respectively, while faecal excretion accounted for 39.7%, 9.3%, and 22.1%, respectively. About 32% of the radioactivity in 0 to 6 hour-urine was parent compound. More than 92% of urinary excretion and more than 93% of faecal excretion occurred within 24 and 48 hours respectively, for all routes of administration. Highest concentration of radioactivity after intraperitoneal administration was seen in the liver, followed by stomach and reproductive organs. No radioactivity was found in organs including liver and kidney 24 hours after intraperitoneal administration, while in the carcass and the skin radioactivity was still detectable after 24 days. The metabolites in tissues and blood were not identified.

   No data on dermal absorption of *Arnica montana* or its constituents are provided. In absence of such data and considering the pattern of use of products containing *Arnicae flos* as well as the lipophilic properties of the main active component helenalin, absorption has to be assumed.

   Little information is available on the pharmacokinetics of the thymol derivatives contained in roots of *Arnica montana*. However, thymol is readily absorbed after oral administration. A small amount is oxidised to thymohydroquinone. Within 24 hours after treatment about 50% of the absorbed substance is excreted via kidney, either unconjugated or as a glucuronide- or sulphate conjugate. The metabolite thymohydroquinone is also excreted in urine.

5. The intraperitoneal LD_{50} in mice for the total extract of *Arnicae flos* is 280 mg/kg bw. Other sources report LD_{50} values for preparations of flowers or roots of *Arnica montana* between 650 and 3000 mg/kg (administration route not known). The lethal subcutaneous dose of a concentrated root extract in mice is given as 3000 mg/kg bw. In view of the lack of reported adverse effects and poisonings, it is assumed that the acute toxicity of the root extract is low.

   The acute oral toxicity of helenalin (aqueous suspension by gavage, or crystalline material in gelatin capsules) was characterised by LD_{50} values of 125 and 150 mg/kg bw for rats and mice respectively, 100 to 125 mg/kg bw in sheep, 90 mg/kg bw in rabbits and 85 mg/kg bw in hamsters. The intraperitoneal LD_{50} in male BDF_1 mice for helenalin was given with 43 mg/kg bw and in outbred female Albino mice as 10 mg/kg bw.

   The LD_{50} of 11,13-dihydrohelenalin is given as 100 mg/kg bw in mice (route not stated).

6. No information on oral repeated dose toxicity of *Arnica montana* was provided. However, repeated intraperitoneal injection of high doses of helenalin to mice increases differential polymorphonuclear leukocyte counts and decrease lymphocyte counts. Serum enzymes and cholesterol levels are increased by helenalin injections at 25 mg helenalin/kg bw/day for 3 days. Helenalin significantly reduces relative liver, thymus, and spleen weights. Histological evaluation revealed substantial effects of multiple helenalin exposures on lymphocytes of the thymus, spleen, and mesenteric lymph nodes, but no histological changes are observed in liver or kidney. Multiple helenalin exposure (25 mg/kg bw/day) significantly inhibits hepatic microsomal enzyme activities and decreases microsomal cytochromes P450 and b5. No effects on white and red blood cell count and hematocrit, nor ulcerogenic action or effects on central nervous system have been noted in Sprague-Dawley rats receiving helenalin at an oral dose of 2.5 mg/kg bw for 3 weeks.

7. No information on reproductive toxicity was provided for *Arnica montana*. For helenalin no antifertility or teratogenic effects were noted in male and female mice receiving the substance at an oral dose of 6 mg/kg bw for 28 days (10 days before mating, through mating and gestation period up to approximately 17 days of pregnancy). As only a summary of the data was available, no adequate evaluation was possible.

8. The mutagenicity of a crude drug extract at 10 to 400 µl (100 µl extract correspond to 100 mg dried flowers) was studied in *Salmonella typhimurium* strains TA 98 and TA 100 with and without metabolic activation. Weak mutagenic activity was noted in TA 98, with and without metabolic activation. In strain TA 100 mutagenic effects were observed with metabolic activation only. A summary of mutagenic effects an alcoholic extract of *Arnicae flos* in the *Salmonella*-microsomal assay was provided. This effect was attributed to the flavonoids, in particular quercetin derivatives. However, another study reports absence of mutagenicity for an extract of *Arnicae flos.*

   The sesquiterpene helenalin was tested with and without metabolic activation in *Salmonella typhimurium* strains TA 100, TA 98, TA 1535 and TA 1537 and showed no mutagenic effect up to concentrations of 1000 µg/plate. Toxicity, precluding evaluation was noted at concentrations of 300 µg/plate and higher. Helenalin was also tested for genotoxicity using 6 strains of *Bacillus subtilis*. It was lethal in strains mc-1 and rec E4, possibly by exerting DNA damage. However, helenalin induced chromosome breaks *in vitro* in Chinese hamster ovary cells.

   On the other hand there is also information that tumor growth of Ehrlich ascites in mice and Walker 256 carcinosarcoma in rats was inhibited by the sesquiterpenes of *Arnica montana* flowers. Also in studies carried out with GLC4 and COLO 320 cell lines the sesquiterpenes helenalin and dihydrohelenalin displayed cytotoxic activity.

   No final conclusion on the mutagenicity of the sesquiterpenes present in *Arnica montana* is possible.

   Thymol not mutagenic in the *Salmonella*-microsomal assay and the *Bacillus subtilis* rec-assay.

9. No carcinogenicity study was available for *Arnica montana* or its main constituents.

   The pyrrolizidine alkaloids tussilagine and isotussilagine, contained in traces in the drug, are reported to be neither hepatotoxic nor carcinogenic.

10. The immunotoxic properties of *Arnica montana* or its components have not been adequately investigated. However, allergenic properties of *Arnica montana* are well known from use in human medicine. There is clear evidence of immunotoxic activity of helenalin-like substances in subacute studies. Helenalin as well as helenalin acetate elicit strong allergic responses in skin of guinea pigs (helenalin 4 times more effective than its acetate) sensitised with an ether extract of the flowers of *Arnica montana* applied in acetone.

11. The effect of *Arnica montana* related residues on human gut flora has not been studied. MIC values in the range of 10 to 100 µg/ml, mainly against Gram positive bacteria, are reported for the sesquiterpene lactones in the summary information provided. *In vitro* antifungal activity of an extract of *Arnica montana* flowers (MICs of 40 to 50 µg/ml for several dermatophytes) is noted.

12. In humans, the acute oral intake of *Arnica montana* tinctures and extracts in traditional medicine has led to several cases of severe poisoning, sometimes with fatal outcome. Oral intake of 30 ml of a 20% *Arnica montana* tincture was reported to produce serious symptoms, oral doses of 60 to 80 g of another tincture (not further specified) were reported to be lethal. Symptoms are irritation of mucous membranes, nausea, dizziness, diarrhoea, fever, epistaxis, arrhythmia, fatal gastroenteritis (due to irritant properties of *Arnica montana*), paralysis of skeletal and cardiac muscles, increase or decrease of pulse rate, dyspnoe, and collapse. In pregnant women, abortion may be induced.

    After dermal administration of *Arnica montana* extracts, skin reactions including dermatitis and necrosis have been observed. *Arnica montana* is a strong sensitiser with the sesquiterpene lactones with an a-methylene group implicated as the contact allergens. Cross-reaction to all compositae plants are known.

    After oral or parenteral use of homeopathic preparations of *Arnica montana* root, no poisoning or adverse effects have been reported. However, skin irritation may be found after dermal use.

    Thymol has been used in human medicine for topical treatment of skin disorders, for inhalation in respiratory disorders and in dental care.

13. In the United States of America *Arnica* is listed as an 'unsafe herb' based on its oral toxicity by the Food and Drug Administration.

Arnica montana root itself has not previously been evaluated by national or international health bodies. Thymol, a main component of *Arnica montana* root has previously been considered by the Committee for Veterinary Medicinal Products (CVMP) and is included in Annex II of Council Regulation (EEC) No. 2377/90. The other thymol derivatives present in *Arnica montana* roots are closely related to thymol. Thymol is also used for flavouring purposes in foodstuffs and beverages. In 1992 the Committee of Experts on Flavouring Substances of the Council of Europe evaluated thymol. The upper limits established for thymol are 50 mg/kg in foodstuffs and 10 mg/kg in beverages.

14. No information on residues of *Arnica montana* and their depletion following treatment of food producing animals was provided. Considering the available pharmacokinetic data on the helenalin derivative 11,13-dihydrohelenalin and the lipophilic properties of the sesquiterpene lactones helenalin and 11,13-dihydrohelenalin, it can be assumed that any residues are likely be found in fatty tissues, such as subcutaneous tissue.

Considering the concentrations at which sesquiterpene lactones residues would be expected to occur in edible tissues following topical use of veterinary medicinal products containing *Arnicae flos* or extracts of *Arnica montana*, it is unlikely that consumers will be exposed to harmful levels of residues of sesquiterpene lactones resulting from treatment of food-producing animals.

Conclusions and recommendation

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

- *Arnica montana* may be contained in the feed of food producing animals; nevertheless, some major constituents possess high acute toxicity,
- after topical use sesquiterpene lactones, the main toxicologically relevant constituents of the aerial parts of *Arnica montana* including the flowers, are unlikely to be present in treated animals at levels posing a risk to the consumer,
- veterinary medicinal products containing *Arnica montana* for topical use or *Arnicae radix* for all routes of administration are used only in a small number of individual animals for infrequent and non-regular treatments;
- *Arnicae radix* has not been connected with any adverse effects,
- thymol, the major component of the essential oils in the roots, is already included in Annex II of Council Regulation (EEC) No 2377/90,
- animals are unlikely to be sent for slaughter immediately after treatment;

the Committee concludes that there is no need to establish an MRL for topical use of *Arnica montana* (*Arnicae flos* and *Arnicae planta tota*) and for *Arnicae radix* and recommends their inclusion in Annex II of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Arnica montana (Arnicae flos and *Arnicae planta tota)*	All food producing species	For topical use only
Arnicae radix	All food producing species	For use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias at concentrations in the products not exceeding one part per ten only