

Pharmaceutical companies
(see Distribution list)

Copies to those involved in the graduated plan procedure

Ref.: 75-3822-V12617-204289/10
Phone: (0228) 99307 3232
Bonn, 22 July 2010

Prevention of drug-associated risks, Stage II
Medicinal products prepared using plants of *Aristolochiaceae* of the *Asarum* genus

For products see Annex –

Notice of the BGA (*Bundesgesundheitsamt* / Federal Health Agency) dated 03 June 1981 (GV4-7251-01-5383/81)

Hearing (letter) of the BfArM dated 07 December 2009 (75-3822-V12617-118994/09)

Comments from pharmaceutical companies

Dear Madam/Sir

In the above-mentioned matter the BfArM issues the following

Notice

The marketing authorisations / registrations of the a. m. medicinal products are revoked with immediate effect.

Reasons

The ruling of the a. m. measures is based on Section 30 sub-section 1 in connection with Section 25 sub-section 2 no. 5 and Section 39 sub-section 2 no. 4 and sub-section 2d in connection with Section 30 sub-section 1 of the German Medicines Act (AMG) in the version of the Announcement of 12 December 2005 (Federal Gazette / BGBl I p. 3394), amended by Article 1 of the Ordinance of 28 September 2009 (BGBl I p. 3172).

In homeopathy it is possible to administer homeopathic preparations from *Aristolochia clematidis*, *Aristolochia curarina*, *Aristolochia serpentaria* (= *Aristolochia officinalis*), *Aristolochia cymbifera e radice* (= *Aristolochia milhomens*), *Aristolochia rotunda*, *Asarum europaeum* and *Asarum canadense*.

Plants of the *Aristolochiaceae* family and preparations derived thereof are also used in traditional Chinese medicine (TCM), for instance various *Aristolochia* spp., like *Aristolochia fangchi*, *Aristolochia manshuriensis*, *Aristolochia contorta* and *Aristolochia debilis*¹, and *Asarum* spp. like the dried roots and the rhizome of *Asarum sieboldii* and *Asarum heterotropoides*.

¹ European Medicines Agency. (23 Nov. 2005) "Public Statement on the Risks associated with the use of Herbal Products containing *Aristolochia* Species" [online], EMEA/HMPC/138381/2005. Available: <http://www.emea.europa.eu/pdfs/human/hmpc/13838105en.pdf>.

The graduated plan procedure of 1981 (see Notice mentioned above), which referred exclusively to medicinal products derived from *Aristolochia*, was to be extended since aristolochic acid I (AA I) was detected in three homeopathic mother tinctures of *Asarum europaeum*, in one mother tincture of *Asarum canadense* and in samples of the TCM drug *Asari radix et rhizoma*. The investigations were made in connection with the revision of the HAB monograph "Asarum europaeum" and the elaboration of the Ph. Eur. monograph "Manchurian Wildginger Root and Rhizome" (*Asari radix et rhizoma*). When investigated by the central laboratory of German pharmacists (*Zentrallaboratorium Deutscher Apotheker*) in 2007, the TCM drugs *Asari radix* and *Asari radix et rhizoma* were detected to contain aristolochic acid in concentrations of 5.1 µg/g and 2.7 µg/g (DAC sample 7, limit of quantification: 6 ppm).

The homeopathic mother tinctures of *Asarum europaeum* and *Asarum canadense* were assayed according to Method C (HPLC/MS/MS) published under "Test for aristolochic acid I in herbal drugs" (2.8.21) in *Pharmeuropa* 19.4 of October 2007. *Asarum europaeum* had AA I concentrations of 43.8 µg/kg, 85.0 µg/kg and 128.4 µg/kg, i.e. 0.044 – 0.13 ppm, and *Asarum canadense* of ca. 127.4 µg/kg or ca. 0.13 ppm. Assays of homeopathic mother tinctures yielding values of over 100 µg/kg (= 0.1 ppm) are considered reliable. In the drug the above HPLC Method C has a sensitivity of 15 µg/kg (= 0.015 ppm).

By comparison, HPLC with UV detection at 254 nm, applied in investigations in connection with the revision of the HAB monograph *Aristolochia clematitis*, showed AA I and AA II concentrations of 0.008%, 0.017% and 0.022%, i.e. maximally 220 ppm, in three *Aristolochia clematitis* mother tinctures (the AA II percentage was about 33%).

Genuine occurrence of aristolochic acid in plants is currently only assumed for the *Aristolochiaceae* family¹. So far, aristolochic acid in species of other families have only been reported for commercially procured samples without proper taxonomic determination, or for adulterated samples².

According to the literature, aristolochic acid (almost only AA I) has been detected in a large number of *Asarum* species^{3,4,5,6}. In *Asarum canadense* (underground parts of various origin) AA I contents ranged from 0.01 – 18.40 ppm⁷ and even up to a maximum of 370 ppm³, related to the dry weight.

² K.M. Wu, J.G. Farrelly, et al. (2007) "Complexities of the herbal nomenclature system in traditional Chinese medicine (TCM): Lessons learned from the misuse of *Aristolochia*-related species and the importance of the pharmaceutical name during botanical drug product development." *Phytomedicine* 14 (4): 273-279

³ Schaneberg, B. T., W. L. Applequist, et al. (2002). "Determination of aristolochic acid I and II in North American species of *Asarum* and *Aristolochia*." *Pharmazie* 57 (10): 686-689

⁴ Zhang, C., X. Wang, et al. (2006). "Simultaneous determination of five aristolochic acids and two aristololactams in *Aristolochia* plants by high-performance liquid chromatography." *Biomed. Chromatogr.* 20 (4): 309-318

⁵ Jong, T. T., M. R. Lee, et al. (2003). "Analysis of aristolochic acid in nine sources of Xixin, a traditional Chinese medicine, by liquid chromatography/atmospheric pressure chemical ionization/tandem mass spectrometry." *J. pharmaceut. biomed.* 33 (4): 831-837

⁶ Schaneberg, B. T. and I. A. Khan (2004). "Analysis of products suspected of containing *Aristolochia* or *Asarum* species." *J. ethnopharmacol.* 94 (2-3): 245-249

⁷ McMillin, D.L., C.D. Nelson, et al. (2003) "Determination of Aristolochic Acid in *Asarum canadense* (Wild Ginger)" [online], Meridian Institute. Available: <http://www.meridianinstitute.com/reports/wgreport.html> [19th March 2008]

In at least 40 other species AA I concentrations reached values of up to 3377 ppm (*Asarum crispulatum*)⁵ related to the dry weight, most of them in the 2-digit ppm range^{3, 5, 8, 9}.

Therefore, it must be assumed that aristolochic acid is present also in the not yet examined species or in the *Asarum* species examined but without detection of aristolochic acid. Consequently, our risk assessment cannot be confined to plants of the *Aristolochia* species used in homeopathy and in TCM according to current knowledge. The entire *Aristolochiaceae* family with the genera *Aristolochia* and *Asarum* should be included in the risk assessment.

Toxicological assessment

Aristolochic acid is the characteristic ingredient in the *Aristolochia* species. According to latest findings it is also present in plant parts of the *Asarum* species. Aristolochic acid occurs, among others, as AA I and AA II, the latter being the demethoxylated form of AA I.

In in-vitro and in-vivo tests aristolochic acid has proved to be genotoxic^{10, 11}. In animal experiments it has shown carcinogenic effects. Its carcinogenicity is associated with the formation of AA-specific DNA adducts. Reductive metabolism activates aristolochic acid by nitroreductases to the nitrenium ion (the finally carcinogenic cyclic N-acylnitrenium ion¹²) forming the N6-adenosine and N2-guanosine adducts. Via mutations in the proto-oncogen *H-ras* these DNA adducts induce tumour development in the forestomach and auditory canal of rats.

Some findings suggest a tumorigenic effect in the human ureter. Aristolochic acid is assumed to be the trigger of the cancers in a Belgian patient group with terminal renal failure (*Chinese herb nephropathy patients (CHN)*), treated in a slimming cure also with Chinese medicinal herbs including *Aristolochia fangchi*). The DNA adduct pattern in the renal tissue of these patients was similar to that in animals that had developed tumours after administration of aristolochic acid.

⁸ Kawamura, T., Y. Osada, et al. (2003) "Contents variation of aristolochic acid in the plants of Aristolochiaceae; About the related plants of Chinese herb Xixin." Nat. Med. 57 (3): 105-109

⁹ Hashimoto, K., M. Higuchi, et al. (1999) "Quantitative analysis of aristolochic acids, toxic compounds, contained in some medicinal plants." J. Ethnopharm. 64 (2): 185-189

¹⁰ Kevekordes et al., 2001: Micronucleus formation in human lymphocytes and in the metabolically competent human hepatoma cell line Hep-G2: results with 15 naturally occurring substances. Anticancer Research 21, 1A, 461-469

¹¹ Robisch et al., 1982: Aristolochic acid is a direct mutagen in Salmonella typhimurium. Mutation Research 105, 4, 201-204

¹² NTP-Report on Carcinogens, Draft Background Document for Aristolochic Acid-Related Exposures: (1) Aristolochic Acid & (2) Botanical Products Containing Aristolochic Acid, U.S. Department of Health and Human Services Public Health Services National Toxicology Program Research Triangle Park, NC 27709, Scheduled Peer Review Date: January 24-25, 2008

Especially the main DNA adduct formed by aristolochic acid, a desoxyadenosine adduct [7-(desoxyadenosine-N6-yl)-aristolactam I, dA-AA I], was detected in the kidneys and ureters of all CHN patients, even when exposure to aristolochic acid during the slimming cure dated back 10 years^{13, 14, 15, 16, 17}.

Two prevalence studies (data from Belgium) and a large number of case reports on urothelial tumours following the intake of products containing aristolochic acid are documenting the carcinogenic effects of aristolochic acid in humans^{12, 17, 18, 19}.

Mechanistic aspects of DNA adducts

DNA-adducts are premutagenic damages and, thus, are seen as precursors of mutations. Such structural changes to the genetic material are often the first step in the multi-stage process of chemical carcinogenesis (initiator effect). The multi-stage concept of carcinogenesis, i.e. the distinction between tumour initiation, tumour promotion and tumour progression in the process of tumour development, is linked with a distinction between genotoxic and non-genotoxic/epigenetic risk factors²⁰.

DNA adduct formation is caused by covalent binding of reactive molecules to the DNA, with the result that modified nucleotides develop. Attachment to DNA components changes the spatial structure of the DNA.

The DNA adduct formers are direct mutagens, which, in the process of replication at the positions of these modifications or in direct neighbourhood, cause base substitutions, deletions, shifts in the reading pattern, and are able to prevent correct DNA replication. These genetic faults are irreversibly fixed by DNA replication. Theoretically, every mutation has the potential to trigger the development of cancer. Therefore it is impossible to define threshold values for direct mutagens²¹.

¹³ Arlt et al., 2002: Aristolochic acid as a probable human cancer hazard in herbal remedies: a review. *Mutagenesis* 17, 265–277

¹⁴ Cosyns et al., 1994. Urothelial malignancy in nephropathy due to Chinese herbs. *Lancet* 344 (8916): 188. (Support not reported. Authors affiliated with University of Louvain Medical School, Belgium)

¹⁵ Mengs et al., 1982 : The carcinogenic action of aristolochic acid in rats. *Archives of Toxicology* 51 (2), 107-119

¹⁶ Mengs, U., 1983: On the histopathogenesis of rat forestomach carcinoma caused by aristolochic acid. *Archives of Toxicology* 52 (3), 209-220

¹⁷ Nortier et al., 2000: Urothelial carcinoma associated with the use of a Chinese herb -Aristolochia fangchi- *N. Engl. J. Med.*, 342, 1686-1692

¹⁸ Cosyns JP, Jadoul M, Squifflet JP, Wese FX, van Ypersele de Strihou C. 1999 Urothelial lesions in Chinese-herb neuropathy. *Am J Kidney Dis* 33 (6): 1011-1017 (Support not reported. Authors affiliated with Universite Libre de Bruxelles, Belgium)

¹⁹ Nortier JL, Vanherweghem JL. 2002. Renal interstitial fibrosis and urothelial carcinoma associated with the use of Chinese herb (Aristolochia fangchi). *Toxicology* 181-182: 577-580 (Support not reported. Authors affiliated with universite Libre de Bruxelles, Belgium)

²⁰ Wörth C. C. T., 2000, Entwicklung eines Nachweis-Verfahrens für DNA-Addukte basierend auf Fluoreszenzderivatisierung und kapillarelektrophoretischer Trennung, Dissertation molekulare Toxikologie, Leiter Prof. Manfred Wießler am Deutschen Krebsforschungszentrum Heidelberg

²¹ Neumann HG (2006a) Die Risikobewertung von Kanzerogenen und die Wirkungsschwelle. Teil I. *Bundesgesundheitsblatt* 49: S. 665–675

Neumann HG (2006b) Die Risikobewertung von Kanzerogenen und die Wirkungsschwelle, Teil II. *Bundesgesundheitsblatt* 49, S. 818–823

Neumann HG (2006c) Die Risikobewertung von Kanzerogenen und die Wirkungsschwelle, Teil III. *Bundesgesundheitsblatt* 49, S. 911–920

Modifying factors

Cells possess repair mechanisms to eliminate adducts. If their repair capacity is insufficient or lacks efficient repair mechanisms for a certain type of damage, DNA damage may have serious effects on the cells: they can degenerate and, eventually, the noxa is able to induce tumour growth.

Persistence of AA-DNA-adducts

The main AA-DNA-adduct, a desoxyadenosine adduct [7-(desoxyadenosine-N6-yl)-aristolactam I, dA-AA I], was detected in the kidneys and ureters of all the CHN patients mentioned above, even when exposure to aristolochic acid during the slimming cure dated back 10 years^{13, 22}.

Difficulty to evaluate DNA-adducts

Adduct formation indicates exposure to genotoxic substances, but not whether the exposure will trigger toxic effects.

Threshold of Toxicological Concern (TTC) is not applicable

Aristolochic acid has been proven to be a direct mutagen and one of the most potent carcinogens^{11, 15, 23, 24, 25}.

The mutagenic activity of aristolochic acid was compared with that of N-methyl-N'-nitro-N-nitrosoguanidine following systemic administration. It was markedly higher in aristolochic acid than in the comparator²⁶. Therefore, aristolochic acid belongs to the group of highly potent genotoxic carcinogens and is explicitly excluded from the TTC approach, in accordance with the *GUIDELINE ON THE LIMITS OF GENOTOXIC IMPURITIES EMEA/CHMP/QWP/251344/2006*:

"Some structural groups were identified to be of such high potency that intakes even below the TTC would be associated with a high probability of a significant carcinogenic risk²⁷. This group of high potency genotoxic carcinogens comprises aflatoxin-like-, N-nitroso- and azoxy-compounds that have to be excluded from the TTC approach."

(For exclusion see also POINTS TO CONSIDER ON NON-CLINICAL SAFETY OF HOMEOPATHIC MEDICINAL PRODUCTS OF BOTANICAL, MINERAL AND CHEMICAL ORIGIN, ADOPTION BY HMA, July 2007: *"Additionally, Aristolochia species are excluded from the TTC approach for homeopathic medicinal products of botanical origin in compliance with the Position Paper on the Risks Associated with the Use of Herbal Products containing Aristolochia species (EMEA/HMPWP/23/00)."*)

²² Arlt et al., 2001: Analyses of DNA adducts formed by ochratoxin A and aristolochic acid in patients with Chinese herbs nephropathy. Mutation Research-Genetic Toxicology and Environmental Mutagenesis 494, 143-150

²³ Schmeisser et al., 1984: Cancer Lett 23: 97-101

²⁴ Pezzuto et al., 1988: Mutat Res 206: 447-454

²⁵ Mengs et al., 1988: Arch Toxicol 61: 504-505

²⁶ Maier et al., 1985: Mutat Res 143: 143-148

²⁷ Kroes et al., Food and Chemical Toxicology 42 (2004): 65-83

Estimation of a virtually safe dose

In accordance with the estimates derived from human data, an acceptable limit for aristolochic acid, comparable to TTC, is 0.36 ng/d, with a residual cancer life-time risk of less than 1:1 000 000.

Derivation of the virtually safe dose on the basis of human data according to Nortier et al. (2000) ¹⁷:

Assuming a

- 50% tumour risk for cumulated intake of ca. 200 g of *Aristolochia fangchi* for ca. 2 years, and
- a content of 0.65 mg of aristolochic acid per gram of *Aristolochia fangchi* (herb),

the intake per patient amounts to 130 mg of aristolochic acid in the course of 2 years.

This is equivalent to a daily exposure of 0.18 mg of aristolochic acid.

Assuming a body weight (b.w.) of 50 kg this is equivalent to a daily dose of aristolochic acid of 0.0036 mg/kg b.w.

On the assumption of a residual risk of 1 : 1 000 000 and a 50% tumour risk, the virtually safe dose of aristolochic acid is 0.36 ng per day.

Calculation of the potency considered safe for homeopathic preparations of *Asarum*

The calculation considers

- the highest known content of aristolochic acid in *Asarum crispulatum* of 3377 ppm (= 750 ppm in the mother tincture = 0.075%). Calculation on the basis of the maximum content is necessary because the data available so far come from as few as three homeopathic mother tinctures of *Asarum europaeum* and one mother tincture of *Asarum canadense*. In view of the high toxic potential of aristolochic acid it is impossible to determine a potency limit alone on the basis of these four samples;
- the highest daily dose for “worst-case“ conditions,
- the liquid dilution/mixture and tablet dosage forms.

Fresh underground parts are the starting material for the mother tinctures of *Asarum europaeum* and *Asarum canadense*. The mother tinctures are prepared according to HAB, manufacturing method 3a. Decimal dilution 1 (D1) is prepared from 3 parts of mother tincture and 7 parts of ethanol 62% (m/m), D2 from 1 part of D1 and 9 parts of ethanol 62% (m/m). The subsequent dilutions are prepared accordingly. Ethanol 43% (m/m) is used from potency D4 and up.

Assuming 3377 µg or 3.377 mg of aristolochic acid in 1 g of dried plant:

With a loss of drying of 60% this is equivalent to 1.35 mg of aristolochic acid in 1 g of fresh plant.

If prepared according to HAB, manufacturing method 3a, 0.55 g of fresh plant is processed in 1 g of mother tincture. Thus, 1 g of mother tincture contains ca. 0.75 mg of aristolochic acid.

Liquid dilution/mixture (dose specification as in monograph):

Doses of 5-10 drops half-hourly to hourly.

10 drops x 24 x 2 = 480 drops

minus 8 hours of sleep: 10 x 8 x 2 = 160 drops

Thus the maximum dose/day is 480 – 160 = 320 drops.

(30 drops, according to drop table, are equivalent to 1 g of liquid dilution)

A maximum daily dose of 320 drops of mother tincture (UT) would amount to 11 g of UT.

11 g UT contain a maximum of 8.25 mg of aristolochic acid.

This is equivalent to ca. 2.48 mg in D1 and ca. 0.248 ng in D8.

Hence, potency D8 is below the virtually safe dose of 0.36 ng/d of aristolochic acid.

By choosing a dose of 320 drops we take account of the possibility that therapists deviate from the current dose recommendation of Commission D of 17 March 2004. Deviations are conceivable with a phrase like: "Unless otherwise prescribed..." in the dosage specification.

Since the pharmaceutical companies have not included any dose limits in the patient package leaflet, we cannot rule out that patients are given higher doses.

Tablets (of 250 mg of active substance each):

Doses of 1-3 tablets half-hourly to hourly.

3 tablets x 24 x 2 = 144 tablets

minus 8 hours of sleep: 3 x 8 x 2 = 48 tablets

Thus the maximum dose/day is 144 – 48 = 96 tablets (equivalent to 24 g of active substance).

Derived from the content of aristolochic acid in the mother tincture, D1 contains 5.4 mg, D8 0.54 ng and D9 0.054 ng of aristolochic acid.

Hence, potency D9 is below the virtually safe dose of 0.36 ng/d of aristolochic acid.

Owing to the high toxic potential of aristolochic acid (see above) and in consideration of the graduated plan procedure for *Aristolochia* species of 1981, the first potency where the risk from the *Asarum* species is acceptable is D11, not lower. This is equivalent to a maximum daily exposure of 3.6 pg/day of aristolochic acid.

In the hearings with the pharmaceutical companies we were repeatedly requested to await the discussion in the Group of Experts for Homeopathic Raw Materials and Stocks of the German Homeopathic Pharmacopoeia Commission before issuing a notice. Unfortunately we cannot comply with this request because there is no suitable, valid and sufficiently sensitive assay for the detection of aristolochic acid in mother tinctures of *Asarum europaeum*. And with the mechanisms of action as they are, it is not acceptable to wait for the development of a future assay.

Summary

Aristolochic acid has been detected not only in homeopathic mother tinctures of *Aristolochia clematitis*, but also of *Asarum europaeum* and *Asarum canadense* as well as in the TCM drug *Asari radix et rhizoma*. These findings are confirmed by statements in the literature on the presence of aristolochic acid in numerous *Asarum* species.

Therefore it is necessary to extend the measures ruled in the graduated plan procedure of 1981 to all medicinal products prepared by using plants of the *Asarum* species.

In view of the high toxic potential of aristolochic acid and taking the highest known content of aristolochic acid in *Asarum crispulatum* as a basis, having converted the virtually safe dose into a potency stage under the conditions of worst-case doses and following the 1981 graduated plan procedure for *Aristolochia* species, we have come to the conclusion that D11 is the potency limit for homeopathic products from *Asarum* species of the *Aristolochiaceae* family. Potencies up to and including D10, related to the finished product, raise safety concerns. Therefore, it is necessary to revoke the authorisations/registrations of products as listed in the Annex.

It is explicitly stated here that the present graduated plan procedure does not only apply to finished medicinal products liable to registration or authorisation in accordance with Section 5 sub-section 1 of the German Medicines Act (AMG). It also applies to the sale of the medicinal drug itself and to those homeopathic medicines that are exempted from the obligation to registration if marketed in quantities of not more than 1000 packages per year, due to Section 38 sub-section 1 AMG. The pharmaceutical companies concerned are obliged to immediately implement the measures for the mentioned products. The competent authorities will control the implementation.

Standard registrations

According to pharmaceutical legislation the present ruling is valid for individually registered medicinal products. Users of respective standard registrations are requested to immediately adopt the above mentioned changes on their own account in order to adapt to the up-to-date findings, in anticipation of a change of the ordinance for standard registrations on the basis of Section 39 sub-section 3 in connection with the provisions of Section 36 sub-section 1 AMG.

Legal remedy

Objections to this Notice may be raised within one month from its announcement. Objections are to be made in writing or orally for record at the Federal Institute for Drugs and Medical Devices, Kurt-Georg-Kiesinger Allee 3, 53175 Bonn,

Yours sincerely
Dr. A. Thiele
on behalf of the BfArM

Annex