European Commission

Pilot project: Proposal for approbation of basic substances, in the context of Regulation (EC) N°1107/2009

EQUISETUM Arvense L.

BASIC SUBSTANCE APPLICATION

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" EQUISETUM Arvense L."

1. PURPOSE OF THE APPLICATION

This report is submitted to support the application for the first approbation of the plant Equisetum arvense L. as a substance in the Parliament and Council Regulation (EC) 1107/2009 as a basic substance.

1.1. NAME AND ADDRESS OF APPLICANTS

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2. IDENTITY OF THE SUBSTANCE/PRODUCT AS AVAILABLE ON THE MARKET AND PREDOMINANT USE

2.1. PREDOMINANT USES OF THE SUBSTANCE OUTSIDE PLANT PROTECTION

Equisetum arvense L. (Equisetaceae, subgenus *Equisetum),* the active substance, is a well-known and widespread pteridophyte distributed in the northern hemisphere. Its sterile stems are used as medicines in various countries, constituting "Equiseti herba" of European Pharmacopeias (DAB 10, Ph. Helv. VII, OAB 90, Ph. Pol. III, Ph. Ross 9 and Ph. Hung.).

2.2. IDENTITY AND PHYSICAL CHEMICAL PROPERTIES OF THE SUBSTANCE AND PRODUCT TO BE USED

2.2.1. Common name of the substance and product and their synonyms/plant nomenclature

Proposed name: *Equisetum arvense L.*

ISO common name (approved or proposed): Not relevant

Synonyms: Equiseti herba (European Pharmacopoeia); Field horsetail, Common horsetail; Prêle des champs (French); Schachtelhalm (German); Coda cavallina (Italian); Paardestaart (Dutch)

2.2.2. Chemical name with CAS, EEC and CIPAC numbers

N° CAS : 71011-23-9
N° CTFA : 8717
N° EINECS/ELINCS : 275-123-8

No chemical denomination can be assigned to *Equisetum arvense L.* aerial part (active substance) because this product is a complex mixture of chemical substances.
Not relevant in the way that the aerial part of the plant is collected, and the active substance is composed of a complex mixture of natural products.

2.2.3. Molecular and structural formula, molecular mass

Molecular formula: not applicable

Structural formula: not applicable

Molecular mass: not applicable

2.2.4. Method or methods of manufacture of the substance and of the product

The sterile stems of the plant are picked in nature, manually, in different areas in Europe, mainly in France and Bulgaria, and the plant is not cultivated today. The plant is perennial. Exceptionally, the plant is imported from China by the manufacturer Martin Bauer S.p.A.
Company MARTIN BAUER S.p.A. 12/11/2008 **Company**, **report** of the quality and conformity certificate of the active substance *Equisetum arvense*. PPM0021084 fg

Company MARTIN BAUER S.p.A. 01/09/2008 Company, report of the quality and conformity certificate of the active substance *Equisetum arvense*. PPM002673

Company MARTIN BAUER S.p.A. 12/11/2008 Company, report of the quality and conformity certificate of the active substance *Equisetum arvense*. PPM0021084

2.2.5. Description and specification of purity of the active substance and product

The active substance is a dried plant. It is a complex mixture of natural compounds, the purity of the active substance cannot be defined.

The active substance is composed of the cut dried aerial parts, sterile stems, of the plant. It consists of fragments of grooved stems and linear leaves, light green to greenish-grey. They are rough to the touch, brittle and crunchy when crushed. The main stems are about 0.8 mm to 4.5 mm in diameter, hollow, jointed at the nodes which occur at intervals of about 1.5 cm to 4.5 cm; distinct vertical grooves are present on the internodes, ranging in number from 4 to 14 or more. Verticils of widely spaced and erect branches, usually simple, each about 1 mm thick with 2 to 4 longitudinal grooves, occur at the nodes. The leaves are small, linear, verticillate at each node, concrescent at the base; they form a toothed sheath around the stem; with the number of teeth corresponding to the number of grooves on the stem. Each tooth, often brown, is lanceolate-triangular. The lowest internode of each branch is longer than the sheath of the stem it belongs to.

The chemical composition of the plant *Equisetum arvense L.* was reported in the monographic document from the "PDR for Herbal Medicines", third edition;

Heber, D. 2004 Horsetail, *Equisetum arvense,* PDR for Herbal Medecines, third edition, Montyale (NJ). ISBN 1-56363-512-7

- flavonoids: 0,6 to 0,9% : apigenin-5-0-glucoside, genkwanin-5-O-glucoside, kaempferol-3,7-di-O-glucoside, kaempferol-3-O-(6'-O-malonyl-glucoside)-7-O-glucoside, kaempferol-3-O-sophoroside, luteolin-5-O-glucoside, quercetin-3-O-glucoside
- caffeic acid ester (up to 1%): including chlorogenic acid, dicoffeoyl-meso-tartaric acid
- silicic acid (5 to 7,7%): to some extent water soluble
- pyridine alkaloids: nicotine (traces), palustrine (in the gamatophytes and in the rhizome styrolpyrone glucosides, including equisetumpyrone)

However, in the literature the variability in phenolics content of *Equisitum arvense* was described and it can be taken into account in the description of the active substance. *(Markus V and Co, July 1994)*

Consequently, based on the European Pharmacopoeia, the maximum content of stems from other Equisetum species and hybrids must be 5% and 2% of other foreign matter in the active substance. In addition, the dried drug should contain at a minimum 0.3% of total flavonoids expressed as isoquercitroside ($C_{21}H_{20}O_{12}$; M = 464.4).

Currie H. A. 2009 Chemical evidence for intrinsic 'Si' within Equisetum cell walls. Phytochemistry 70 () 2089–2095

Currie H. A. 2007 Silica in Plants: Biological, Biochemical and Chemical Studies Annals of Botany 100: 1383–1389

Silica content is fully described in these papers.

General information on Equisetum arvense content can be found in a very recent article.

Asgarpanah J. et al. 2012 Phytochemistry and pharmacological properties of *Equisetum arvense* L. Journal of Medicinal Plants Research Vol. 6(21), pp. 3689-3693

Equisetum arvense L. is known as Horsetail. *E. arvense* extracts are important areas in drug development with numerous pharmacological activities in many countries. For a long time, *E. arvense* has been used in traditional medicines for the treatment of brittle fingernails, loss of hair and for rheumatic diseases. *E. arvense* has recently been shown to have antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, antidiabetic, antitumor, cytotoxic and anticonvulsant activities. Apigenin, luteolin, equisetumoside A, equisetumoside B and equisetumoside C, nicotine, palustrine and palustrinine are phytochemical compounds which are reported from this plant. Due to the easy collection of the plant and being widespread and also remarkable biological activities, this plant has become medicine in many countries. This article presents comprehensive analyzed information on the botanical, chemical and pharmacological aspects of *E. arvense*.

2.2.6. Identity of inactive isomers, impurities and additives

The active substance *Equisetum arvense L.*, dried cut plant, is not containing additive, preservative nor added chemical materials. The product is not irradiated but treated by dry-steam. It is GMO-free. There are no allergens based on supplier and internal information in the company. The product doesn't contain animal material. Therefore the question of BSE is not relevant. Heavy metal content, pesticides content, mycotoxines and microbiology were quantified and checked for each batches in the following way:

Loss on drying: maximum 10 per cent.

Ash insoluble in hydrochloric acid: minimum 3.0 per cent and maximum 15.0 per cent.

Total ash: minimum 12.0 per cent and maximum 27.0 per cent.

Heavy metals: The limits are for the different metals:

Pb	=< 10 ppm
Cd	=< 1.0 ppm
Hg	=< 0.1 ppm

Radioactivity: The limits are < 600 Bq/kg

Pesticides residues in the plant:
The vegetal drug has to satisfy to the limits presented in the point 2.8.13 of the European Pharmacopoeia .

The list of pesticides analysed and not detected:
Organophosphorous: Azinphos ethyl, azinphos methym, chlorfenvinphos, chlormephos, chlorpyrifos, chlorpyrifos methyl, diazinon, dichlorvos, dimethoate, disulfoton, ethion, fenchlorphos, fenitrothion, phenthoate, fonofos, phorate, phosalone, isofenphos, malathion, methidathion, parathion, parathion methyl, pirimiphos methyl, sulfotep, tetrachlorvinphos.

Herbicides: alachlor, atrazine, simazine, terbutylazine, trifluralin.
Fumigants: methyl bromide, carbon sulphide, carbon tetrachloride, bromopropylate, piperonyl butoxide.

Chlorinated compounds: aldrin + dieldrin, chlordane + oxychlordane, endosulfan, endcrin, heptachlor, heptachlorepoxid, hexachlorobenzene, lindane, methoxychlor, perthane.

Fungicides: dithiocarbamates, quintozene, pentachloroaniline.

Pyrethroids: cypermethrin, deltamethrine, fenvalerate, permethrin, pyrethrins.

Mycotoxins: The units are ppb and the limits are :

Aflatoxin B1	=< 5 ppb
Aflatoxin B1, B2, G1, G2	=< 10 ppb

Microbiology
Limits proposed are based on the results obtained for the batch PPM0026731/805 (see 1.3.11) as this batch is considered as a worst case. The units are cfu/g, and the limits are:

Aerobic bacteria	=< $5X10^{e5}$
Fungi (yeasts/moulds)	=< $5X10^{e4}$
Enterobacteriaceae	=< 10^{e3}
Escherichia coli	absent in 1 g

The units are g and the limits are absent in: Salmonella absent in 10 g

European Pharmacopoeia 2008, Equisetum Stem, Equiseti Herba. European Pharmacopoeia 6.0., 01/2008:1825

2.2.7. Methods of analysis

2.2.7.1. Methods of analysis for determination of the active substance as manufactured

The *Equisetum arvense L.* dried plant was identified in the European Pharmacopoeia in the following way:
The active substance being a plant, the aerial part of the plant, the purity can be define only with the specific tests and analyses regarding

- a: the absence of other parts of the same plant like rhizomes
- b: the absence of other Equiseti plants (for example *Equ. palustre*, *Equ. telmateia*..) (TLC test described in IIA 1.10) Distinction can be made between *Equisetum arvense L.* and *Equisetum palustris* and other species following visual identification (Document: Equisetum – Scouring Ruches and Horsetails)
- c: and the absence of other plants (foreign matter)

Identification:

a. It consists of fragments of grooved stems and linear leaves, light green to greenish-grey. They are rough to the touch, brittle and crunchy when crushed. The main stems are about 0.8 mm to 4.5 mm in diameter, hollow, jointed at the nodes which occur at intervals of about 1.5 cm to 4.5 cm; distinct vertical grooves are present on the internodes, ranging in number from 4 to 14 or more. Verticils of widely spaced and erect branches, usually simple, each about 1 mm thick with 2 to 4 longitudinal grooves, occur at the nodes. The leaves are small, linear, verticillate at each node, concrescent at the base; they form a toothed sheath around the stem; with the number of teeth corresponding to the number of grooves on the stem. Each tooth, often brown, is lanceolate-triangular. The lowest internode of each branch is longer than the sheath of the stem it belongs to.

b. Reduced to a powder, this powder is greenish-grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the epidermis in surface view; composed of rectangular cells with wavy walls and paracytic stomata with the 2 subsidiary cells covering the guard cells and having conspicuous radial ridges; in transverse sectional view the epidermis is crenate, with the protuberances formed from the contiguous walls of 2 adjacent, U-shaped cells. Fragments of large-celled parenchyma and groups of long, non-lignified fibres with narrow lumens are visible, together with scattered small, lignified vessels with spiral or annular thickening.

c. **Foreign matter**: maximum 5 per cent of stems from other Equisetum species and hybrids and maximum 2 per cent of other foreign matter.

2.2.7.2. Analytical methods for determination of relevant impurities

For the determination and the quantification of other *Equisetum* species and hybrids:
The general method is presented in: Eur. Ph. (*2.2.27*).
A thin-layer chromatography is proposed and described in the European Pharmacopoeia 6.0. (page 1795).
Examine the chromatograms obtained in the test for other Equisetum species and hybrids.
Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	2 red fluorescent zones
Caffeic acid: a greenish-blue fluorescent zone	
	2 greenish-blue fluorescent zones
	An orange fluorescent zone
Hyperoside: an orange fluorescent zone	
	2 greenish-blue fluorescent zones
Rutin: an orange fluorescent zone	
Reference solution	**Test solution**

TESTS
Other Equisetum species and hybrids. Thin-layer chromatography Eur. Ph. (*2.2.27*).
Test solution. To 1.0 g of the powdered drug (355) Eur. Ph. (*2.9.12*) add 10 ml of *methanol R*. Heat in a water-bath at 60°C for 10 min with occasional shaking. Allow to cool. Filter.
Reference solution. Dissolve 1.0 mg of *caffeic acid R*, 2.5 mg of *hyperoside R* and 2.5 mg of *rutin R* in 10 ml of *methanol R*.
Plate: TLC silica gel plate R.
Mobile phase: anhydrous formic acid R, glacial acetic acid R, water R, ethyl acetate R (7.5:7.5:18:67 V/V/V/W.
Application: 10 pl, as bands.
Development: over a path of 10 cm.
Drying: at 100-105°C.
Detection: spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Then spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for 30 min. Examine in ultraviolet light at 365 nm.
Results: the chromatogram obtained with the test solution shows no yellow or greenish-yellow fluorescent zone shortly above the starting line.

Loss on drying: determined on 1.000 g of the powdered drug by drying in an oven at 105°C for 2 hours

Analytical methods for the determination of active substance and significant/relevant impurities in the decoction

Heavy metal: The method reference is AAS-ETA (Codex Herbarum)

Pesticides: The method for the determination is Gas Chromatography according to the Eur. Ph. 6th.

Mycotoxins: The method reference is in according with Dir. 98/53/EC.
For the aflatoxin B1 : immunoassay FU XI° Ed. (Pharm. Use)
For the aflatoxin B1, B2, G1, G2 : immunoassay FU XI° Ed. (Pharm. Use)

Microbiology
The method references are Internal EHIA (European Herbal Infusions Association, www.ehia-online.org).

2.2.7.3. Analytical methods for determination of residues

Analytical methods for residue determination in plants, soil, water, air, food of animal origin and body tissues

Determination of residues has not been realized nor studied in treated plants, human and animal food as the soluble compounds extracted from the plant horsetail, and the plant itself, are highly biodegradable in water and soil and similar to the natural background level. So the use of the active substance in agriculture cannot produce residues on plants and in soil, water and air. Therefore no residue definition and no LMR have been set

2.3. CURRENT, FORMER AND IN CASE PROPOSED TRADE NAMES OF SUBSTANCES/ PRODUCTS AS PUT ON THE MARKET

Not relevant

2.4. MANUFACTURER OF THE SUBSTANCE/PRODUCTS

Not relevant

2.5. TYPE OF PREPARATION OF THE SUBSTANCE/PRODUCT

Dispersible concentrate (DC) (decoction)

2.6. DESCRIPTION OF THE RECIPE FOR THE PRODUCT TO BE USED

The active substance is a basic substance, therefore no formulation will be submitted but a recipe of the decoction was described.
The decoction made of *Equisetum arvense L.* boiling water fluid extract has been prepared on the information given by the French organic and biodynamic associations for the recipe. After extraction, the preparation is made of 10^{th} dilution of the extract with water (spring water or rainwater. In each case, the solvent for extraction and preparation is natural water (spring water or rainwater) and the pH is 6.5.

The recipe retained for this registration:

Mode of preparation : boiling water extraction then dilution	**aerial part of dry plant/solvent (g/L) in the mother extraction**	**aerial part of dry plant/solvent (g/L) in the preparation**
Macerate 200 g of the aerial part of *Equisetum arvense L.* dry plant in 10 litres of natural or rain cold water for 30 min and allow boiling for 45 minutes. After cooling down, then proceeds the dilution by 10 (with natural or rain cold water).	**20**	**2**

The preparation made of dry or fresh plant has to be applied some maximum 24 hours after the preparation because the water extract is sensible to oxygen and to avoid the potential contamination and multiplication of microorganisms which may occur during the storage.

2.7. FUNCTION ON PLANT PROTECTION

Elicitor, fungicide via the stimulation of natural defense mechanisms.

3. USES OF THE SUBSTANCE AND ITS PRODUCT

3.1. FIELD OF USE

The horsetail plant, *Equisetum arvense*, is intended to be used in fields for plant protection on grapevines and apple trees.

3.2. EFFECTS ON HARMFUL ORGANISMS OR ON PLANTS

Equisetum arvense is intended to control foliar fungi diseases like *Venturia inaequalis, Plasmopara viticola, Erysiphe necator* and *Podosphaera leucotricha.*

Equisetum arvense has long been known in botanical folklore as having a preventive effect on fungal plant diseases. The traditional hot water extract from *Equisetum arvense* has long been used by organic and biodynamic farmers.
Equisetum arvense water extract is intended to be used as plant strengthener and preventive treatment of pathogenic fungi. In this way, we are presenting some papers about the effect of the natural silica (silicic acid) used for the control of powdery mildews and fungal diseases in some cultures. No detailed Mode of action is clearly understood at the moment.
Equisetum arvense water extract is a useful new plant protection product of particular value for the suppression mildew in conjunction with other organic farming tools.
Equisetum arvense extract is a foliar stimulator of natural defences and fungicide for use as a spring post-emergence treatment in all cultivars of grapevine and apple trees.

The effect would be based on the high percentage of silica in the plant that works on lowering the impact of moisture. Silicon would reduce the effects of excessive water around plants that would lead to fungus. It would act also as an activator of plant defense mechanisms.

Reynolds A. G. et al. 1996 Use of Potassium Silicate for the Control of Powdery Mildew [*Uncinula necator* (Schwein) Burrill] in *Vitis vinifera* L. Cultivar Bacchus. Am. J. Enol. Vitic., Vol. 47, No. 4, p421-428

Dagostin S. et al. 2011 Are there alternatives to copper for controlling grapevine downy mildew in organic viticulture? Crop Protection 30, 776-788

Daiana Garcia E. et al. 2011 Mould growth and mycotoxin production as affected by *Equisetum arvense* and *Stevia rebaudiana* extracts Food Control 22 (2011) 1378-1384

Cherif M et al. 1992 Silicon induced resistance in cucumber plants against *Pythium ultimum*. Physiological and Molecular Plant Pathology, 41(6):411-425

The effect of the amendment of nutrient solutions with soluble potassium silicate on the response of cucumber (cv. Corona) root and hypocotyl tissues infected by *Pythium ultimum* was examined by light and electron microscopy, and by energy dispersive X-ray analysis (EDX). Plants were grown in 0 or 1.7 mM Si-amended nutrient solutions, and root and hypocotyl samples were collected at different times after inoculation with P. ultimum. By 48 h after infection, striking differences in the expression of defence reactions were observed between Si-amended and Si-free cucumber plants. Treatment of plants with Si markedly stimulated the accumulation of an electron-dense, phenolic-like material in infected host tissues, and significantly increased the percentage of cells filled with this material. Fungal hyphae colonizing occluded host cells were seriously damaged, and were often reduced to empty hyphal shells. Additionally, Si-treated cucumber plants responded to *P. ultimum* infection by forming electron-dense layers along primary and secondary cell walls, as well as over pit membranes of xylem vessels. EDX analysis failed to reveal the presence of silica deposits in P. ultimum-infected plants grown in Si-supplemented media. Our results suggest that a relationship exists between Si treatment, resistance to *P. ultimum* attack, and expression of plant defence mechanisms.

Fauteux F. 2005 Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiology Letters 249 1-6

Silicon (Si) is a bioactive element associated with beneficial effects on mechanical and physiological properties of plants. Silicon alleviates abiotic and biotic stresses, and increases the resistance of plants to pathogenic fungi. Several studies have suggested that Si activates plant defense mechanisms, yet the exact nature of the interaction between the element and biochemical path ways leading to resistance remains unclear.

Fauteux F. 2006 The protective role of silicon in the Arabidopsis–powdery mildew pathosystem. PNAS vol. 103 _ no. 46 17554–17559

The role and essentiality of silicon (Si) in plant biology have been debated for >150 years despite numerous reports describing its beneficial properties. To obtain unique insights regarding the effect of Si on plants, we performed a complete transcriptome analysis of both control and powdery mildew-stressed *Arabidopsis* plants,

with or without Si application, using a 44K microarray. Surprisingly, the expression of all but two genes was unaffected by Si in control plants, a result contradicting reports of a possible direct effect of Si as a fertilizer. In contrast, inoculation of plants, treated or not with Si, altered the expression of a set of nearly 4,000 genes. After functional categorization, many of the upregulated genes were defense-related, whereas a large proportion of down-regulated genes were involved in primary metabolism. Regulated defense genes included R genes, stress-related transcription factors, genes involved in signal transduction, the biosynthesis of stress hormones (SA, JA, ethylene), and the metabolism of reactive oxygen species.

Kuepper G. 2004 ORGANIC ALTERNATIVES FOR LATE BLIGHT CONTROL IN POTATOES, PEST MANAGEMENT TECHNICAL NOTE, p1-8

Foliar feeding has been associated with disease resistance. Two materials that have acquired such a reputation are kelp-based products and the Biodynamic™ preparation #508—made from the primitive plant horsetail (*Equisetum arvense*).

Epstein E. 2009 Silicon: its manifold roles in plants, Annals of Applied Biology ISSN 0003-4746, p1-6

The title of this essay declares that silicon does have roles in plants and all participants in this conference know that that is so. This knowledge, however, is not shared by the general community of plant biologists, who largely ignore the element. This baffling contrast is based on two sets of experience. First, higher plants can grow to maturity in nutrient solutions formulated without silicon. That has led to the conventional wisdom that silicon is not an essential element, or nutrient, and thus can be disregarded. Second, the world's plants do not grow in the benign environment of solution culture in plant biological research establishments. They grow in the field, under conditions that are often anything but benign. It is there, in the real world with its manifold stressful features, that the silicon status of plants can make a huge difference in their performance. The stresses that silicon alleviates range all the way from biotic, including diseases and pests, to abiotic such as gravity and metal toxicities. Silicon performs its functions in two ways: by the polymerization of silicic acid leading to the formation of solid amorphous, hydrated silica, and by being instrumental in the formation of organic defence compounds through alteration of gene expression.

In parallel, essential oil was demonstrated to have antimicrobial activity.

Radulovic N. 2006 Composition and Antimicrobial Activity of Equisetum arvense L. Essential Oil, Phytother. Res. 20, 85–88

The 1:10 dilution of the essential oil of *Equisetum arvense* L. was shown to possess a broad spectrum of a very strong antimicrobial activity against all tested strains.

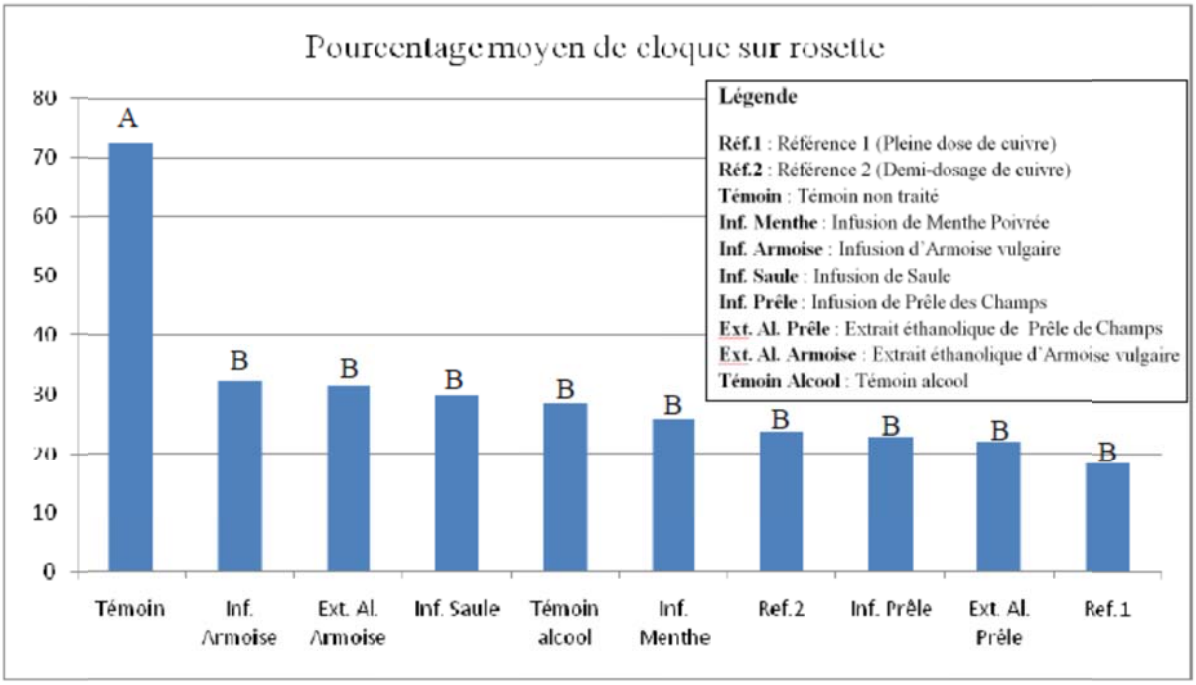
On cucumber, basis of silica use can be found in

Wolff S.A. 2012 Foliar applications of silicon fertilisers inhibit powdery mildew development in greenhouse cucumber. Journal of Food, Agriculture & Environment Vol.10 (1): 355-359.

Foliar application of potassium silicate and other Si-based fertilisers reduces the development of powdery mildew in greenhouse- cultivated cucumber, although the basic mechanisms are not known. Procedures for application and inoculation seem to influence on the results; strengthening the theories suggesting a more local protective effect of foliar applied Si. On the whole, our results strengthen previous reports on the mildew restraining effects of Si amendments to cucumber plants, and represent foliar applications of Si-based fertilisers as an attractive alternative for powdery mildew control in cucumber production.

Regarding fruit trees, assays were done in France, small fungicide effect is observed with equisetum aqueous extract (Prêle) treatments (column 8) on peach leaf curl *Taphrina deformans.*

ONDET S.J. et al. 2011 Stratégie de maîtrise de la Cloque du pêcher par phytothérapie GRAB, in CASDAR 2009, Evaluation des caractéristiques et de l'intérêt agronomique de préparations simples de plantes, pour des productions fruitières, légumières et viticoles économes en intrants. AAP CAS DAR 2009, n° 9046



Regarding fruit trees and vegetables, use of equisetum aqueous extract, for fungicide effect is observed world widely.

PATHAK R. K. et al. BIODYNAMIC PRODUCTION OF FRUIT AND VEGETABLES AT CISH, LUCKNOW, presentation at FAO, from Central Institute for Subtropical Horticulture Rehmankhera, Lucknow- 227 107, India

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Heine G. et al. 2007 The effect of silicon on the infection by and spread of *Pythium aphanidermatum* in single roots of tomato and bitter gourd. Journal of Experimental Botany, Vol. 58, No. 3, pp. 569–577

3.3. SUMMARY OF INTENDED USES

Crop and/or situation (a)	Member State	Example Product name as available on the market	FGI (b)	Target (c)	Product**		Application				Application rate per treatment			Total rate	PHI (days) (m)	Remarks (*)
					Type (d-f)	Conc of a.i. g/kg (i)	Method kind (f-h)	Growth stage and season** (j)	Number min max (k)	Interval between applications (min)	g a.i./hl min max (g/hl)	Water l/ha min max	g a.i./ha min max (g/ha) (l)	kg a.i./ha min max (kg/ha) (l)		
Fruit trees Apple fruit Malus pumila, Malus domestica Peach-tree *Prunus persica*	France FR All Member States	Homogenate of *Equisetum arvense* L.	F	Foliar fungi like scab disease *Venturia inaequalis,* Powdery mildews: *Podosphaera leucotricha* Peach leaf curl *Taphrina deformans*	Dispersible Concentrate (DC)***	2	Foliar application spraying	From green leaf tip (BBCH 53) to flowers fading (BBCH 67) Spring	2- 6	7 days	200	500 to 1000	1000 to 2000	2 to 12	None	Plant homogenate extracted with hot water and filtered to be used 24 h after preparation
Grapevine Vitis vinifera				Downy mildews: *Plasmopara viticola,* Powdery mildews: *Erysiphe necator*				From 1st shoots (BCH10) to cluster tightening (BBCH57) Spring to summer				100 to 300	200 to 600	0.4 to 3.6		
Cucumber roots Cucumis sativus			G	Powdery mildews: *Podosphaera xhantii* Root fungi like common *root rot*, seedling *blight Pythium* spp.			Root Feeding application and foliar application spraying	From (9th leaf unfolded on main stem) (BBCH19) to 9 or more primary side shoots visible (BBCH49)	6	3-4 days		300	600	3.6	15 days	
Tomato *Lycopersicum esculentum*			F	early blight: *Alternaria solani* Septoria blight *Septoria lycopersici*			Foliar application spraying	First inflorescence visible (BBCH 51) to BBCH 59 Summer	2	14 days		300	600	1.2	15 days	

**** The product cannot be applied in case of hot temperature. It is used in case of rainy period**
***** The product is a plant homogenate extracted with hot water and filtered (decoction)**

* For uses where the column „Remarks“ in marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s).

(a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)

(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds

(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated

(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) Indicate the minimum and maximum number of application possible under practical conditions of use

(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha

(m) PHI - minimum pre-harvest interval

4. CLASSIFICATION AND LABELLING OF THE SUBSTANCE

Not applicable: *Equisetum arvense L.* is a plant and it is used as a decoction.

5. IMPACT ON HUMAN AND ANIMAL HEALTH

5.1. EFFECTS HAVING RELEVANCE TO HUMAN AND ANIMAL HEALTH ARISING FROM EXPOSURE TO THE SUBSTANCE/ITS PRODUCTS OR TO IMPURITIES CONTAINED IN THE SUBSTANCE/PRODUCT OR THEIR TRANSFORMATION PRODUCTS

General information on Equisetum arvense can be found in a very recent article.

Asgarpanah J. et al. 2012 Phytochemistry and pharmacological properties of *Equisetum arvense* L. Journal of Medicinal Plants Research Vol. 6(21), pp. 3689-3693

Equisetum arvense L. is known as Horsetail. *E. arvense* extracts are important areas in drug development with numerous pharmacological activities in many countries. For a long time, *E. arvense* has been used in traditional medicines for the treatment of brittle fingernails, loss of hair and for rheumatic diseases. *E. arvense* has recently been shown to have antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, antidiabetic, antitumor, cytotoxic and anticonvulsant activities. Apigenin, luteolin, equisetumoside A, equisetumoside B and equisetumoside C, nicotine, palustrine and palustrinine are phytochemical compounds which are reported from this plant. Due to the easy collection of the plant and being widespread and also remarkable biological activities, this plant has become medicine in many countries. This article presents comprehensive analyzed information on the botanical, chemical and pharmacological aspects of *E. arvense*.

Recommendation: The most common posology of all countries is 3 times daily a tea prepared with 2-3 g herbal substance (Daily dose: 6-9 g herbal substance).

EMEA, 2007, Equisetum *arvense* L., Herba, ASSESSMENT REPORT for the DEVELOPMENT of COMMUNITY MONOGRAPHS and for INCLUSION of HERBAL SUBSTANCE(S), PREPARATION(S) or COMBINATIONS THEREOF in the LIST. EMEA/HMPC/394895/2007

Maeda H, 1997 Occurrence of dermatitis in rats fed a cholesterol diet containing field horsetail (*Equisetum arvense* L.). J Nutr Sci Vitaminol. 43(5):553-63

Potential Side Effects of Horsetail
Because of its native action, intake of horsetail in excessive amounts may lead to nausea, increased frequency of bowel movements, increased urination, loss of potassium stores, and muscle weakness. People with kidney disorders and diabetes should avoid horsetail. People who have thiamine (vitamin B1) deficiency or poor nutrition or are pregnant should also avoid horsetail, as it may affect levels of thiamine. Avoid taking horsetail together with other diuretics, steroids and laxatives. When taken in appropriate doses, it is traditionally considered to be safe. Its adverse side effect includes skin rash, dermatitis. Uncommonly, its side effects include brain and heart diseases

Sandhu N.S. 2010 Pharmacognostic Evaluation Of *Equisetum arvense* Linn. International Journal of PharmTech Research Vol.2, No.2, pp 1460-1464

Pharmacognostical studies were carried out on the sterile stems of *Equisetum arvense* Linn, which showed the presence of xylem vessels, cortex, parenchyma, stomata, and silica granules. Physicochemical parameters such as water, ether and alcohol soluble extractive values were found to be 15.45%, 3.52 % and 4.32 % w/w. The total ash value, acid insoluble ash and water soluble ash were found to be 22 %, 11 % and 8 % w/w respectively. Moisture content and volatile oil content was found to be 15 % and 1.5 % respectively. The loss on drying was found to be 12.5 % w/w. Foaming index calculated was found to be 100. These investigations will be helpful in correct identification and standardization of plant and to differentiate it from the closely resembled species.

Horsetail can produce toxic effects on its prolonged use. Silicates produce digestive problems, especially when used for long. Alkaloids although do not appear in strong concentrations, a prolonged use, can take place by accumulating them in the organism which may facilitate premature childbirth, nervous disorders, headaches, loss of appetite, swallowing problems, etc. These intoxications force to a treatment that restores the thiamine deficiency, although in the case of the animals, they are no longer recoverable in many occasions.

Bioavailability of silicon from choline-stabilised orthosilicic acid Human studies.
The petitioner provided data to prove that incubated dilutions of ch-OSA in water contain primarily silicon in the orthosilicic acid form (Vanden Berghe, 2000). The absorption of silicon from ch-OSA was studied in a cross-over protocol with 14 healthy subjects (8 females and 6 males, aged 22-34 years). None of them had taken silicon supplements for 3 months before the start of the study. Each fasting subject received orally successively 20 mg silicon in the form of ch-OSA, 20 mg stabilised monomeric silicic acid, herbal silica (533 mg of a dry Equisetum arvense extract containing 8% w/w of silicon dioxide), colloidal silicic acid (2 mL of a solution containing 28 g of H_2SiO_3/L) or a placebo (10 mL mineral water) with 1 week wash-out between each supplement or placebo.
http://www.efsa.europa.eu/en/scdocs/doc/ans_ej948_Choline_stabilised_orthosilic_acid_op_Updated_en.pdf

5.2. TOXICOKINETICS AND METABOLISM IN HUMANS

Graefe E.U. and Veit M. Urinary metabolites of flavonoids and hydroxycinnamic acids in humans after application of a crude extract from *Equisetum arvense*. Phytomedicine: international journal of phytotherapy and phytopharmacology 1999, 6(4), pp 239-246.

Materials and Methods
11 healthy volunteers (9 males, 2 females) aged 23 to 37 y (average body mass index 22 kg/m^2) received a flavonoïd-free diet over 8 days. No vegetables, fruit, herbs, spices or beverages with plant ingredients were allowed during the study. As proteins are known to bind polyphenols, the extract was administered 1 h prior to or at least 2 h after the meals. The diet was supplemented by the tea preparation (1 g *Equisetum arvense L.* extract/250 mL water, equivalent to 0.8 g crude extract) 5 times a day over a period of 3 days. Each cup of tea provided 11 µmol quercetin, 6 µmol kaempferol and 49 µmol caffeic acid. 24 h urine samples were collected and analyzed by HPLC-DAD.

Table 1. Quantification of the administered compounds in the extract.

Compounds	% dry weight
Quercetin-3-0-7-0-diglucoside	0.083
Quercetin-3-0-glucoside	0.488
Quercetin-3-0-(6"-0-malonglucoside)	0.041
Kaempferol-3-0-7-0-diglucoside	0.351
Chlorogenic acid	0.444
Monocaffeoyl-*meso*-tartaric acid	0.926
unknown caffeic acid esters	0.399
Dicaffeoyl-*meso*-tartaric acid	0.655

Results
As a previous experiment showed that no intact flavonoïd glycosides or aglycones could be detected in urine, the analysis focused on the detection of flavonoïd metabolites in urine. 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxytoluene which are described as metabolites of quercetin after oral administration in humans and rats could not be detected ; the amount of excretion of homovanillic acid, which is generally regarded as one of the main quercetin metabolites but is also an endogenous metabolite of catecholamines, was 4 ± 1 mg/d and did not increase significantly. In contrast to quercetin metabolites, most of the metabolites putatively originating from hydroxycinnamic acid esters (ferulic acid, feruloylglycine, dihydroferulic acid, dihydrocaffeic acid, *m*-

hydroxyphenylpropionic acid, and hippuric acid) did show increased excretion. Hippuric acid, the glycine conjugate of benzoic acid and *p*-hydroxyphenylacetic acid increased twofold after 2 days of administration of the extract. Thus the degradation to benzoic acid derivatives rather than phenylacetic acid derivatives seems to be a predominant route of metabolism.

Table 2. Metabolites of flavonoids and hydroxycinnamic acids detected in human urine of 11 volunteers. The percentage of metabolites excreted as glucuronide or sulfate conjugates was determined by enzymatic hydrolysis. The cumulative renal excretion over a period of 5 days is given as the median value. The individual ranges demonstrate the observed variation among human subjects.

Metabolite	% conj.	Putatively originated from (Scheline, 1991):	Cumulative amount [mg] excreted **median** (individual range)
homovanillic acid (**2**)	7	quercetin	**2.1** (0.2–15.6)
m-hydroxyphenylacetic acid (**4**)	n.d.*)	quercetin	**0.5** (0–2.2)
p-hydroxyphenylacetic acid (**7**)	10	kaempferol	**6.9** (0.1–24.7)
ferulic acid (**12**)	> 90	caffeic acid	**2.9** (1.2–38.6)
feruloylglycine (**11**)	16	caffeic acid	**9.1** (4.8–19.8)
dihydroferulic acid (**10**)	60–80	caffeic acid	**3.3** (1.1–45.1)
dihydrocaffeic acid (**8**)	n.d.*)	caffeic acid	**2.0**+)
m-hydroxyphenylpropionic acid (**13**)	60	caffeic acid/quercetin	**3.9** (0.6–5.5)
hippuric acid (**5**)	0	quinic acid	**39.9** (4.4–45.53)

*) not detectable
+) could only be detected in one subject

The extract was repeatedly administered in therapeutically relevant doses to simulate phytotherapeutic conditions. Each cup of tea provided 11 µmol quercetin and 6 µmol kaempferol only. These doses are far below the doses administered in studies in which intact flavonoïds could be detected in plasma or urine (usually ranging from 200 to 400 µmol for a single dose).

Conclusion
After repeated oral administration at therapeutic dosage (5 times a day over 3 days), the main components of *Equisetum arvense L.*, flavonoïd glycosides are rapidly and completely metabolised to benzoic acid derivatives and excreted in urine. It has been demonstrated otherwise that the half-life of quercetin is short, approximately 1-2 h, thus accumulation of flavonoïds is improbable, even after regular intake of a herbal tea (usual recommendation 3 times a day).

5.3. ACUTE TOXICITY

Miwa Y et al. A safety toxicology study of *Equisetum arvense L.* Pharmacometrics. 2009, 76: 61-69 (Japanese)

In an acute oral toxicity study in male and female rats, the test substance at doses of 800, 2000 and 5000 mg/kg bw caused no clinical abnormalities, no body weight changes or mortalities. No animals with any suggestion of toxicity signs were noted at necropsy. Thus it was concluded that single dose administration of *Equisetum arvense L.* at **up to 5000 mg/kg** in rats has no toxicological effects.

5.4. SHORT-TERM TOXICITY

Tago Y et al. Evaluation of the subchronic toxicity of dietary administered Equisetum arvense in F344 rats. J Toxicol Pathol, 2010, 23: 245-251

Materials and methods
Groups of 10 F344 rats (males and females) received diets containing *Equisetum arvense L.* at doses of 0; 0.3; 1 and 3 %, respectively for 13 weeks. The test item consisted of *Equisetum arvense L.* powder extracted with hot water. Dosage selections were based on an estimated intake for humans of 5 mg daily as a supplement; to ensure a safety factor of 100-fold, the 1 % dose was set to feed at an approximate dosage level of 500 mg/kg and 3 and 0.3 % doses were chosen as the high and low dose groups using a common ratio of about 3. The animals were observed daily for clinical signs and mortality. Body weight and food consumption were measured weekly. Fresh urine sample were collected from all animals at week 13. At the end of the experiment, the animals were

euthanazied; blood was taken at sacrifice and all organs were excised and fixed for histopathological examination. All organs and tissues in the control and high dose groups were examined.

Table 3. Intake of *Equisetum arvense L.* during the experiment

	Food consumption (g/rat/day)		Intake of *Equisetum arvense*				Total intake of Equisetum arvense (g/rat)	
			(g/rat/day)		(g/kg bw/day)			
	Male	Female	Male	Female	Male	Female	Male	Female
0%	13.4	8.6	-	-	-	-	-	-
0.3%	14.0	8.5	0.04	0.03	0.18	0.17	3.78	2.30
1%	13.6	8.8	0.14	0.09	0.59	0.60	12.28	7.94
3%	13.5	9.0	0.40	0.27	1.79	1.85	36.44	24.17

Results
No death or obvious clinical signs were noted in any of the animals throughout the experimental period. There was no difference in food consumption. The body weights and cumulative body weight gains in all treatment groups were similar to those of the controls. There were no significant differences among the groups in urinalysis, hematology, or serum biochemistry data and organ weights. No treatment-related macroscopic changes were observed in any of the animals at sacrifice. Microscopic examination revealed no histopathological lesions associated with treatment.

Conclusion
Under the conditions of the study, no adverse effects was observed after administration in the diet, and the **NOAEL** was > 3 % in both genders corresponding to **> 1.79 g/kg bw/day** for males and **>1.85 g/kg bw/day** for females, respectively.

5.5. GENOTOXICITY

In a reverse mutation test, the number of revertant colonies on the plates treated with *Equisetum arvense L.* was not increased for *S. Typhimurium* TA100, TA98, TA1535 or TA1537 or E. Coli WP2uvrA: the test substance was not found to have mutagenic potential.
In a chromosomal aberration test with Chinese hamster lung cells, the incidence of cells with chromosomal aberrations was lower than 5% both by the short treatment method and the continuous treatment method; the test substance was not found to have chromosomal aberration potential.
In the micronucleus test in rats, the incidence of MNPCE was not significantly increased: the test substance was not found to have mutagenicity potential in vivo.

Miwa Y et al. A safety toxicology study of *Equisetum arvense L.* Pharmacometrics, 2009, 76: 61-69 (Japanese)

Conclusion
On the basis of these studies, Equisetum arvense L. can be considered as non genotoxic.

5.6. LONG-TERM TOXICITY

No data.

5.7. REPRODUCTIVE TOXICITY

No data.

5.8. NEUROTOXICITY

No data available, however no neurotoxicity has been reported after administration of *Equisetum arvense L.* in acute or short-term studies.

5.9. TOXICITY STUDIES ON METABOLITES

Equisetum arvense L. being a plant, it can be anticipated that the main metabolites formed in crops or in the environment will be metabolites of compounds commonly found in fruits and vegetables (flavonoïds, tannins); therefore no additional or specific information is required.
Residues in urine are described in this study, were 5 g of dried plants, extracted in 1250 ml were daily absorbed orally for 3 days. Flavonoid glucosides are degraded in common flavonoid metabolites, such as hippuric acid, feruloylglycine, *p*-hydroxyphenylacetic acid, *m*-hydroxyphenylpropionic acid, dihydroferulic acid, ferulic acid, homovanilic acid and *m*-hydroxyphenylacetic acid.

5.10. MEDICAL DATA: ADVERSE EFFECTS REPORTED IN HUMANS

Two cases of adverse effects putatively attributed to *Equisetum arvense L.* are reported in the literature; however it is not clear if *Equisetum arvense L.* is the causing agent of the effects.

A case of allergic dermatitis ascribed to nicotine contained in *Equisetum arvense L.* was reported by Sudan in 1985. A man with a medical history of atopic reactions with nicotine as a hapten in tobacco smoke developed dermatitis of his right hand and of the face, an hour after contact with *Equisetum arvense L.* The clinical signs were similar to those induced after passive inhalation of tobacco, a condition called "seborrhoeic dermatitis". A more rapid reaction which necessitated local application of epinephrine and oral antihistamines was induced at rechallenge with fresh plant.
RMS'comment: According to the author, the nicotine content of *Equisetum arvense L.* amounts about 0.00004%, which could be sufficient to cause an allergic reaction. However this case report is very poorly documented, it dates back to 1985 and nicotine is not currently considered as a sensitising agent. In addition, tobacco smoke contains a large array of compounds that can induce allergy; it is likely that some of them might cross-react with *Equisetum arvense L.* compounds. No other cases of Equisetum induced dermatitis have been reported to date.

A case of autism spectrum disorder in a child potentially caused by prenatal exposure to high doses *Equisetum arvense L.* and alcohol in combination is reported A year prior to conception the mother began a weight loss diet and ingested 1200 mg/day of *Equisetum arvense L.* as an herbal medicine. During the periconceptional stage, the mother ingested approximately 20 to 40g of ethanol per day, while the father ingested around 40 to 60g of ethanol per day during the first nine days of embryonic development. The mother reported a significant weight loss during the pregnancy and a deficiency of B-complex vitamins. *Equisetum arvense L.* contains thiaminase an enzyme that destroys thiamine (vitamin B1), and also caffeic acid, chlorogenic acid, and tannic acid which have been found to interact with thiamine: thus they can oxidize the thiazole ring, rendering it unable to be absorbed; in addition, 2 flavonoïds contained in *Equisetum arvense L.*, quercetin and rutin, have also been described as thiamine antagonists. However no thiamine depletion after *Equisetum arvense L.* use as an herbal medicine has been described in humans. By contrast, it has been reported that up to 9% of children born to mothers that consume alcohol during pregnancy are autistic.
RMS' comment: it is likely that thiamine and folic acid deficiency could have been exacerbated by the 3 risk factors in combination: alcohol intake, weight loss and high doses *Equisetum arvense L.* use during early pregnancy, resulting in neurotoxicity in the child. However no other cases of *Equisetum arvense L.* related thiamine deficiency in humans have been found in the literature.

Ortega-Garcia, J.A. et al 2011, Prenatal exposure of a girl with autism spectrum disorder to 'horsetail' (*Equisetum arvense*) herbal remedy and alcohol: a case report. Journal of Medical Case Reports, 5, 129 Published

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5.11. ADDITIONAL INFORMATION RELATED TO THERAPEUTIC PROPERTIES OR HEALTH CLAIMS

Equisetum arvense L. has a long-standing use as an herbal medicine:

- Uses described in traditional folk medicine include: symptomatic treatment of chronic swelling of the legs, slow-healing sprains and fractures, irritable skin conditions, gout, rheumatism, arthritis, hepatitis, sore throat, dermatological problems and haemorrhoids. In folk medicine *Equisetum arvense L.* is used

as an analgesic, antihypertensive, clotting agent, haemostatic, depurative, astringent, diuretic and anti-inflammatory.

- *Equisetum arvense L.* is included in several pharmacopoeias in Europe and Japan. It is used internally for kidney and bladder diseases, oedema and as an adjuvant in slimming diets. It is applied as irrigation therapy for infectious and inflammatory diseases of the genitourinary tract, and kidney stones and used externally as supportive treatment for slow healing wounds.

The potential pharmacological properties of *Equisetum arvense L.* have been investigated in vitro and in vivo in the last decade such as antinociceptive and anti-inflammatory properties anticonvulsant, hepatoprotective, antimicrobial properties, etc. However these experiments have not yet led to clinical applications in humans.

Do Monte et al. 2004 Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense L.* in mice. Pharmacological research, 49, 239-243. Published

WHO, 2010, monographs on medicinal plants commonly used in the newly independent states, Published

In addition, a lot of research has been undertaken recently to investigate the potential of *Equisetum arvense L.* as a new functional food ingredient with beneficial effects in various clinical conditions and on lipid metabolism. The antioxidant and radical scavenging properties have been especially investigated.

Myagmar B.E., Aniya Y. 2000 Free radical scavenging action of medicinal herbs from Mongolia, Phytomedicine, vol. 7(3), 221-229 Published

Mimica Ducik et al. 2008 Phenolic compounds in field horsetail (Equisetum arvense L.) as natural antioxidants. Molecules, vol. 13, 1455-1464. Published

Equisetum arvense L. is available as a dietary supplement in the United States under the Dietary Supplement Health and Education Act of 1994 (DSHEA).
However the Efsa Panel on Dietetic products failed to establish a relationship between the consumption of *Equisetum arvense L.* and health claims.

Efsa. Scientific opinion on the substantiation of health claims related to *Equisetum arvense L.* and invigoration of the body (ID 2437), maintenance of skin (ID 2438), maintenance of hair (ID 2438), maintenance of bone (ID 2439), and maintenance or achievement of a normal body weight (ID 2783) pursuant to Article 13 of Regulation (EC) No 1924/20061. EFSA Journal 2009; 7(9): 1289

In 2009, the Efsa Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation 1924/2006. The opinion addresses the scientific substantiation of health claims in relation to *Equisetum arvense L.* and invigoration of the body, maintenance of skin, maintenance of hair, maintenance of bone, and maintenance or achievement of a normal body weight.
The Panel considered that *Equisetum arvense L.* has not been sufficiently characterised for the 5 health claimed effects and concluded that a cause and effect relationship has not been established between the consumption of *Equisetum arvense L.* and health claims.

5.12. ADDITIONAL INFORMATION RELATED TO USE AS FOOD

The sporophyte of *Equisetum arvense L.* (tsukushi) is consumed as food in sweetened vinegar, cooked food, and chopped fish, while nutritive caulis (sugina) is well known as Sugina tea in Japan and is drunk as a health drink.

Nagai et al. 2005, Antioxidative activities of water extract and ethanol extract from field horsetail (tsukushi) *Equisetum arvense L.* Food Chem. 91:389–394. Published

5.13. ACCEPTABLE DAILY INTAKE, ACUTE REFERENCE DOSE, ACCEPTABLE OPERATOR EXPOSURE LEVEL

Equisetum arvense L. is considered as a food constituent; it is consumed raw or cooked in several countries or for preparing herbal tea. Setting reference values for *Equisetum arvense L.* is therefore considered as unnecessary.

5.14. IMPACT ON HUMAN AND ANIMAL HEALTH ARISING FROM EXPOSURE TO THE ACTIVE SUBSTANCE OR IMPURITIES CONTAINED IN IT

No plant protection product is associated with *Equisetum arvense L.* as the plant will be used as a decoction with no co-formulant added. Therefore no further testing or additional data is required.
As no reference values have been allocated and are considered as unnecessary, a risk assessment for the operator, bystander or worker is not required.

6. RESIDUES

Equisetum arvense L. is a food constituent, therefore the potential residues in crops and animal products resulting from application of the decoction are considered as negligible regarding other uses. No residue definition is needed. Setting of MRL is not necessary.

7. FATE AND BEHAVIOUR IN THE ENVIRONMENT

The active substance is based on a widespread macrophyte and is a complex mixture of natural components. It is reasonable to consider that these different natural substances are degraded in the environment in accordance with the known metabolic pathways of living organic matter. This biodegradation will lead to simple organic or mineral compounds which are also present in the natural environment. *Equisetum arvense* extracts are totally degradable in fields as major compounds are silica for the inorganic part and natural organic matter for the rest.

EMEA, 2007, Equisetum *arvense* L., Herba, ASSESSMENT REPORT for the DEVELOPMENT of COMMUNITY MONOGRAPHS and for INCLUSION of HERBAL SUBSTANCE(S), PREPARATION(S) or COMBINATIONS THEREOF in the LIST. EMEA/HMPC/394895/2007

It contains about 10 % inorganic constituents with two-third silicic acid (or silicates respectively) of with 10 % are water-soluble. Flavonoids (0.2-0.9 %) are present with mostly caempferol and quercetin glycosides and their malonyl esters. There are apparently two chemotypes. Asian and North American varieties contain luteolin-5-glycoside, which is absent from European plants. Also caffeic acid derivates and small amounts of a styrylpyrone glycoside, polyenic acid, sterols, rare dicarboxylic acids and traces of alkaloids including nicotine have been detected. All these compounds are known in nature and present in plants.
The extract is supposed to be at maximum concentration. The hot water extract made from the dried plant is applied immediately, if not, it is conserved at 5°C. It is used maximum 3 days after the preparation, so degradation occurs rapidly in aerobic conditions.

No further information is required.

8. EFFECTS ON NON-TARGET SPECIES

8.1. EFFECTS ON TERRESTRIAL VERTEBRATES

8.1.1. Birds

Considering the natural origin of the active substance, no toxicity to birds is expected. Moreover, Equisetum sp. can enter the diet of herbivorous birds. As an example, adult trumpeter swans (*Cygnus buccinator*) and their young were found to feed on submerged aquatics and on horsetail (*Equisetum fluviatile* and *E. arvense*) in Alaska. Horsetails were the first emergent macrophytes to be consumed by adults during the incubation period

(up to 60%) and the post-hatching period (up to 80%), and represented more than 80% of the diet of the cygnets, as they are a source of protein.

Todd A. et al. 1994, Feeding ecology of trumpeter swans breeding in central Alaska. Journal of Wildlife Managemen, 58, pp774-780

In addition, isoquercitin a component of *Equisetum arvense L.* extract was recently tested as medication against avian flu virus.

Kim et al. 2010, Inhibition of influenza virus replication by plant-derived isoquercetin. Antiviral Research, 88, pp227-235

No risk assessment for wild birds is therefore deemed necessary.

8.1.2. Mammals

Symptoms of Equisetum poisoning are seen primarily in young, rapidly growing horses, but cases of poisoning have also been reported in cows and sheep. The development of symptoms of Equisetum poisoning initiates lowly. The first signs may be a general, scruffy physical appearance, weight loss (without a particular loss of appetite), diarrhea and slightly uncoordinated movements. If not treated, the disease will progress to a point where the horse will show a loss of muscular control, staggering gait and extreme balance issues. The horse is prone to become uneasy and nervous due to its inability to control muscle movement. It may lie down and not be able to get up, may seizure, and may ultimately die from exhaustion within approximately 1– 2 weeks

Bebbington A. 2007 TOXICITY OF EQUISETUM TO HORSES Fact Sheet, Ministry of Agriculture Food and Rural affairs, Ontario http://www.omafra.gov.on.ca/english/livestock/horses/facts/07-037.pdf

The presence of *Equisetum* in pasture is not a primary concern, as consumption at pasture is usually limited by the plant's high silicate content and the abundance of other palatable forage options. However, ingestion of contaminated hay can result in poisoning.

8.2. EFFECTS ON AQUATIC ORGANISMS

Equisetum sp are widely represented in natural wetlands. Some of the representative species, such as *Equisetum fluviatile*, can be the aquatic macrophytes the most consumed by omnivorous fish such as *Rutilus rutilus* and *Leusiscus idus* (Brabrand, 1985). The use of *Equisetum arvense L.* is therefore not expected to have any adverse impact on aquatic ecosystems, and no risk assessment is deemed necessary.

Braband A. 1985, Food of roach (*Rutilus rutilus*) and ide (*Leuciscus idus*): Significance of diet shift for interspecific competition among omnivorous fish. Oecologia, 66:461-467

8.3. EFFECTS ON BEES AND OTHER ARTHROPODS SPECIES

8.3.1. Effects on bees

A project has been conducted in France in order to determine the contact acute toxicity for bees of natural preparations from plants, including one with *Equisetum arvense L.*The bees were sprayed using a Potter tower in order to mimic the treatment of the crops. Several spray concentrations were tested corresponding to the application rate, twice the application rate and dilutions of the application rate. For bees treated with *Equisetum arvense L.* horsetail, the mortality after 4 days never exceeded 4%, and no significant difference compared to the control was observed.

CASDAR 2009, Evaluation des caractéristiques et de l'intérêt agronomique de préparations simples de plantes, pour des productions fruitières, légumières et viticoles économes en intrants. AAP CAS DAR 2009, n° 9046.

The use of *Equisetum arvense L.* is not expected to have any adverse impact on bee colonies, and no risk assessment is deemed necessary.

8.3.2. Effects on other arthropods

Paynter Q. et al 2008 Prospects for biological control of field horsetail *Equisetum arvense* L. in New Zealand. Landcare Research Contract Report LC0708/100

We found records of 38 arthropod species that feed on field horsetail, of which some are clearly unsuitable for use as biological control agents on the basis of inadequate host- specificity (Appendix 2). However, according to literature host-records, 26 species are apparently sufficiently host-specific to be considered for use against field horsetail in New Zealand. We examine, below, whether this shortlist can be further refined, based on the likelihood that an agent will be sufficiently specific and damaging.

Considering the natural origin of the active substance and the feeding activity of arthropods on *Equisetum arvense*, no further information concerning other arthropods is required, and no risk assessment is deemed necessary.

8.4. EFFECTS ON EARTHWORMS AND OTHER SOIL MACRO-ORGANISMS

Considering the natural origin of the active substance, no specific information concerning the toxicity for soil macro-organisms is required. Moreover, a study conducted on nematode populations in abandoned fields including some that were invaded by *Equisetum* plants, shoved no differences on number of species, biomass and number of individuals compared to populations from oak forests. No risk assessment is deemed necessary.

Hanel A. 2010, An outline of soil nematode succession on abandoned fields in South Bohemia. Applied Soil Ecology, 46, pp355–371.

8.5. EFFECTS ON SOIL MICRO-ORGANISMS

Considering the natural origin of the active substance, no specific information concerning the impact on soil micro-organisms is required.

8.6. EFFECTS ON OTHER NON-TARGET ORGANISMS (FLORA AND FAUNA)

E. arvense used in mulches or organic amendments was observed to be slightly active on weed.

Hogue E.J. 2005, Improving Crop Yield and Soil Quality with Mulches, Organic Amendments and Cover Crops. Final Report WTFRC Project # AH-02-214

Favourable results with the use of the *Equisetum arvense* (field horsetail) for in-row weed control indicates potential for native allelopathic and non-allelopathic species as living mulches.

8.7. EFFECTS ON BIOLOGICAL METHODS OF SEWAGE TREATMENT

Not applicable.
This biodegradation will lead to simple organic or mineral compounds which are also present in the natural environment. *Equisetum arvense* extracts are totally degradable in fields as major compounds are silica for the inorganic part and natural organic matter for the rest.

9. OVERALL CONCLUSIONS WITH RESPECT OF ELIGIBILITY OF THE SUBSTANCE TO BE APPROVED AS BASIC SUBSTANCE

Describe in synthesis fulfilment of criteria

(a) is not a substance of concern; and

(b) does not have an inherent capacity to cause endocrine disrupting, neurotoxic or immunotoxic effects; and

(c) is not predominantly used for plant protection purposes but nevertheless is useful in plant protection either directly or in a product consisting of the substance and a simple diluent; and

(d) is not placed on the market as a plant protection product.

Equisetum arvense L. is a plant which can be fully characterized according to the methods of the European Pharmacopoeia.

Equisetum arvense L. has an extremely low toxicological profile:

- Oral LD50 in rats > 5000 mg/kg bw;
- A NOAEL > 1850 mg/kg bw/d was set in a 90-day oral study in rats;
- It is not genotoxic *in vitro* or *in vivo*;
- It is not known to cause endocrine disrupting, neurotoxic or immunotoxic effects;
- Although it has a long-standing use as a traditional herbal medicine, no clear adverse effects have been reported;
- *Equisetum arvense L.* is consumed as food in Japan;
- As a naturally occurring and widespread plant, no specific information concerning neither its fate and behaviour in the environment nor its toxicity for wild animals is required. Moreover, data showing its lack of toxicity for birds, fish, bees and nematods were found in the literature;
- It does not give rise to residues of concern in crops, animal products or in the environment;
-

Therefore it is not considered as a substance of concern.

Equisetum arvense L. is not predominantly used for plant protection purposes but is used as an elicitor in plant protection, as a decoction.

Equisetum arvense L., as a plant is not placed on the market as a plant protection product.*Equisetum arvense L.* is commonly consumed in Japan as food in sweetened vinegar, cooked food, and chopped fish, while nutritive caulis (sugina) is well known as Sugina tea in Japan and is drunk as a health drink. *Equisetum arvense L.* is available as a dietary supplement in the United States under the Dietary Supplement Health and Education Act of 1994 (DSHEA).
Equisetum arvense L. fulfils the criteria of a 'foodstuff' as defined in Article 2 of Regulation (EC) No 178/2002, therefore it shall be considered as a basic substance.

The RMS is of the opinion that all criteria for considering *Equisetum arvense L.* as a basic substance are fulfilled.

ANNEX I LIST REFERENCES RELIED ON

Include here all references studies and assessment reports cited in the various chapter of application model.

Author(s)	Year	Title Source Company, report N° GLP or GEP status Published or not
SECTION 1: Purpose of the application		

Author(s)	Year	Title Source Company, report N° GLP or GEP status Published or not
SECTION 2: Identity of the substance/product as available on the market and predominant use		
Company MARTIN BAUER S.p.A.	2008	**Company**, **report** of the quality and conformity certificate of the active substance *Equisetum arvense*. PPM002673 01/09/2008
Company MARTIN BAUER S.p.A.	2008	**Company**, **report** of the quality and conformity certificate of the active substance *Equisetum arvense*. PPM0021084 12/11/2008
Heber D.	2004	Title: Horsetail,*Equisetum arvense* Source: PDR for Herbal Medecines, third edition, Montyale (NJ). ISBN 1-56363-512-7
Currie H. A.	2009	Title: Chemical evidence for intrinsic 'Si' within Equisetum cell walls Source: Phytochemistry 70 () 2089–2095
Currie H. A.	2007	Title: Silica in Plants: Biological, Biochemical and Chemical Studies Source: Annals of Botany 100: 1383–1389
Asgarpanah J. et al.	2012	Title: Phytochemistry and pharmacological properties of *Equisetum arvense* L. Source: Journal of Medicinal Plants Research Vol. 6(21), pp. 3689-3693
European Pharmacopoeia	2008	Title: Equisetum Stem, Equiseti Herba Source: European Pharmacopoeia 6.0., 01/2008:1825.

Author(s)	Year	Title Source Company, report N° GLP or GEP status Published or not
SECTION 3 : Uses of the substance and its product		
Reynolds A. G. et al.	1996	Title: Use of Potassium Silicate for the Control of Powdery Mildew [*Uncinula necator* (Schwein) Burrill] in *Vitis vinifera* L. Cultivar Bacchus. Source: Am. J. Enol. Vitic., Vol. 47, No. 4, p421-428
Dagostin S. et al.	2011	Title: Are there alternatives to copper for controlling grapevine downy mildew in organic viticulture? Source: Crop Protection 30, 776-788
Daiana Garcia E. et al.	2011	Title: Mould growth and mycotoxin production as affected by Equisetum arvense and Stevia rebaudiana extracts Source: Food Control 22 (2011) 1378-1384
Cherif M et al.	1992	Title: Silicon induced resistance in cucumber plants against Pythium ultimum. Source: Physiological and Molecular Plant Pathology, 41(6):411-425
Fauteux F.	2005	Title: Silicon and plant disease resistance against pathogenic fungi. Source: FEMS Microbiology Letters 249 1-6
Fauteux F.	2006	Title: The protective role of silicon in the Arabidopsis–powdery mildew pathosystem. Source: PNAS vol. 103 _ no. 46 17554–17559

Kuepper G.	2004	Title: ORGANIC ALTERNATIVES FOR LATE BLIGHT CONTROL IN POTATOES, Source: PEST MANAGEMENT TECHNICAL NOTE, p1-8
Epstein E.	2009	Title: Silicon: its manifold roles in plants Source: Annals of Applied Biology ISSN 0003-4746, p1-6
Radulovic N.	2006	Title: Composition and Antimicrobial Activity of Equisetum arvense L. Essential Oil Source: Phytother. Res. 20, 85–88
Wolff S.A.	2012	Title: Foliar applications of silicon fertilisers inhibit powdery mildew development in greenhouse cucumber. Source: Journal of Food, Agriculture & Environment Vol.10 (1): 355-359.
Ondet S.J. et al.	2011	Title: Stratégie de maîtrise de la Cloque du pêcher par phytothérapie GRAB, in Source: CASDAR 2009, Evaluation des caractéristiques et de l'intérêt agronomique de préparations simples de plantes, pour des productions fruitières, légumières et viticoles économes en intrants. AAP CAS DAR 2009, n° 9046
Pathak R. K. et al.	-	Title: BIODYNAMIC PRODUCTION OF FRUIT AND VEGETABLES AT CISH, LUCKNOW, Source: Presentation at FAO, from Central Institute for Subtropical Horticulture Rehmankhera, Lucknow- 227 107, India
Heine G. et al.	2007	Title: The effect of silicon on the infection by and spread of *Pythium aphanidermatum* in single roots of tomato and bitter gourd. Source: Journal of Experimental Botany, Vol. 58, No. 3, pp. 569–577

Author(s)	**Year**	**Title** **Source** **Company, report N°** **GLP or GEP status** **Published or not**
SECTION 4: Classification and labelling of the substance		
		Title: Source:

Author(s)	**Year**	**Title** **Source** **Company, report N°** **GLP or GEP status** **Published or not**
SECTION 5 : Impact on human and animal health		
Asgarpanah J. et al.	2012	Title: Phytochemistry and pharmacological properties of *Equisetum arvense* L. Source: Journal of Medicinal Plants Research Vol. 6(21), pp. 3689-3693
EMEA	2007	Title : Equisetum *arvense* L., Herba Source: ASSESSMENT REPORT for the DEVELOPMENT of COMMUNITY MONOGRAPHS and for INCLUSION of HERBAL SUBSTANCE(S), PREPARATION(S) or COMBINATIONS THEREOF in the LIST. EMEA/HMPC/394895/2007
Maeda H.	1997	Title: Occurrence of dermatitis in rats fed a cholesterol diet containing field horsetail (*Equisetum arvense* L.). Source: J Nutr Sci Vitaminol. 43(5):553-63
Sandhu N.S.	2010	Title: Pharmacognostic Evaluation Of *Equisetum arvense* Linn. Source: International Journal of PharmTech Research Vol.2, No.2, pp 1460-1464
Sandhu N.S.	2010	**Title:** EQUISETUM ARVENSE: PHARMACOLOGY AND PHYTOCHEMISTRY - A REVIEW. **Source:** Asian Journal of Pharmaceutical and Clinical Research Vol. 3, Issue 3, 146-150
Graefe E.U. and Veit M.	1999	Title: Urinary metabolites of flavonoids and hydroxycinnamic acids in humans after application of a crude extract from *Equisetum* arvense. Source: Phytomedecine, vol6 (4) pp 239-246.
Miwa Y et al.	2009	Title: A safety toxicology study of *Equisetum arvense* L Source: Pharmacometrics, , 76, pp 61-69
Tago Y.	2010	Evaluation of the subchronic toxicity of dietary administered *Equisetum arvense* in F344

et al.		rats. J Toxicol Pathol, 23: 245-251
Ortega-Garcia, J.A. et al	2011	Title: Prenatal exposure of a girl with autism spectrum disorder to 'horsetail' (*Equisetum arvense*) herbal remedy and alcohol: a case report. Source: Journal of Medical Case Reports, **5**, 129
Do Monte et al.	2004	Title: Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense L.* in mice. Source: Pharmacological research, 49, 239-243
WHO	2010	Title: monographs on medicinal plants commonly used in the newly independent states.
Myagmar B.E. Aniya Y.	2000	Title: Free radical scavenging action of medicinal herbs from Mongolia Source: Phytomedicine, vol. 7(3), 221-229
Mimica Ducik et al.	2008	Title: Phenolic compounds in field horsetail (*Equisetum arvense L.*) as natural antioxidants. Source: Molecules, vol. 13, 1455-1464
EFSA	2009	Title: Scientific opinion on the substantiation of health claims related to *Equisetum arvense L.* and invigoration of the body (ID 2437), maintenance of skin (ID 2438), maintenance of hair (ID 2438), maintenance of bone (ID 2439), and maintenance or achievement of a normal body weight (ID 2783) pursuant to Article 13 of Regulation (EC) No 1924/20061 Source: EFSA Journal; **7**(9), p 1289
Nagai et al.	2005	Title: Antioxidative activities of water extract and ethanol extract from field horsetail (tsukushi) Source: *Equisetum arvense L.* Food Chem. (2005) 91:389–394

Author(s)	**Year**	**Title** **Source** **Company, report N°** **GLP or GEP status** **Published or not**
SECTION 6 : Residues		
		Title: Source:

Author(s)	**Year**	**Title** **Source** **Company, report N°** **GLP or GEP status** **Published or not**
SECTION 7 : Fate and Behaviour in the environment		
EMEA	2007	Title : Equisetum arvense L., Herba Source: ASSESSMENT REPORT for the DEVELOPMENT of COMMUNITY MONOGRAPHS and for INCLUSION of HERBAL SUBSTANCE(S), PREPARATION(S) or COMBINATIONS THEREOF in the LIST. EMEA/HMPC/394895/2007

Author(s)	**Year**	**Title** **Source** **Company, report N°** **GLP or GEP status** **Published or not**
SECTION 8 : Effects on non-target species		
Todd A. et al.	1994	Title: Feeding ecology of trumpeter swans breeding in central Alaska. Source: Journal of Wildlife Management 1994, 58, pp774-780.
Kim et al.	2010	Title: Inhibition of influenza virus replication by plant-derived isoquercetin Source: Antiviral Research, 2010, 88, pp227-235
Bebbington A. et al	2007	Title: TOXICITY OF EQUISETUM TO HORSES Source: Fact Sheet, Ministry of Agriculture Food and Rural affairs, Ontario http://www.omafra.gov.on.ca/english/livestock/horses/facts/07-037.pdf

Braband A.	1985	Title: Food of roach (*Rutilus rutilus*) and ide (*Leuciscus idus*): Significance of diet shift for interspecific competition among omnivorous fish. Source: Oecologia 1985, 66:461-467.
CASDAR	2009	Title: Evaluation des caractéristiques et de l'intérêt agronomique de préparations simples de plantes, pour des productions fruitières, légumières et viticoles économes en intrants Source: AAP CAS DAR 2009, n° 9046.
Paynter Q. et al	2008	Title: Prospects for biological control of field horsetail *Equisetum arvense* L. in New Zealand. Source: Landcare Research Contract Report LC0708/100
Hanel A.	2010	Title: An outline of soil nematode succession on abandoned fields in South Bohemia Source: Applied Soil Ecology 2010, 46, pp355–371.
Hogue E.J.	2005	Title: Improving Crop Yield and Soil Quality with Mulches, Organic Amendments and Cover Crops Source: Final Report WTFRC Project # AH-02-214