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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ARISTOLOCHIA

SUMMARY REPORT

1. *Aristolochia* is a plant genus of the family *Aristolochiaceae.* Homeopathic preparations of these plants are complex mixtures of compounds containing several aristolochic acids (nitrophenanthrene carboxylic acids) and aristolactams (phenanthrene-lactam carboxylic acids) as main medicinal principle, together with the quarternate aporphin base magnoflorine (a dibenzo-quinolinium derivative). Additionally, various essential oils, tannins, resin-like substances, clematitin, ß-sitosterin and flavonol glycoside are found in the extract of herbs and/or roots. A minimum of 2.9% of dry matter has been established for the herbal D 0 ("Mother tincture"). Tinctures from roots possibly contain higher concentrations of active substances.

   The proportion of aristolochic acids (composed of aristolochic acid I and II at a ratio of 65-77 % : 21-35%) in *Aristolochia* tincture is estimated to be 300 µg/ml at D 0.

2. The substance, at concentrations of D 1 (1:10) to C 30 ($1:100^{30}$), is used parenterally or orally at dose levels of a few drops up to 10 ml one to four times a day (corresponding to 0-1 µg/kg bw daily) for several days in horse, cattle, sheep, goat and swine for regulation of sexual functions, immunostimulation, antiphlogistic effects and others.

3. The pharmacodynamic properties of *Aristolochia* have not been investigated according to current standards. Aristolochic acids are reported to stimulate defence mechanisms against infections and inflammation in several mammalian species, including humans. Aristolochic acid stimulates the phagocytic activity of peripheral granulocytes in healthy volunteers at oral doses of 3 times 0.3 mg/person and day given for 10 days. This is a daily dose of approximately 0.015 mg/kg bw for a 60 kg person. Anti-inflammatory effects have been attributed to *Aristolochia* drugs and have been demonstrated for aristolochic acids in the respective animal models.

4. Pharmacokinetic data, including metabolism, are available only for a few constituents of *Aristolochia*. Pharmacokinetics of aristolochic acid I and II have been studied in rats, mice, guinea pigs, dogs and humans after oral treatment. The doses studied were in the range of 0.6 to 85 mg/kg bw. Most of the results relate to rats. Following oral administration, aristolochic acid I was readily absorbed from the gastrointestinal tract. After oral administration of aristolochic acid I to rats, about 91% of the dose was recovered in the excreta, equally divided in urine and faeces. Parent compound or conjugates of parent compounds were not detected in rat excreta. More than 80% of the radioactivity was identified as aristolactam Ia or conjugates thereof. Minor urinary and faecal metabolites of aristolochic acid I were aristolactam I, aristolochic acid Ia, aristolic acid I lacking the nitro-group and 3,4-methylenedioxy-8-hydroxy-1-phenanthrene carboxylic acid. Following oral administration of aristolochic acid II, 13.5% of the dose was recovered in excreta, most of it in faeces. Aristolactam II was the main single component (4.6% in urine and 8.9% via faeces). Additionally, aristolactam Ia and 3,4-methylenedioxy-1-phenanthrene carboxylic acid (aristolic acid II, lacking the nitro group) were detected. However, the main proportion of the dose could not be accounted for. Metabolites, which have lost the nitro group (aristolic acid derivatives) are, probably, formed by gut bacteria, since they have not been observed after intravenous application.

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In other laboratory animal species, the metabolic pattern was not reported in detail. It appeared that in mice, metabolism was similar to that observed in the rat. Urine samples of guinea pigs, rabbits, dogs and man were reported to yield not all of the metabolites found in the rat. In rabbits and dogs, small amounts of parent compounds were detected in urine.

In human volunteers treated with daily doses of 0.9 mg aristolochic acids/person for three days, only aristolactam I and II were identified in urine on day 3. Faecal excretion was not examined. Further, aristolochic acids-like substances of not exactly defined identity were detected at relatively high concentrations in bile, urine, cerebrospinal fluid and saliva of human volunteers given aristolochic acids in capsules at a daily dose of 1.35 mg/person for three days. Three nursing mothers treated with oral doses of 1.35 mg aristolochic acids for three days were reported to excrete 2.5-5% of the dose into the breast milk.

No data on the distribution of aristolochic acids or metabolites in the tissues of laboratory animals were available.

5. Data on the acute toxicity of the plant drug *Aristolochia* are not available. In rats, the oral LD_{50} of aqueous solutions of the active ingredient aristolochic acids is 203.4 mg/kg bw in males and 183.9 mg/kg bw in females. The LD_{50} after intravenous administration is reported as 82.5 mg/kg bw and 74.0 mg/kg bw in males and females respectively. In mice, the oral LD_{50} of aristolochic acids is 55.9 in males and 106.1 mg/kg bw in females. The LD_{50}**,** after intravenous administration, is reported as 38.4 mg/kg bw and 70.1 mg/kg bw in males and females respectively. Rabbits are reported to be more sensitive to the toxic action of aristolochic acids, deaths being observed already after single intravenous doses of 1-5 mg/kg bw. Intragastric and intravenous administration of lethal doses caused sedation, piloerection, abnormalities of co-ordination, dyspnoea, kyphotic posture and occasionally tremor, followed by prone posture and apathy. The animals died within 15 days. The toxic mechanisms have not been examined in detail, but nephropathies and capillary toxicity are normally encountered after single acutely toxic doses in all mammalian species examined.

   For the *Aristolochia* ingredient magnoflorine, an intravenous LD_{50} of 20 mg/kg bw and intraperitoneal LD_{50} of 19.6 mg/kg bw (for the chloride) and a subcutaneous LD_{50} of 138 mg/kg bw has been reported for mice. Information on acute oral toxicity was not available. Data on the toxic mechanisms are lacking.

6. In a non-GLP subacute toxicity study using only 14 male Wistar rats per dose level, animals were treated daily by gavage with doses of 0, 0.2, 1.0, 5.0, 25.0 mg aristolochic acids/kg bw as an aqueous solution. Treatment was for four weeks. The body weights of the animals receiving the highest dose decreased and 2 animals died. Seven animals per dose group were examined for haematological and clinical chemical parameters. At the highest dose of 25 mg/kg bw, mean corpuscular volume and reticulocytes decreased, total serum protein and glucose decreased and urine protein, as well as glucose, increased in comparison to control. Atrophy of thymus and spleen, hepatocellular basophilia, forestomach inflammation, erosion, and hyperplasia as well as nephrosis and degeneration of testes were observed. In the bladder, moderate urothelial hyperplasia and mild cystitis were found. Similar changes, but less severe, were observed in animals receiving a daily dose of 5 mg/kg bw. Mild changes only were found at 1 mg/kg bw.

7. No GLP conform subchronic toxicity studies are available for *Aristolochia* or its main ingredients. However, in a pilot carcinogenicity study in rats, in which aristolochic acids were administered as sodium salt orally per stomach tube at dose levels of 0, 0.1, 1, and 10 mg/kg bw, haematological and clinical-chemical changes were not reported in the animals during the first 3 months, until sacrifice. At this time point, animals receiving the highest dose of 10 mg/kg bw already bore papillomas at the site of the forestomach. About half of the animals of this group exhibited hyperplasia of renal pelvis and nearly all showed hyperplasia of urinary bladder. In the lower dose group, nearly all animals exhibited papillomas and a few animals showed hyperplasias of the forestomach or of the urinary bladder. The animals of the lowest dose group showed no corresponding pre-carcinogenic organ changes. An

inadequate number of rats (n=9 to 10 per dose) was studied. An exact study design was not given and detailed data were not reported.

8. No study on the chronic toxicity of *Aristolochia* was available. Two inadequate oral gavage carcinogenicity studies were performed with aristolochic acids in rodents (see paragraph 13).

9. Specific studies on the effects of *Aristolochia* on male and female fertility have not been submitted. *Aristolochia indica*, containing several aristolochic acids together with other ingredients, was reported to exert antifertility effects in male mice, when given orally at 75 mg of the water soluble part of a chloroform extract/kg bw at three day intervals, 7 times in 19 days. Severe degenerative damage has been observed in the testes of 8-week old male rats given aristolochic acids as sodium salt at a single oral dose of 200 mg/kg bw. Fifteen male rats per dose group received aristolochic acids as sodium salt at daily doses of 1 or 25 mg/kg bw for 4 weeks. At the higher dose level, animals showed reduced weight of testes compared to controls. Degenerative changes were seen in nearly all seminiferous tubules of the testes, the severity of damage, however, being less than after the single dose treatment with 200 mg/kg bw. At the dose of 1 mg/kg bw no changes could be detected in testes.

   Emmenagogue and abortefacient properties of *Aristolochia* species have been demonstrated in animals and man. It could not be clarified which of the ingredients of *Aristolochia* may be responsible for the respective effects.

10. No study on embryo- and foetotoxic or teratogenic effects of *Aristolochia* or one of its main ingredients is available. Multigeneration studies are lacking.

11. Mutagenic activity of several ingredients of *Aristolochia* was reported. Not all of the ingredients of *Aristolochia* were examined, but all of the aristolochic acids tested so far in Ames tests, tests examining sister chromatid exchange and structural chromosome aberration in human lymphocytes, a mouse bone marrow micronucleus test, a granuloma pouch assay in rats, examining gene mutations at the HGPRT-locus, and in several mutagenicty tests in *Drospohila melanogaster*, have shown direct mutagenic properties. The addition of S9-mix in the Ames test either led to similar results or decreased the mutagenic effect.

    DNA-adduct formation by several aristolochic acids or their sodium salts has been demonstrated *in vitro* and *in vivo*. For aristolochic acid I and II, it has been shown that adducts are formed via an activated aristolactam-species. The adduct formation occurs exclusively by binding of the aristolochic acid to exocyclic amino groups of purine bases, predominantly deoxyadenosine. The respective adducts may persist lifelong.

12. Aristolochic acids given orally were found to be carcinogenic in rats and mice.

    In male and female rats, the time interval to tumour development decreased dose dependently. Daily doses of 0.1 to 10 mg/kg bw given for three months led to tumours like neoplasms of the forestomach, bladder and kidney in most of the animals. At the highest dose, tumours were already observed after 6 months. Animals receiving the lower or medium dose exhibited similar neoplastic changes after a longer latency period (6 to 12 months for the 1 and 0.1 mg/kg bw group). Four of four male rats and one of five female rats receiving the lowest dose of 0.1 mg/kg bw for 12 months had developed cancer of the forestomach at 16 months, the last time point of sacrifice. In another study, using the same protocol but aristolochic acid I instead of aristolochic acids, a dose of 10 mg/kg bw given for 3 months induced tumours of the forestomach, the small intestine and the ear duct within 6 months.

    A dose of 5 mg/kg bw aristolochic acids given orally per stomach tube for 3 weeks to female mice elicited tumours in all treated animals within 1 year.

The carcinogenicity studies did not comply with acceptable standards. No threshold dose or dose/effect relationship could be established. No other ingredient of *Aristolochia* has been tested for carcinogenic properties.

13. No information on *Aristolochia* tolerance in the target species are available.

14. No special studies investigating the neurotoxic effects of *Aristolochia* are available.

15. No adequate studies on the skin and eye irritant properties are available.

16. Information on microbiological properties of *Aristolochia* are not available.

17. No studies on immunotoxic properties of *Aristolochia* meeting current requirements are available.

18. In 8 of 10 human cancer patients receiving intravenous doses of 1 mg aristolochic acids/kg bw daily for 3 days or longer, elevated blood urea nitrogen levels were reported persisting for 2 months or more. The glomerular filtration rate decreased already after 2 days on therapy. At the same time, haematological parameters did not change significantly. Several patients died with acute toxic nephrosis.

    Recently, an epidemic of irreversible nephropathy has been observed, occasionally followed by transitional cell carcinoma development in the renal pelvis, ureter and bladder in young women taking "Chinese herb-slimming drugs" for several months. The epidemic was explained by the inadvertent intake of unspecified amounts of *Aristolochia* ingredients contained in *Aristolochia* fang-chi, mixed into the medicinal product. In all of 5 women undergoing kidney transplantation after Chinese herb nephropathy, the respective DNA-adduct deoxyadenosine-aristolochic acid was detected in renal tissue. The ingested doses may be calculated to be in the range of a few µg per kg bw daily.

    Additionally, it has been hypothesised that "Balkan nephropathy", progressing to kidney tumours in humans, may be caused by flour containing aristolochic acid as impurities in these regions.

19. No NOEL could be established from any pharmacological or toxicological data available and no ADI can be derived.

20. Data on pharmacokinetics and residues of *Aristolochia* in target animals have not been submitted. No data have been provided on the amount of ingredients possibly absorbed after oral application of the intended therapeutic doses, nor is information available on the distribution of the substance in the target tissues, including milk, nor on the elimination from the injection site.

Conclusions and recommendation

Having considered that:

- the substance *Aristolochia* has not been adequately defined in respect to different ingredients and impurities,
- no adequate toxicity studies have been submitted and no final conclusions can be drawn concerning the subchronic and chronic as well as reproductive toxicity of its ingredients,
- the substance contains ingredients, which are severely nephrotoxic in humans at µg per kg doses of aristolochic acids,
- the substance contains ingredients which have been shown to exert strong mutagenic effects in bacterial and mammalian cell systems,
- there was evidence that some of the ingredients are potent carcinogens in rats and mice at low dose levels and also in humans at µg per kg bw levels;

The Committee therefore considers that residues of *Aristolochia* constitute a hazard for the health of the consumer and recommends its inclusion in Annex IV to Council Regulation (EEC) No. 2377/90.