European Medicines Agency
Post-authorisation Evaluation of Medicines for Human Use

London, 6 March 2008
Doc. Ref: EMEA/HMPC/104918/2006

COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

Final

ASSESSMENT REPORT ON *ECHINACEA PURPUREA* (L.) MOENCH., HERBA RECENS

Herbal substance(s)	*Echinacea purpurea* (L.) Moench., herba recens, succus, succus siccum,
Rapporteur	Prof. Dr. Samo Kreft, M.Pharm

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 75 23 70 51
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

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1 Introduction

Assessment report reviews the scientific data, particularly the pharmacological and clinical data, available for the *Echinacea purpurea* (L.) Moench (syn: *Rudbekia purpurea* L.), herba and the herbal medicinal products thereof.

The monograph in European Pharmacopoeia defines the herbal substance "Purple coneflower herb" as:
Dried, whole or cut flowering aerial parts of *Echinacea purpurea* (L.) Moench.
Content: minimum 0.1 per cent for the sum of caftaric acid ($C_{13}H_{12}O_9$; M_r 312.2) and cichoric acid $C_{22}H_{18}O_{12}$; M_r 474.3) (dried drug).

US Pharmacopeia contains the monograph "*Echinacea purpurea* Aerial Parts", which defines the drug as: "consists of the aerial parts of *Echinacea purpurea* (L.) Moench (Fam. Asteraceae). It is harvested during the flowering stage. It contains not less than 1.0 percent of chichoric acid, and not less than 0.01 percent of dodecatetraenoic acid isobutylamides ($C_{16}H_{25}NO$) on the dry basis." (Giancaspro 2004)

There is no pharmacopoeial monograph for the fresh plant.

Preparations from plant parts of *Echinacea* species rank among the medicines longest used in the American people's medicine. *Echinacea* was well known for a long time by the Indian tribes in Nebraska and Missouri. The immunostimulating effect was later also used by the European immigrants. At the beginning of the 20th century, *Echinacea* was the best-selling American medical plant in the United States (Foster 1996). In the lasts decades *Echinacea*-containing medicinal products became among the most popular medicinal products in Europe.
There are three species of *Echinacea* mostly used in phytotherapy: *E. purpurea, E. angustifolia* and *E. palida*, but there are also some reports of the use of *E. simulata* and *E. paradoxa* (Bauer & Foster 1991).
E. purpurea is sometimes adulterated with other *Echinacea* species (HagerROM 2004) and quite often mislabelled (Gilroy et al. 2003). Much of the early research reported for *E. angustifolia* and *E. purpurea* was probably actually conducted on *E. pallida* and studies published prior to 1987 must be viewed with suspicion in terms of the actual species being evaluated (Bauer et al. 1988c).

Information about the legal status of products containing *Echinacea purpurea*, herba in Member States (and Associate Members and Observer States):

Member state	products, indications	Legal status
Austria	- Echinacea “Bioforce” drops (Aponova Pharma) - Echinacea “ratiopharm”tablets (Ratiopharm Arzneimittel) - Echinacin “Madaus” Capsetten oral gum (Madaus) - Echinacin “Madaus” pressed juice (Madaus) - Echinacin “Madaus” tablets (Madaus) - Echinacin “Madaus” drops (Madaus) - Sanvita Imun-Solution (Sanamed) **Indications:** Internal use: Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common colds) and also of the urogenital tract. External use: Semi-solid preparations with a minimum of 15% of pressed juice. Dermal wound-healing, after vaccination, insect bites. **Posology** *Echinacea purpureae* herba Internal use: Adult daily dose: 6-9 ml of pressed juice; other equivalent preparations at comparable dosage; Children: Proportion of adult dose according to age or body weight.	authorised 1991 authorised 2004 authorised 1998 authorised 2000 authorised 2004 authorised 1994
Belgium	Medicinal product: Oral solution **Indication:** Upper respiratory tract infections (serious pathologies excluded) **Posology:** 800mg juice /g; -adults : 2.5 ml + 1.25 ml after 2 hours, then 3 times 2.5 ml per day; maximum 15 ml/day -2 to 12 years : 1 drop per kg bw per day -below 2 years: prescription only. *E. purpurea* extract registered as homeopatic mother tincture. Food supplement Oral solution, capsules, tablets, syrup, buccal spray Claims on packaging: improved breathing, favourable influence on throat No clear compositions or unambiguous posology available. Amounts of variable markers are declared, such as echinacoside, echinacin, echinacein, phytosterols, chicoric acid, polysaccharides	April 2000 From January 1990 onwards
Czech Republic	4 oral and 1 topical preparation. (DR. RENTSCHLER, Germany; LEK, Slovenia; 3x MADAUS, Germany)	

Finland	five products containing *E. purpurea* on a market. Pharmaceutical forms: oral drops, solution (three products), tablet (one product) and lozenge (one product). **Indications**: for one product it is 'To relieve symptoms of cold.' and for other two products it is 'For symptoms of cold, temporary cough and irritation of throat. To treat small wounds.' Indication for lozenges and tablets is 'To relieve symptoms of cold.' **Posology**: Varies from product to product. For two oral drop products the posology is 'For internal use with water 10-20 drops 2-5 times daily. For small wounds 1-2 drops/few drops several times a day into the wound.' For the third oral drop product the posology is '40 drops 3 times daily.' For lozenges the posology is '1 lozenge 3 times daily' and for tablets 'For adults with water 2 tablets 3-5 times daily about 15 minutes before meals.'	Two products have been registered first time in 1990 and 1991 (two oral drop products) and the marketing authorisations have been granted for two products in 1996 (oral drops and lozenges) and for one product in 1997 (tablets).
France	No authorized herbal medicinal products containing *Echinacea* are on the market.	No
Germany	85 herbal medicinal products containing *E. purpurea* are on the market. They exist in various pharmaceutical forms for oral use: syrup, oral liquid (expressed juice), effervescent tablet, oral gum, soft capsule, tablet, film-coated tablet, coated tablet. **Indications**: Adjuvant in (frequently occuring) recurrent respiratory tract infections and/or urinary tract infections. **Posology:** according to Komission E. One product for external use. **Indication**: Herbal medicinal product traditionally used as mild acting adjuvant in wound healing. Echinacin® Salbe Madaus: 100 g contains 16 g of expressed juice	Marketing authorization Marketing authorization for traditional use (according to German Drug Law)
Hungary	4 oral and 1 topical preparation. **Indication:** Prevention and adjuvant therapy of common cold and influenza; Prophylaxis and adjuvant therapy of recurrent respiratory and urogenital infection; Adjuvant in wound healing.	First oral preparation first registered in 1992, topical preparation first registered in 2000
Ireland	No products containing *Echinacea* currently authorised.	

Italy	no medicinal products containing only *Echinacea* as active substance are currently authorised. Food supplements are on the market: Pharmaceutical forms: liquid form, tablets, capsules, syrup, spray. Part of plant used in the preparation: leaves, apical flowers, stalk, root, whole plant. **Indications:** may be helpful during the cold season it may be helpful to enhance natural defenses during the cold season. **Posology:** it varies according to the different products, and to the part of the plant used in the preparation. Apical flowers: the range goes from 25 mg to 144 mg Whole plant: from 0.14 mg to 7,500 mg Leaves: from 20 mg to 450 mg Root: from 3 mg to 2,430 mg Leaves + root: 4 mg Leaves + stalk: 200 mg Methods of administration: oral.	No
Latvia	1. Echinacea – Ratiopharm 100 mg, Lutschtabletten. Herba Echinaceae purpureae. Ratiopharm GmbH, Germany. ATC code: V03AX. 2.Echinacis Madaus Capsetten, Lozenges. Herba Echinaceae purpureae. Madaus AG, Germany. ATC code: V03AX. 3.Echinacis Madaus Liquidum, Liquidum. Herba Echinaceae purpureae. Madaus AG, Germany. ATC code: V03AX. 4.Futura Echinacea, Chewable tablets. Extr. Echinaceae purpureae. Dansk Droge A/S, Denmark. ATC code:V03AX. 5.Immunal tablets, Tablets 80 mg. Herba, succus Echinaceae purpureae L. siccum. Lek Pharmaceuticals d.d., Slovenia. ATC code: V03AX. 6.Immunal, Solution (peroral use). Herba, succus Echinaceae purpureae L. siccum. Lek Pharmaceuticals d.d., Slovenia. ATC code: L03A. 7.Tinctura Echinaceae, Tinctura. Rhizomata et radices Echinaceae. JSC “Riga Pharmaceutical Plant”, Latvia. ATC code: L03A. Products containing *E. purpurea* are also used as food supplements.	Marketing authorization

Poland	1) Echinaceae purpureae herbae succus sicc. 2) Echinaceae purpureae herbae extractum 3) Echinaceae purpureae herbae extractum spissum 4) Echinaceae purpureae herbae extractum 5) Echinaceae purpureae herbae extractum 6) Echinaceae purpureae herbae succus 7) Echinaceae purpureae herbae extractum siccum (4.5-3.5:1); water Pharmaceutical Form 1) tablets, 100mg 2) ointment, 50mg/g 3) coated tablets, 100mg 4) oral drops, 800mg/ml 5) tablets 6) oral drops 7) tablets, 100mg **Indications:** 1) Supplementory in upper airways infections. After medical consultation also in urinary tract infections. 2) traditionally in troubles with small wound healing 3) Recurrent upper airways infections (common cold), therapy and prophylaxis. 4) Supplementory in recurrent upper airways infections. Prophylaxis of common cold. 5) Supplementory in upper airways infections. Prophylaxis against common cold. 6) Common cold. Recurrent infections of upper airways. 7) Common cold. Supplementory in upper airways cattarhs and sinusitis.	1) 2000 2) 1994 3) 1999 4) 1993 5) 2003 6) 1999 7) 2004
Portugal	*Echinacea purpurea* extract - 16g /100g ointment, **Indications**: Inflammatory conditions of the skin (eczema), burns. (two products) In combination: *Pygeum africanum* extract – 25 mg *Sabal serrulata* dry extract – 200 mg *Echinacea angustifolia* dry extract – 100 mg Tablets, **Indications**: Prostatic hypertrophy at a early stage and urinary disturbances. Trospium chloride – 60 mg *Echinacea angustifolia* extract – 60 mg *Sabal serrulata* extract – 60 mg Capsules, Indications: Urinary Antispasmodic	Marketing authorization at 1996 and 1997, respectively. Marketing authorization at 1987 Marketing authorization at 1988

Romania	ADDITIVA ECHINACEA effervescent tablet containing 125 mg dried pressed juice (38-56:1) of *E. purpurea* fresh herb. **Indications**: immunity stimulation,support treatment for recurrent respiratory infections. Posology and administration: Adults: one effervescent tablet (dissolved in 150 ml water)-3 times daily.	
Slovenia	3 herbal medicinal products containing *E. purpurea* are on the market. One of the products (Echinaforce, Bioforce) contains also 5% of *E. purpurea* root. **Indications**: to increase the defence capacity of the body; for prevention and quicker recovery from cold and influenza; to relieve the symptoms of disease in mild viral and bacterial respiratory tract infections (runny nose, sore throat, coughing, fatigue); in long-term antibiotic treatment of chronic infections leading to a weakening of patient's cellular immune reaction. Food supplements are on the market	Marketing authorization. One of the products (Immunal, Lek) is on the market for more than 20 years.
Sweden	a) ethanol extract (1:3), b) ethanol/water (62-70%) extract, herb (1:12), root (1:11) c) dry ethanol/water (57.3%) extract corresponding to fresh herb 140 mg, fresh root 8 mg d) fresh herb, dried pressed juice 1 ml corresponding to 2 g herb e) fresh herb, pressed juice 160 mg/g ointment **Indications:** ad a) – d) Traditionally used for the relief of cold symptoms. ad e) Traditionally used for treatment of sores on the lips and on other small superficial wounds such as chapping in the corner of the mouth or on the fingertips. Risks are known.	ad a) 2004 ad b) before 1978 ad c) 2004 ad d) 1995 ad e) before 1984

2 Pharmacology

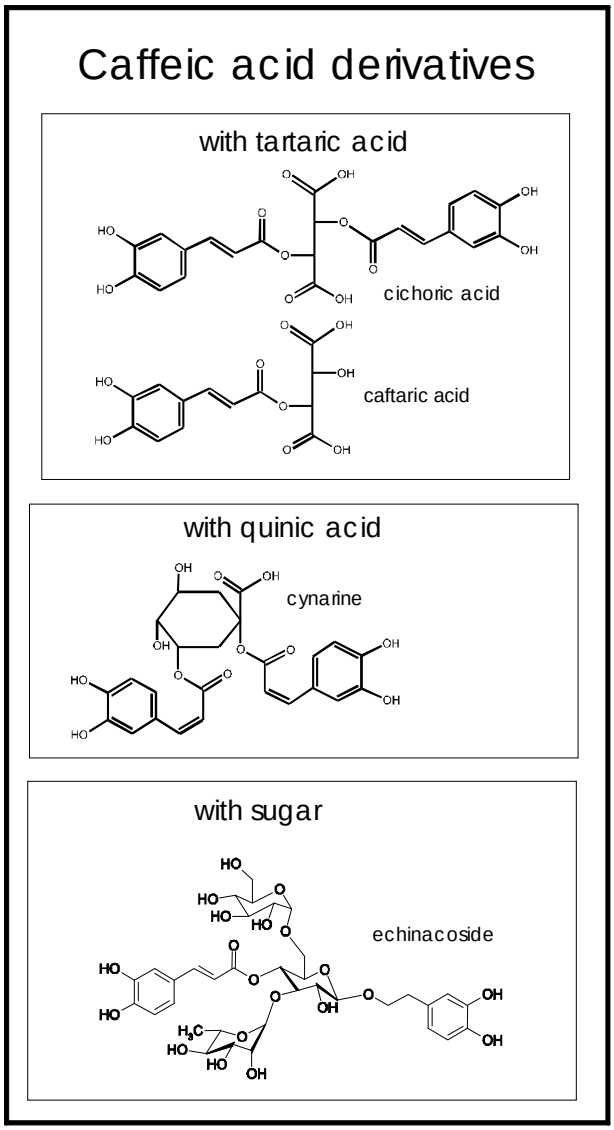
2.1 Pharmacokinetics

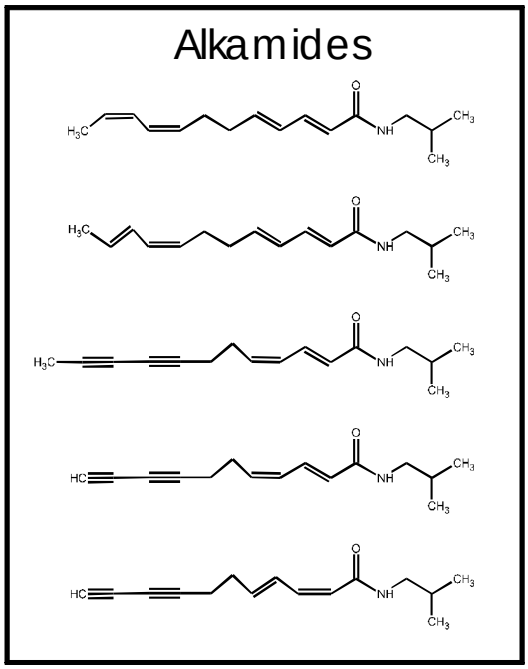
2.1.1 Phytochemical characterisation

The search for active substances of *Echinacea* is not finished. At present mainly three groups of constituents are regarded as effective principles (ESCOP 2003): caffeic acid derivatives, alkamides, polysaccharides. Melanins were recently purposed as a new active constituent of *Echinacea purpurea* (Pasco et al. 2005, Pugh et al. 2005).

The caffeic ester derivative cichoric acid (2,3-O-dicaffeoyl-tartaric acid) is the major compound of this class found in the aerial parts of *Echinacea purpurea* with a concentration range of 1 to 5%, followed by caftaric acid (2-O-caffeoyl-tartaric acid) (Kreft 2005; Manček & Kreft 2005; Bauer et al. 1988b). Comercial product can contain as little as 0.13 mg/g of cichoric acid and 0.14 mg/g of caftaric acid (Gotti et al. 2002). Other derivatives like 2-O-feruloyl-tartaric acid and 2-O-caffeoyl-3-coumaroyl-

tartaric acid are present in small quantities. Cynarine and echinacoside are characteristic for other *Echinacea* species and are practically not present in the aerial pars of *Echinacea purpurea* (Gotti et al. 2002).
Further characteristic constituents are a series of alkamides with the isomeric dodeca-2E,4E-8Z,10E/Z-tetraenoic acid isobutylamide as main compound.
The volatile oil (0.08–0.32%) contains, among other compounds, borneol, bornyl acetate, pentadeca-8-(Z)-en-2-one, germacrene D, caryophyllene and caryophyllene epoxide. Polysaccharides (PS) such as PS I, a 4-O-methyl-glucuronoarabinoxylan with an average MW of 35,000 D, and PS II, an acidic arabinorhamnogalactan of MW 450,000 D, have been isolated from *Echinacea purpurea* herb. A xyloglucan (MW 79,500 D) has also been isolated from the herb and a pectin-like polysaccharide from the expressed juice.





Poysaccharides

4-O-methyl-glucurono-arabino-xilan (m.w. 35 000)

Arabino-rhamno-galactan (m.w. 450 000)

2.1.2 Absorption, metabolism and excretion

So far no *in vivo* pharmacokinetic investigations were performed on the *Echinacea purpurea* expressed juice. A reason can be found in the fact that the expressed juice is a mixture of many substances. Pharmacokinetic of caffeic acid and related hydroxycinnamates (Bourne & Rice-Evans 1998; Westendorf & Czok 1978) and of alkamides was studied *in vivo* and *ex vivo* (in cell cultures).

Absorption of alkamides after oral application of *E. purpurea* was studied (Dietz et al. 2001). One hour after oral application of 65 ml of *Echinacea purpurea* concentrated tincture, containing 4.3 mg of dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides, it was possible to detect 44 ng of this alkamide per ml of human blood. An HPLC quantification method has been developed. Since the

volume of distribution of the substance in the body is at least 5 litre, it can be calculated that at least 5% of alkamide was absorbed one hour after oral application.

Matthias et al. (2005a) have examined serial plasma samples from 9 healthy volunteers who ingested tablets manufactured from ethanolic liquid extracts of *Echinacea angustifolia* and *Echinacea purpurea* immediately after a standard high fat breakfast. Caffeic acid conjugates could not be identified in any plasma sample at any time after tablet ingestion. Alkamides were rapidly absorbed and were measurable in plasma 20 min after tablet ingestion and remained detectable for up to 12 h. Concentration-time curves for 2,4-diene and 2-ene alkamides were determined. The maximal concentrations for the sum of alkamides in human plasma were reached within 2.3 h post ingestion and averaged 336 +/- 131 ng eq/ml plasma. No obvious differences were observed in the pharmacokinetics in 2 additional fasted subjects. This single dose study provides evidence that alkamides are orally available and that their pharmacokinetics are in agreement with the one dose three times daily regimen already recommended for *Echinacea*.

Studies of transport of alkamides trough a cultured monolayer of colonic cells (Jager et al. 2000) were performed on human adenocarcinoma colonic cell line Caco-2 (ATCC) as a model to assess the epithelial transport of dodeca-2 E,4 E,8 Z,10 E/ Z-tetraenoic acid isobutylamides. 30 minutes after apical loading of 25 microg/ml, about 15% of these alkamides were detectable on the basolateral side. Close monitoring of the transport during 6 hours revealed a nearly complete transport to the basolateral side after 4 hours and no significant metabolism was observable. Transport experiments performed at 4^{o}C showed only a slight decrease in transport, which is a strong hint that dodeca-2 E,4 E,8 Z,10 E/ Z-tetraenoic acid isobutylamides cross biological membranes by passive diffusion. Nearly the same results were obtained after preincubation of the Caco-2 cells with lipopolysaccharides (LPS) or phorbol 12-myristate-13-acetate (PMA) to mimic an inflammatory status. These results support the assumption that the alkamides can be easily transported from the intestinum and hence may contribute to the *in vivo* effects of *Echinacea* preparations.

Permeability of alkylamides and caffeic acid conjugates trough Caco-2 cell monolayer model was studied again in 2004 (Matthias et al. 2004). Caffeic acid conjugates (caftaric acid, echinacosides and cichoric acid) permeated poorly through the Caco-2 monolayers although their potential metabolite, cinnamic acid, diffused readily with apparent permeability of 10^{-4} cm/s. Alkylamides were found to diffuse with apparent permeability ranging from $3* 10^{-6}$ to $3* 10^{-4}$ cm/s. Compounds with apparent permeability is considered to $> 1* 10^{-6}$, are considered to have almost complete intestinal absorption.

Alkamide content in plasma samples obtained from a randomized, open, single-dose, crossover study after oral administration of a 60% ethanolic extract from the roots of *E. angustifolia* to 11 healthy subjects was analysed by liquid chromatography electrospray ionization ion-trap mass spectrometry (Woelkart et al. 2005). The maximum concentration of dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides, the main alkamides in the roots of *E. angustifolia*, appeared already after 30 minutes and was 10.88 ng/ml for the 2.5-ml dose.

Matthias et al. (2005b) have investigated the metabolism of the alkylamides by human liver microsomes. No significant degradation of alkylamides was evident in cytosolic fractions. Time- and NADPH-dependent degradation of alkylamides was observed in microsomal fractions suggesting they are metabolised by cytochrome P450 (P450) enzymes in human liver. Pure synthetic 2-ene alkylamides inhibited the degradation of 2,4-diene alkylamides. These findings suggest that *Echinacea* may affect the P450-mediated metabolism of other concurrently ingested pharmaceuticals.

The dose-dependent pharmacokinetics of caffeic acid was studied on rabbits (Uang & Hsu 1997). Three different doses (5, 10 and 25 mg/kg) were administered intravenously to six rabbits each. The concentration-time profiles for caffeic acid could be fitted by a two-compartment model for each dose. The results showed that total-body clearance and elimination rate constant from the central compartment (k10) after a 5 mg/kg dose were greater than those after the other two doses. Furthermore, the terminal elimination half-life (beta half-life) and mean residence time (MRT) after a 5 mg/kg dose were less than after the other doses. The AUC value increased linearly with dose within the range of 10-25 mg/kg. Most of the unchanged caffeic acid was excreted in the urine within 2 h.

The percentage of unchanged caffeic acid excreted in the urine was 63, 60, and 55% after doses of 5, 10 and 25 mg/kg, respectively, which was not significantly different. However, significant differences in the renal clearances and renal excretion rate constants were observed with a 5 mg/kg dose compared to the other doses. On the other hand, nonrenal clearances and nonrenal excretion rate constants showed no dose-related differences. The differences observed in total-body clearance, k10, beta half-life, and MRT between a 5 mg/kg dose and the other doses can be explained on the basis of the differences in renal clearance and renal excretion rate constants.

2.2 Pharmacodynamics

2.2.1 Animal studies

Immunomodulatory effect

Several different candidate substances from *Echinacea* have been identified that may contribute to its immunomodulatory effects, including polysaccharides of various sizes, caffeic acid derivatives, alkamides and melanins. The most studied compounds are the polysaccharides, with supporting evidence coming from studies conducted both in vitro and in vivo. Research on the alkamides also indicates a major role for these compounds. Less evidence exists for the immunostimulatory actions of the caffeic acid derivatives. It is very likely that a combination of these and other unknown agents contribute to the overall therapeutic activity of *Echinacea* products.

Polysaccharides

Echinacea polysaccharides were isolated from aerial parts and roots of *E. purpurea*. Further purification yielded a protein-free preparation called EPS (Stimpel et al. 1984) and two polysaccharides; PSI, 4-O-methylglucuronoarabinoxylan (35,000 Da) and PSII, a 50,000 Da acidic arabinorhamnogalactan (Proksch & Wagner 1987). These polysaccharides did not influence *in vitro* T and B cell proliferation or cytokine production but instead affected the *in vitro* phagocytosis, chemotaxis, and production of cytokines observed in granulocytes and macrophages (Stimpel et al. 1984; Wagner et al. 1985). These polysaccharides also enhanced the cytotoxic action of macrophages toward tumor P815 cells (Stimpel et al. 1984). Later work repeated and extended earlier studies by using an arabinogalactan isolated from *E. purpurea* grown in tissue culture (Wagner et al.-, 1988). This polysaccharide enhanced macrophage activation and intracellular killing of *Leishmania enriettii* (Luettig et al. 1989).

The concentrations of *Echinacea* polysaccharides required to obtain the *in vitro* effect on immune cells discussed above were extremely high. In studies using EPS (Stimpel et al. 1984) concentrations of 1,000 microg/ml were required to enhance macrophage cytotoxicity. In addition, this high concentration of EPS was required to enhance macrophage IL-1 production to levels 50% of those achieved using maximal concentrations of Salmonella lipopolysaccharide (LPS). Concentrations 250 microg/ml of the purified arabinogalactan isolated from cultures of suspension cells of *E. purpurea* (Wagner et al. 1988) were required to enhance macrophage production of cytokines to levels equal to (interferon-beta) or 20% (IL-1) of those achieved with maximal concentrations of *E. coli* lipopolysaccharide (10 microg/ml).

Animal studies using i.v. EPS demonstrated enhanced phagocytosis (Wagner et al. 1985). Arabinogalactan injected i.v. into mice exhibited enhanced resistance against systemic infections with *Listeria monocytogenes* and *Candida albicans* in both normal (Roesler et al. 1991) and immunocompromised (Steinmuller et al. 1993) animals. Oral administration of a polysaccharide fraction from *E. purpurea* aerial parts had no effect on lung macrophage function in normal rats (Goel et al. 2002). Polysaccharides purified from *E. purpurea* tissue culture injected i.v. into patients undergoing chemotherapy for gastric cancer showed a lessening of leucopoenia (Melchart et al. 2002).

Caffeic Acid Derivatives

Cichoric acid is present in roots of *E. purpurea* (0.6%-2.1%) and aerial parts of *E. purpurea*, *E. angustifolia* and *E. pallida* at concentrations of 1.2-3.1% (Bauer et al, 1988b). In an *in vitro*

granulocyte assay, cichoric acid concentrations between 10 and 100 ng/ml caused strong stimulation of phagocytosis and in mice it enhanced carbon clearance (Bauer et al, 1989).
Echinacoside is not present in *E. purpurea* root or aerial parts (Bauer et al, 1988b; Pietta et al. 1998).

Alkamides

The major lipophilic components of *Echinacea* are the alkamides. The aerial parts of all three species contain alkamides (Bauer et al. 1988b). Fifteen major alkamides were identified in roots of *E. angustifolia* and 11 in *E. purpurea* roots (Bauer et al. 1988b).
The following studies were performed to determine if the phagocytic activity exhibited by extracts from *Echinacea* was predominantly due to polar or non-polar compounds. Ethanolic extracts from all three species and from both roots and aerial parts were separated into a polar (water) and non-polar (chloroform) fraction. The fractions were tested for phagocytosis in the granulocyte smear test (*in vitro*) and carbon clearance (*in vivo*). In essentially every case the non-polar (chloroform) fractions were the most active (Bauer et al. 1988a; Bauer et al. 1989). A further purified non-polar fraction enriched for alkamides (isolated from *E. purpurea* and *E. angustifolia* roots) enhanced phagocytosis in the carbon clearance test by 1.5 to 1.7 fold (Bauer et al. 1989).
A purified alkamide fraction administered orally to rats was found to enhance the phagocytic activity and phagocytic index of lung alveolar macrophages. In addition, alveolar macrophages collected from alkamide-treated rats produced more TNF-alpha and nitric oxide after stimulation with LPS in vitro (Goel et al. 2002). The upregulation of TNF-alpha mRNA by *Echinacea* alkylamides was found to be mediated by cannabinoid receptor CB2, increased cAMP, p38/MAPK and JNK signalling, as well as NF-jB and ATF-2/CREB-1 activation (Gertsch et al. 2004).

Melanin

This new active constituent of *Echinacea purpurea* was purposed recently (Pasco et al. 2005, Pugh et al. 2005).
It was shown that melanin is an immunostimulatory compound that is a major component of immunostimulant medicinal plants. While melanin is present in commonly consumed vegetables, its specific activity is several orders of magnitude less than melanin extracted from these medicinal plants. The major reason that this agent has eluded detection is its solvent-specific requirement for extraction/solubility. Ingestion of melanin by mice for four days increases production *ex vivo* of interferon-gamma by spleen cells and IgA and interleukin-6 by Peyer's patch cells. The isolated melanin was an amorphous dark colour pigment (reddish brown and similar to pheomelanin), general insoluble in most solvents, bleaching by oxidizing agents (H_2O_2), and pheomelanin-like solubility in alkali and phenol. Elemental analysis indicated about 50% carbon, about 13% nitrogen, about 7% hydrogen, about 0.8% sulphur and about 0.08% phosphorus. NF-kappa B/luciferase reporter gene based monocite activation assay was used to screen for immunomodulatory activities of extracts. The EC50 value for *Echinacea* melanin was 1 microg/ml with maximal activation occurring at 10 microg/ml. Maximal activation with this melanin is equal to that of maximally activating concentrations of *E. coli* LPS (10 microg/ml). Monocyte activation by *Echinacea* melanin substantially increased the expression of cytokine mRNAs characteristic of this state. *Echinacea* melanin induced IL-1 mRNA expression to the same extent as maximally activating concentrations of LPS.

Juice, extract, crude fractions

Treatment with 1.0 or 5.0 mg/ml of lyophilised expressed juice of *Echinacea purpurea* (Echinacin) induced a dose-dependent and highly significant increase ($p<0.001$) in the percentage of phagocytosing granulocytes from 79% to 95% and stimulated the phagocytosis of yeast particles significantly ($p<0.01$) about 50%. With the highest tested dose of 12.5 mg/ml both the number of phagocytosing granulocytes and the phagocytosis index decreased (Stotzem et al. 1992). Also the phagocytosis of isolated peritoneal macrophages from mice and macrophages of the isolated perfused rat liver was significantly stimulated after i.p. and/or p.o. application of *Echinacea purpurea* expressed juice.
The dry residue of an ethanolic tincture (1:10) showed a maximum phagocytosis stimulation of 33% in the *in vitro* granulocyte test in the concentration of 0.001%. Higher dilutions did not show any effect (Bauer et al. 1988a). In the *in-vivo* carbon clearance test on mice p.o. administration of 0.5 ml

Echinacea extract in 30 ml isotonic saline solution over 3 days leads to 3-fold increased phagocytosis in relation to the control group.
The water-soluble fraction increases the carbon clearance by the factor 1.9 (Bauer et al. 1988a, Bauer et al. 1989) and the lipophilic alkamide fraction by the factor 1.7. In the granulocyte test the water-soluble fraction (10^{-3}%) causes a 42% phagocytosis stimulation and the chloroform fraction (10^{-3}%) 37%.
In vitro and *in vivo* phagocytosis stimulation could be proven for the ethanolic extract and the mother tincture. The carbon clearance test of an extract, manufactured from the herb with ethanol (1:10), showed on mice after p.o. application an increased coal elimination by the factor 1.4 in relation to control. The chloroform-soluble fraction was stronger (factor 2.1); the water-soluble fraction was weaker (factor 1.3) (Bauer et al. 1989). A mother tincture of *Echinacea purpurea*, manufactured according the German homoeopathic pharmacopoeia, stimulated in a dosage of p.o. 3 times daily 0.17 ml/kg kg over 2 days the carbon clearance around the factor 2.1.

The immunostimulating effect of *Echinacea purpurea* was studied on natural killer (NK) cells since these cells are active in spontaneous, non-specific immunity against neoplasms and virus-mediated infections (Currier & Miller 2000). Aging mice was selected as a model animal, since at this stage of life, like humans, the above-mentioned afflictions increase in frequency. It was previously found that neither the cytokine, interleukin-2, nor the pharmacological agent, indomethacin, both potent stimulators of NK cell numbers/function in younger adult mice, is effective in stimulating NK cells in elderly mice. The study of Currier & Miller (2000) was designed to assess the numbers/production of NK cells in the spleen and bone marrow of aging, normal mice, after *in vivo* dietary administration of *E. purpurea* (14 days), or, after injection of thyroxin, a stimulant of NK cell function (10 days). Immunoperoxidase labelling techniques, coupled with haematologic tetrachrome staining were used to identify NK cells in both the spleen (primary site of NK cell function) and the bone marrow (site of NK cell generation). Double immunofluorescence staining, employing propidium iodide, was used to assess NK cell lytic function. The results revealed that *E. purpurea*, but not thyroxin, had the capacity to increase NK cell numbers, in aging mice, reflecting increased new NK cell production in their bone marrow generation site, leading to an increase in the absolute numbers of NK cells in the spleen, their primary destiny. The *E. purpurea*-mediated increase in NK cell numbers was indeed paralleled by an increase in their anti-tumor, lytic functional capacity.

The mechanism of activation of human peripheral blood NK cells by *Echinacea* water soluble extracts was studied (Gan et al. 2003). The study examined *in vitro* the effects of soluble extracts of *E. purpurea* on natural killer (NK) cells present in human peripheral blood mononuclear cells (PBMC). Flow cytometric methods were used to examine activation, cytotoxicity, NK-target binding, and killer cell frequency. Treatment of PBMC with *Echinacea* overnight resulted in the activation of CD69 expression and increase in mean fluorescence intensity in both the CD16+ and CD16+CD56+ NK subsets. However, the frequency of CD16+ cells was decreased as well as the mean fluorescence intensity was down-regulated. NK cytotoxicity was increased 100% at the concentration of 0.1 microg/ml of *Echinacea* in a short time (4 h) assay. At the single cell level the frequency of CD56+ NK-target conjugates increased and a plateau was reached after 30-60 min of incubation. Likewise, the frequency of CD56+ killer cells in the conjugates was also significantly increased by *Echinacea*. There was recruitment of non-conjugated CD56+ cells into CD16+ NK-target conjugates and activation of the NK-target non-killer conjugates into killer cells.

Antimicrobial effect

Cichoric acid from *Echinacea purpurea* expressed juice was found to reduce a yield of VSV (Vesicular Stomatitis virus) in mouse L-929-cells. 125 microg/ml cichoric acid after 4 h incubation reduced the infectivity of VSV by more than 50% (Cheminat et al. 1988). For trideca-1,11-dien-3,5,7,9-tetrain and trideca-1-en-3,5,7,9,11-pentain, both main alkylamides from *Echinacea purpurea*, a bacteriostatic and fungistatic effect is described. Total growth inhibition of *Escherichia coli* was found in concentrations of 100 and/or 50 microg/ml, against *Pseudomonas aeruginosa* with a concentration of 5.0 and/or 1,000 microg/ml (Schulte et al. 1967a, Schulte et al. 1967b). For both constituents the following inhibiting concentrations were determined: *Aspergillus niger* >0.01% and/or 0.1%; *Candida*

albicans 0% and/or 0.2%; *Staphylococcus aureus* 0.005% and/or 0.01%; *Pseudomonas aeruginosa* 0.005% and/or 0.1% and *Escherichia coli* 0.0005% and/or 0.005% (Reisch et al. 1967).
For the first time Orinda et al. (1973) reported about a virustatic effect of an *Echinacea* expressed juice. He showed that expressed juice in presence of DEAE Dextran mouse-L-929 cells protected against the cytopathic effect of Encephalomyocarditis virus (EMC virus) and Vesicular Stomatitis virus (VSV). The virustatic effect was quantitatively measured by 2 methods (Wacker & Hilbig 1978): the Plaque reduction method in the cell culture and the colour method by Finter. The extract of *Echinacea purpurea* was fractionated by TLC and the virustatic activity of the fractions was tested. The virustatic activity was distributed to all fractions of the entire TLC. The antiviral principle could not be inactivated by a two-hour treatment at 60 to 80°C.
Both a decoction and a 30% ethanolic extract of *Echinacea purpurea* inhibits the intracellular propagation of ECHO9 HILL virus in a monkey kidney cell culture. A 50% virus inhibition was still observed with dilution rates between 1:6 to 1:15 (Skwarek et al. 1996).

Isobutylamides and polyacetylenes from *Echinacea purpurea* have phototoxic antimicrobial activity against fungi, including clinically relevant pathogenic fungi. A hexane extract of *Echinacea* inhibit growth of yeast strains of *Saccharomyces cerevisiae, Candida shehata, Candida kefyr, Candida albicans, Candida steatulytica* and *Candida tropicalis* under near UV irradiation (phototoxicity) and to a lower extent without irradiation in the conventional antifungal activity (Binns et al. 2000).

Other effects

The healing of standardised, surgery made skin wounds on guinea pigs was accelerated by *Echinacea* ointment (Kinkel et al. 1984). The wound area at 6. and 9. day after surgery was significantly ($p<0,05$) smaller than those of the untreated control animals. In comparison with the control group, the clinical picture was significantly improved in the group treated with *Echinacea* ointment already on day 3 ($p<0,05$).
10 and/or 30 microlitre of an extract from *Echinacea purpurea* fresh plants, manufactured with 90% ethanol, (final ethanol concentration 65%), with a dry residue of 10.5 mg/ml, significantly inhibited the concentration of collagen lattices populated with C3H10T1/2-fibroblasts *in vitro*. Ethanol of equal concentration did not have influence. Dependent on the time of the addition of the extract the elongation of fibroblasts and the cell processes leading to the cross-linking of the collagen were inhibited. If the extract was applied 1 h after the beginning of cross-linking, no more influence could be determined. The authors discussed the significance of these observations in relation to the process of wound healing (Zoutewelle & Van Wijk 1990). *Echinacea*-Fibrin grafts stimulated the healing tendency in guinea pig. Compared with pure fibrin grafts, healing tendency of the wound areas increased and less marked leucocytic infiltration were observed in *Echinacea*-Fibrin grafts (Tünnerhoff & Schwabe 1956).

Caffeic acid derivates protected collagen from free radical-induced degradation in a dose dependent manner; the IC50 for cichoric acid was 16.5 microM (Maffei Facino 1995).
The radical scavenging activity of *Echinacea* methanolic extracts and isolated phenolic compounds was evaluated *in vitro* with a spectrophotometric method based on the reduction of an alcoholic 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution at 517 nm in the presence of a hydrogen donating antioxidant (Pellati et al. 2004). Among the pure compounds, echinacoside had the highest capacity to quench DPPH radicals (EC50 = 6.6 microM), followed by cichoric acid (8.6 microM) and cynarin (11.0 microM). Chlorogenic acid, caffeic acid and caftaric acid had lower activity (18.9 microM, 19.1 microM and 20.5 microM, respectively).
The average EC50 values for E. *purpurea, E. pallida* and *E. angustifolia* were 134, 167 and 231 microg/ml, respectively.

Dried pressed juice from *Echinacea purpurea* whole plants significantly ($p< 0.05$) elevates a SOD (superoxide dismutase) activity when given to a mice in a dose of 360 mg/kg every other day for 3 weeks (Mishima et al. 2004).

Alkamides from the roots of *Echinacea purpurea* were examined for anti-inflammatory activity in an *in vitro* model system (Clifford et al. 2002). Cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-

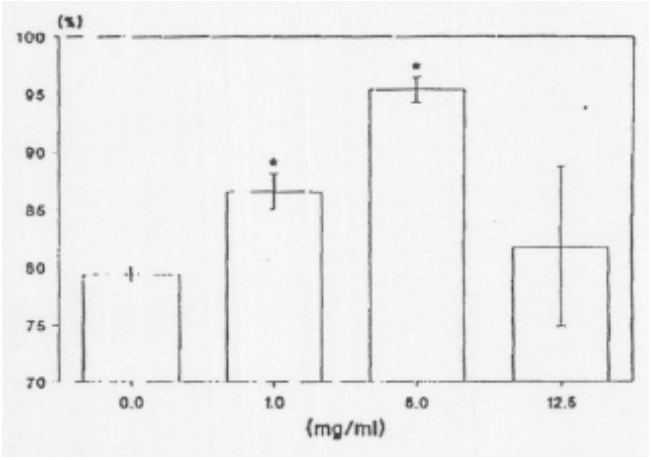
II) inhibitory activities were assessed at pH 7 for alkamides isolated from *E. purpurea* roots to compare inhibitory activities between the two cyclooxygenase isozymes. At 100 microg/ml, several *E. purpurea* alkamides inhibited COX-I and COX-II enzymes in the range of 36-60% and 15-46%, respectively, as compared to controls.

5-lipoxygenase-inhibiting activity of extracts of five wild and three commercially used species of the genus *Echinacea* were investigated to characterise anti-inflammatory activity of *Echinacea* (Merali et al. 2003). The inhibition of the 5-lipoxygenase (5-LOX) enzyme of the arachadonic acid pathway was determined by HPLC detection of a direct metabolic product (LTB4) of 5-LOX derived from stimulated rat basophilic cells. Root extracts of the three commercial species of *Echinacea* (*E. purpurea, E. pallida var. angustifolia, E. pallida var. pallida*) inhibited the 5-LOX enzyme.

Mosquitocidal activity was assessed at 100 and 10 microg/ml, with 100% mortality against *Aedes aegyptii* larvae noted for several *E. purpurea* alkamides at 100 microg/ml.

2.2.2 Human studies

Addition of 1.0 or 5.0 mg/ml of lyophilised expressed juice of *Echinacea purpurea* (Echinacin) to human blood *in vitro* induced a dose-dependent and highly significant increase ($p<0.001$) in the percentage of phagocytosing human granulocytes from 79% to 95% and stimulates the phagocytosis of yeast particles significantly ($p<0.01$) about 50%. With the highest tested dose of 12.5 mg/ml both the number of phagocytosing granulocytes and the phagocytosis index decreased (Stotzem et al. 1992).



Influence of *Echinacea purpurea* on % of phagocytosing human granulocytes in vitro (mean +/- SD). * significant difference as compared with control. $p < 0.001$ (Stotzem et al. 1992).

Regulation of human immune gene expression as influenced by a commercial blended *Echinacea* product was studied (Randolph et al. 2003). *Echinacea* preparation was given to healthy volunteers and gene expression in their blood cells was examined. Additionally, gene expression in human immune cells (THP-1) were exposed to *Echinacea* extracts (250 microg/ml) *in vitro* was studied. Gene expression was studied by measuring the amount of respective mRNA with quantitative PCR. Expression of interleukin-1alpha, interleukin-1beta, TNF-alpha, intracellular adhesion molecule, interleukin-8 and interleukin-10 genes increased up to 10-fold in *Echinacea* treated THP-1 cells. *In vivo*, many lymphokines were down regulated, but the expression of interferon-alpha steadily rose, consistent with an antiviral response.
The results of this study are consistent with the results of an older study, where the cytokine production by normal human peripheral blood macrophages at *in vitro* stimulation with commercial preparations of *Echinacea* was measured with ELISA (Burger et al. 1997). *Echinacea* stimulated immune cells produced significantly higher amount of interleukin-1, TNF-alpha, interleukin-6 and interleukin-10.

Echinacea herb and root powders were also found to significantly enhance the viability and/or proliferation of human peripheral blood mononuclear cells *in vitro* (Rininger et al. 2000).

Stimulation of phagocytic activity and production of cytokines by oral application of a commercially available *Echinacea* preparation was also studied in humans *in vivo* (Schwarz et al. 2002). Forty healthy male volunteers (ages 20-40 years) participated in the study. They received either a freshly expressed juice of *Echinacea purpurea* herbs or placebo juice using a double-blind placebo-controlled crossover design with two treatment periods of 14 days and a wash-out period of 4 weeks in between. Endpoints for immune stimulation were phagocytic activity of polymorphonuclear leukocytes and monocytes measured by flow-cytometry, production of tumor necrosis factor alpha (TNF-alpha) and interleukin-1beta (IL-1beta) by LPS-stimulated blood monocytes. *Echinacea purpurea* herbs did neither enhance phagocytic activity of polymorphonuclear leukocytes nor that of monocytes when compared with placebo. Similarly as in the study of gene expression (Randolph et al. 2003) also in this study the production of TNF-alpha and IL-1beta by immune cells isolated from volunteers treated with *Echinacea purpurea* herbs did not increase. *Echinacea purpurea* herbs decreased serum ferritin concentration ($p = 0.0005$). All other laboratory and safety data remained unchanged.

Ability of *Echinacea purpurea* to prevent or to relieve experimental infection with rhinovirus type 39 (RV-39) was evaluated in a randomised, double-blind, placebo-controlled clinical trial (Sperber et al. 2004). Forty-eight previously healthy adults received *Echinacea* or placebo, 2.5 ml 3 times per day, for 7 days before and 7 days after intranasal inoculation with RV-39. Symptoms were assessed to evaluate clinical illness. Viral culture and serologic studies were performed to evaluate the presence of rhinovirus infection. A total of 92% of *Echinacea* recipients and 95% of placebo recipients were infected. Colds developed in 58% of *Echinacea* recipients and 82% of placebo recipients ($p = 0.114$, by Fisher's exact test). Administration of *Echinacea* before and after exposure to rhinovirus did not significantly decrease the rate or the severity of infection; however, the trend of beneficial effect of *Echinacea* was shown in nearly all measured parameters: mean score of sore throat was 1.04 in *Echinacea* group and 2.45 in placebo group, mean score of congestion was 1.87 in *Echinacea* group and 2.66 in placebo group, mean score of headache was 0.63 in *Echinacea* group and 0.92 in placebo group. Mean total symptom score was lover in *Echinacea* group than in placebo group on every individual day of the trial.
Similarly, effectiveness of *Echinacea* for prevention of experimental Rhinovirus colds was not statistically significant in an earlier study (Turner et al. 2000). Infection occurred in 44 and 57% and illness occurred in 36 and 43% of the *Echinacea*- and placebo-treated subjects, respectively.

2.3 Interactions

No pharmacodynamic or pharmacokinetic drug interactions of whole *Echinacea purpurea* herb extract or isolated constituents have been reported in humans (ESCOP, WHO, Komission E). Theoretically it can be expected, that *Echinacea* preparations can interact with immunomodulatory therapy (immunostimulatory and immunoinhibitory). However, no clinical cases of drug interactions have been reported (Izzo & Ernst 2001).

In *in vitro* test *E. purpurea* demonstrated mild inhibition of CYP3A4 activity with 7- benzyloxy-4-trifluoromethylcoumarin as the model substrate, but mild inducing effects in the presence of the model substrate resorufin benzyl ether. Little effect on CYP2D6 and moderate inhibition of CYP2C9 was seen with *E. purpurea* (Yale & Glurich 2006).

Clinical trial using 12 healthy volunteers (6 women, 6 men) revealed that the effects of *E. purpurea* **root** on CYP activity were minor (Gurley et al. 2004).

In another trial on 12 healthy volunteers *Echinacea purpurea* **root** administration significantly increased the systemic clearance of midazolam by 34%, and significantly reduced the midazolam area under the concentration-time curve by 23%. In contrast, the oral clearance of midazolam was not significantly altered. The oral availability of midazolam after *Echinacea* dosing was significantly. Hepatic availability and intestinal availability were significantly altered in opposite directions. *Echinacea* dosing significantly reduced the oral clearance of caffeine. The oral clearance of

tolbutamide was reduced by 11%. The oral clearance of dextromethorphan in 11 CYP2D6 extensive metabolizers was not affected by *Echinacea* dosing. *E. purpurea* root reduced the oral clearance of substrates of CYP1A2 but not the oral clearance of substrates of CYP2C9 and CYP2D6. *Echinacea* selectively modulates the catalytic activity of CYP3A at hepatic and intestinal sites. The type of drug interaction observed between *Echinacea* and other CYP3A substrates will be dependent on the relative extraction of drugs at hepatic and intestinal sites (Gorski et al. 2004).

Ethanolic extracts from fresh *Echinacea purpurea* and *Spilanthes acmella* and dried *Hydrastis canadensis* were examined with regard to their ability to inhibit cytochrome P450(2E1) mediated oxidation of p-nitrophenol in vitro. In addition, individual constituents of these extracts, including alkylamides from *E. purpurea* and *S. acmella*, caffeic acid derivatives from *E. purpurea*, and several of the major alkaloids from *H. canadensis*, were tested for inhibition using the same assay. *H. canadensis* (goldenseal) was a strong inhibitor of the P450(2E1), and the inhibition appeared to be related to the presence of the alkaloids berberine, hydrastine and canadine in the extract. These compounds inhibited 2E1 with K(I) values ranging from 2.8 microM for hydrastine to 18 microM for berberine. The alkylamides present in *E. purpurea* and *S. acmella* also showed significant inhibition at concentrations as low as 25 microM, whereas the caffeic acid derivatives had no effect. Commercial green tea preparations, along with four of the individual tea catechins, were also examined and were found to have no effect on the activity of P450(2E1) (Raner et al. 2007).

3 Clinical Efficacy

3.1 Clinical studies

Most of the clinical studies are related to the immunological effect and recurrent infections of the upper respiratory tract.

Table 1: The list of the placebo-controlled clinical studies with p.o. use of expressed juice of *Echinacea purpurea* herb on the treatment of recurrent infections of the upper respiratory tract. These studies were used to asses the indications, posology and safety.

authors, year	**patients (age)**	**indication**	**formulation**	**Dose (mode of administration)**	**control**	**efficacy**	**safety**	**comment**
Yale & Liu 2004	128 (18-62 years)	Treatment of the common cold	freeze-dried pressed juice from the aerial portion of *E. purpurea*	100 mg 3 times per day	placebo	**Not significant**	well tolerated	lower dosing than in other trials
Hoheisel et al. 1997	120 (38 ± 11 years)	Treatment of URTI "first signs of cold" "justifiable initial signs of acute upper respiratory infection"	*E. purpurea* expressed juice, (Echinagard)	20 drops (1-2 ml) every 2 hours for the first day and thereafter three times daily. (in half glass of water)	Placebo "identical in color and ethanol concentration"	**Significant:** median time taken to improvement was 4 days (*Echinacea*) and 8 days (placebo)	No specific adverse events. Tolerability of placebo and *Echinacea* was equal.	
Schulten et al. 2001	80 (39 ± 12 years)	Treatment of URTI "incipient infection of upper respiratory tract (subjective sensation of having a cold and at least one of the following symptoms: sneezing, rhinorrhea, congestion of the nose, sore throat, cough, headache, malaise, chilliness"	*E. purpurea*, (Echinacin, EC3lJO) pressed juice from fresh flowering purple coneflower (1.7-2.5:1). stabilised by ethanol.	5 ml twice daily	Placebo "indistinguishable in terms of appearance, taste, smell, colour and packaging"	**Significant:** In *Echinacea* the median time of illness was 6 days and in placebo 9 days	well tolerated	
Taylor et al. 2003	407 children (5.5 ± 2.7 years	Treatment of URTI	*E. purpurea* herba dried expressed juice (extract?), (31-54:1) dissolved to give 1:1 extract.	3.75 ml twice daily for children 2 to 5 years old and 5 ml twice daily for children 6 to 11 years old.	placebo "was identical in appearance and similar in taste and smell"	**Not significant**	no difference in overall rate of adverse events, rash occurred in 7.1% in *Echinacea* and 2.7% in placebo	dosage is not clear, the product was not standardized, late beginning of treatment

Table 2: The list of other clinical studies with expressed juice or extract of *Echinacea purpurea* herb or combinations on the immunological effect. This studies were used for assessment of safety.

authors, year	**appl.**	**patients (age)**	**indication**	**formulation**	**dose**	**control**	**efficacy**	**safety**	**comment**
Turner et al. 2000	p.o.	117	prevention of experimental rhinovirus colds	not clear (contained 0.16% cichoric acid with almost no echinacosides or alkamides)	300 mg 3 times daily	placebo	**Not significant, but important trend:** Infection occurred in 44 and 57% and illness occurred in 36 and 43% of the *Echinacea*- and placebo	No side effects were observed	prevention, unknown species
Sperber et al. 2004	p.o.	48 (18-64 years)	Prevention of experimental rhinovirus colds	*E. purpurea* herba expressed juice,	2.5 ml 3 times per day	placebo	**Not significant, but important trend:** Colds in 58% of *Echinacea* and 82% of placebo	Not different to placebo	prevention, poor statistical power
Grimm & Müller 1999	p.o.	108 (40 ± 16 years)	Prevention of URTI	*E. purpurea* fluid extract, (Echinacin-Liquidum)	4 ml twice daily	placebo	**Not significant, but important trend:** The average number of colds and respiratory infections per patient was 0.78 in the *Echinacea* group, and 0.93 in the placebo group.	Side effects were observed in 20% of *Echinacea* group and in 13% of placebo group. The majority of adverse events was mild and transient.	prevention
Baetgen 1984	i.m.	170 children (0-13 years)	Treatment of pertusis	Echinacin	2 ml/day in children 1 ml/day in infants	active (antibiotic)	**No statistical analisys.** reduction of disease in 34% of patients in *Echinacea* group and in 10% in antibiotic group	well tolerated	

authors, year	appl.	patients (age)	indication	formulation	dose	control	efficacy	safety	comment
Baetgen 1988	i.m.	1280 children (0-19 years)	Treatment of URTI	Echinacin (Madaus)	2 ml/day in children 1 ml/day in infants	active (antibiotic)	**No statistical analisys.** reduction of disease in 46% of patients in *Echinacea* group, in 16% in antibiotic group and in 25% in patients receiving combination of *Echinacea* and antibiotic.	"The injections with Echinacin were remarkably well tolerated"	
Barrett et al. 2002	p.o.	148 students	Treatment of URTI	*E. purpurea* herb (25%) and root (25%) an *E. angustifolia* root (50%) provided by Shaklee Tecnica (Pleasanton, California)	4 capsules (containing 247 mg of *Echinacea*) 6 times during the first 24 hours of the study, and then 4 capsules 3 times daily.	placebo	**Not significant**	Not different to placebo	mixture of 2 species
Heinen-Kammerer et al. 2005	p.o.	995	treatment of chronic recurrent respiratory disease	*E. purpurea* fluid extract, (Echinacin)		standard therapy	The risk of recurrent illnes was 2.3 fold lower and the duration of relapse 1.4 days shorter in *Echinacea* group		non-randomised study
Lindenmuth & Lindenmuth 2000	p.o.	95	early symptoms of cold or flu	Herbal tea (*Echinacea* species?)	5 to 6 cups per day	placebo	**significant** difference between the *Echinacea* and placebo for all 3 questions measured (efficacy, number of days the symptoms lasted, and number of days for change): $p < 0.001$	no negative effects reported by any of the subjects in either group	

authors, year	appl.	patients (age)	indication	formulation	dose	control	efficacy	safety	comment
Melchart et al. 2002	i.v.	50	counteraction of the undesired effects of chemotherapy	fraction isolated from the *Echinacea purpurea* herb cell cultures	2 mg of polysaccharides per day	matched historical controls	53% increase in median number of leukocytes, no effects on phagocytic activity of granulocytes or on lymphocyte subpopulations	Sixty-eight adverse events including two deaths were observed, most likely due to chemotherapy and the general condition of the patients	open prospective study with matched historical controls
Vonau et al. 2001		50	recurrent genital herpes	extract of *Echinacea purpurea* (Echinaforce)		placebo (cross-over)	Not statistically significant		
Brinkeborn et al. 1999	p.o.	246 (41 ± 14 years)	Treatment of URTI "immediately after the onset of the first symptoms of common cold"	*E. purpurea* 95% herba, 5% radix, dry extract (Echinaforce)	3 times daily 2 tablets containing 6.78 mg of extract.	Placebo "could almost not be distinguished bz smell or taste"	**Significant:** index of 12 symptoms reduced for 63% in *Echinacea* and for 29% in placebo	Treatments were well tolerated. In *Echinacea* group the frequency of adverse events was not significantly higher than in the placebo group.	
Goel et al. 2004	p.o.	282 (18 – 65 years)	Treatment of URTI "first symptoms of related to common cold… A cold is the recent onset of unexplained tiredness (fatigue), muscle aches and pains that are not related to … You may also get headaches, feel feverish and get chills with or without fever…"	water-ethanol extract of fresh herbs of *E. purpurea* containing 0.25 mg/ml of alkamides, 2.5 mg/ml of cichoric acid and 25 mg/ml of polysaccharides (Echinilin, Natural Factors Nutritional Products, ind. Vancouver, BC, Canada)	4 ml 10 times per day on first day and 4 ml 4 times per day for next 6 days.	placebo	**Significant:** total daily symptom scores were 23.1% lower in the *Echinacea* than in placebo	Not different to placebo	

3.1.1 Placebo-controlled trials on *E. purpurea* expressed juice for treatment of URTI

Yale & Liu (2004) tested the efficacy of a standardized preparation of *E. purpurea* in reducing symptom severity and duration of the common cold, in a randomised, double-blind, placebo-controlled trial recently. 128 patients received either 100 mg of freeze-dried pressed juice from the aerial portion of *E. purpurea* or a lactose placebo 3 times daily until cold symptoms were relieved but not longer than 14 days. Symptoms (sneezing, nasal discharge, nasal congestion, headache, sore or scratchy throat, hoarseness, muscle aches, and cough) were scored subjectively by the patient and recorded daily. Patients were enrolled within 24 hours of cold symptom onset. No statistically significant difference was observed between treatment groups for either total symptom scores (P range, 0.29-0.90) or mean individual symptom scores (P range, 0.09-0.93). The time to resolution of symptoms was not statistically different (P = 0.73).

Hoheisel et al. (1997) carried out a randomised double-blind placebo-controlled trial on 120 patients with initial symptoms of acute, uncomplicated upper airways infection. The peroral treatment with either *Echinacea purpurea* expressed juice or placebo lasted for up to 10 days. The dosage was 20 drops every 2 hours for the first day and thereafter three times daily 20 drops. Only two patients were excluded for protocol violation and all completed the study. The time taken to improvement was significantly shorter (P<0.0001) in the *Echinacea* group than in the placebo group. In the sub-group of patients with „real" cold, i.e. fully expressed disease, the median time taken to improvement was 4 days (*Echinacea* group) and 8 days (placebo group). Average termination of treatment due to improvement was after 6 days (*Echinacea* group) and after 10 days (placebo group). No specific adverse events were reported. The findings demonstrate that early initiation of treatment with the expressed juice of *Echinacea purpurea* can reduce the development of the disease and significantly shorten the duration of the common cold and reduce the length of the treatment period required.

Schulten et al. (2001) recruited a total of 80 adult male or female patients with first signs of a cold in a randomised double–blind placebo-controlled trial. The number of days of illness with a complete picture of the common cold (defined by the modified Jackson score of at least 5 points and experience of rhinorrhea and/or a subjective sensation of having a cold) was the primary end-point. In the verum group the median time of illness was 6.0 days compared to 9.0 days in the placebo group, assigning zero time for patients without a complete picture (one-sided p = 0.0112). EC31J0 was well tolerated and clinically effective in alleviating symptoms more rapidly than placebo in patients with a common cold.

Taylor et al. (2003) studied the efficacy of *Echinacea purpurea* in reducing the duration and/or severity of URTI symptoms in children. The design of this study was randomised, double-blind, placebo-controlled trial of healthy children 2 to 11 years old recruited from a regional practice-based network and an alternative medical center in 4-month periods from 2000 through 2002. Patients were randomised to receive either *Echinacea* or placebo for up to 3 URIs over a 4-month period. Study medication was begun at the onset of symptoms and continued throughout the URTI, for a maximum of 10 days. Data were analysed on 707 URIs that occurred in 407 children, including 337 URIs treated with *Echinacea* and 370 with placebo. There were 79 children who completed their study period without having an URTI. The median duration of URIs was 9 days (95% confidence interval, 8-10 days); no difference in duration between URIs treated with *Echinacea* or placebo was found (P = 0.89). No difference in the overall estimate of severity of URTI symptoms between the 2 treatment groups (median, 33 in both groups; P = 0.69) was found either. There were also no statistically significant differences between the 2 groups for peak severity of symptoms (P = 0.68), number of days of peak symptoms (1.60 in the *Echinacea* group and 1.64 in the placebo group; P = 0.97), number of days of fever (0.81 in the *Echinacea* group vs. 0.64 in the placebo group; P = 0.09), or parental global assessment of severity of the URTI (P = 0.67). Overall, there was no difference in the rate of adverse events reported in the 2 treatment groups; however, rash occurred during 7.1% of the URIs treated with *Echinacea* and 2.7% of those treated with placebo (P = 0.008). The authors of the study

concluded that *Echinacea purpurea*, as dosed in this study, was not effective in treating URTI symptoms in patients 2 to 11 years old, and its use was associated with an increased risk of rash.

There were many critics on this study published in scientific literature (Kim et al. 2004; Firenzuoli & Gori 2004; Washam 2004; Le Tourneau 2004; Blumenthal 2004) and one answer of the authors (Taylor et al. 2004). The main critics were that the dosage of *Echinacea* extract in the product was not stated and the product was not standardized. Additional weakness of the study was that placebo group used significantly more vitamins and mineral supplements. The patients started to take medication very late. The parents were asked to call coordinator of the study, when at least 2 symptoms of a URTI developed, and they started to take medication after the coordinator confirmed that the child met criteria for having a URTI. Barrett (2004) published a comment supporting the quality of Taylor's study.

Weber et al. (2005) made a follow up of the patients from the study of Taylor et al. (2003). The aim of this study was to determine whether *Echinacea purpurea* given to children for the treatment of acute upper respiratory tract infection (URI) was effective in reducing the risk of subsequent URI. A total of 524 children ages 2 to 11 years were enrolled in the study. Children were monitored for URIs over a 4-month observation period during the fall/winters of 2000-2001 and 2001-2002. At entry the children were randomized to receive *Echinacea* or placebo to treat acute URIs during the observation period. The occurrence of a second URI and the number of days between the end of the first URI and the start of the second URI was ascertained. Survival and Cox regression analyses were used to determine whether children who took *Echinacea* for their URIs were less likely to develop subsequent URIs. Among the 401 children with at least one URI treated with study medication, 69.2% of those receiving placebo developed a second URI versus 55.8% of those who received *Echinacea*. Use of *Echinacea* was associated with a 28% decreased risk of subsequent URI ($p = 0.01$, 95% confidence interval 8%-44% decreased risk). *Echinacea purpurea* may be effective in reducing the occurrence of subsequent URIs in children.

3.1.2 Non-controlled trial

Götte & Roschke (2001) made observation in children with recurring infections of the upper respiratory tract to asses the tolerability and efficacy of Echinacin. A good to very good tolerability and efficacy was found in children above the age of two years and in young adults. 338 paediatricians treated 1,327 children and young adults with recurring infections of the upper respiratory tract. Only children who had been affected by at least two infections of the respiratory tract during the past twelve months were included in the observation of use. The children had to be at least 2 years of age and show recurring signs and symptoms of a respiratory tract infection. At conclusion of the treatment, the tolerability was assessed with the aid of the recorded adverse drug reactions and the global assessment by the physician and the parents of the patients. The symptoms considered were specified on the observation form (sneezing, running nose, blocked nose, sore throat, cough, headache, feeling of illness and chill). Furthermore, the physician and parents evaluated the duration of the respiratory tract infection in historical comparison to the duration of illness without treatment with the juice. A total of 1,322 children and young adults - males and females - were included in the assessment of efficacy. All patients who had taken the juice at least once were included. A total of 5 patients were excluded from the analysis of tolerability, because they had not shown up for the final examination and no data regarding the assessment of tolerability had been recorded for them. The evaluation of efficacy involved 1,192 children and young adults, 579 females and 609 males. In four cases, the data on sex were missing. On average, the juice was administered to the patients over a period of 11 days. The results obtained from this observation of use, with doses adjusted according to age, revealed a good to very good tolerability and efficacy. In more than 95% of cases, the physician and parents globally assessed the tolerability as good or very good. More than 60% of the treating physicians and parents reported that the duration of the respiratory tract infection was shorter in comparison to the duration of disease without treatment with the juice. The efficacy was rated by the physician as well as by the parents as very good or good in more than 80% of the cases. In comparison to treatment without the juice, the physician observed in this respiratory tract infection a shorter duration of disease in more

than 60% and the course of disease was rated as less severe in more than 70% of all patients. The parents of the children and young adults made comparable assessments.

3.1.3 Trials on other indications (prevention) and other preparations (extract) or combinations.

Brinkeborn et al. (1999) evaluated the efficacy and safety of different doses and preparations of *Echinacea purpurea* in the treatment of common cold in a randomised, double-blind, placebo-controlled study. 246 of 559 recruited healthy, adult volunteers caught a common cold and took 3 times daily 2 tablets of different *Echinacea purpurea* preparations. Group I got a preparation of 95% herba and 5% radix, group II got the same preparation as group I but at 7 times higher concentration, group III got a special *Echinacea purpurea* radix preparation an group IV got placebo tablets. Duration of treatment was until the patients felt healthy again but not longer than 7 days. Primary endpoint was the relative reduction of the complaint index defined by 12 symptoms during common cold according to the doctor's record. The preparations of group I and II were significantly more effective than the special root extract or placebo. Treatment with 7-fold higher dosage of group II was only slightly more effective than dosage of group I. The index of 12 symptoms reduced for 63%, 64% and 45% in group I, II and III, respectively and only for 29% in group IV (placebo). All treatments were well tolerated. Among the *Echinacea* groups the frequency of adverse events was not significantly higher than in the placebo group.

Barrett et al. (2002) assessed the efficacy of dried, encapsulated, whole-plant *Echinacea* as early treatment for the common cold in a randomised, double-blind, placebo-controlled community-based trial at University of Wisconsin, on 148 students with common colds of recent onset. Each active capsule contained a dried mixture of *E. angustifolia* root (50% [123 mg]), *E. purpurea* root (25% [62 mg]), and *E. purpurea* herb (25% [62 mg]). *Echinacea* capsules also contained thyme (49 mg) and peppermint (31 mg) to disguise taste and flavor, as well as citric acid (3 mg) as a preservative. The placebo capsules contained 333 mg of alfalfa. The patients took four capsules six times during the first 24 hours of the study, and four capsules three times each day thereafter until symptoms resolved, for a maximum of 10 days. Severity and duration of self-reported symptoms of upper respiratory tract infection were recorded. No statistically significant differences were detected between the *Echinacea* and placebo groups for any of the measured outcomes. Trajectories of severity over time were nearly identical in the two groups. Mean cold duration was 6.01 days in both groups as a whole, 5.75 days in the placebo group, and 6.27 days in the *Echinacea* group (between-group difference, -0.52 day [95% CI, -1.09 to 0.22 days]). After controlling for severity and duration of symptoms before study entry, sex, date of enrolment, and use of nonprotocol medications, researchers found no statistically significant treatment effect (adjusted hazard ratio, 1.24 [CI, 0.86 to 1.78]). Multivariable regression models assessing severity scores over time failed to detect statistically significant differences between the *Echinacea* and placebo groups.

Goel et al. (2004) studied the efficacy of a well standardized formulation containing alkamides, cichoric acid, and polysaccharides at concentrations of 0.25, 2.5, and 25 mg/ml, respectively, in reducing the severity and duration of symptoms of a naturally acquired common cold. The preparation (Echinilin, Natural Factors Nutritional Products, Inc., Vancouver, BC, Canada) is prepared from freshly harvested *Echinacea purpurea* plants. In a randomised, double-blind, placebo-controlled trial, 282 subjects aged 18 to 65 years with a history of two or more colds in the previous year, but otherwise in good health, were recruited. The subjects were randomised to receive either *Echinacea* or placebo. They were instructed to start the *Echinacea* or placebo at the onset of the first symptom related to a cold, consuming 10 doses the first day and four doses per day on subsequent days for 7 days. Severity of symptoms (10-point scale: 0, minimum; 9, maximum) and dosing were recorded daily. A nurse examined the subjects on the mornings of days 3 and 8 of their cold. A total of 128 subjects contracted a common cold (59 *Echinacea*, 69 placebo). The total daily symptom scores were found to be 23.1% lower in the *Echinacea* group than in placebo in those who followed all elements of the study protocol ($P < 0.01$). Throughout the treatment period, the response rate to treatments was greater in the *Echinacea* group. The average of every individual symptom (runny nose, sore throat, stuffy nose, fatigue, headache, chills) except in cough, was significantly lower in *Echinacea* group

compared to placebo. In cough, the difference was not significant. No differences in white blood cell differential count were observed between the treatment groups.

In their next study **Goel et al. (2005)** administered EchinilinTM or placebo to volunteers at the onset of their cold for a period of 7 days, with eight doses (5 mL/dose) on day 1 and three doses on subsequent days. Fasting blood samples were obtained before and during their colds. The decrease in total daily symptomatic score was more evident in the echinacea group than in the placebo group. These effects of echinacea were associated with a significant and sustained increase in the number of circulating total white blood cells, monocytes, neutrophils and NK cells. In the later part of the cold, the echinacea treatment suppressed the cold-related increase in superoxide production by the neutrophils. These results suggest that EchinilinTM, by enhancing the non-specific immune response and eliciting free radical scavenging properties, may have led to a faster resolution of the cold symptoms.

Baetgen (1984) compared the therapy i.m. injection of 1-2 ml of diluted *Echinacea purpurea* expressed juice alone or in combination with antibiotics on three successive days for the treatment of pertussis, in a retrospective study of 170 children. In about one third of cases (35%) the duration of pertussis could be reduced to five days by giving i.m. injections of *Echinacea* expressed juice. In 81% of cases the reduction of duration was to 10 days.
A combination of *Echinacea* and antibiotic is not equally effective. Out of this group of 77 patients only 9% improved within 5 days and 53% within 10 days of treatment. But the combination is superior to treatment with an antibiotic alone. Only 10% of the patients treated with antibiotics alone improved within 5 days and 46% within 10 days. Injections of *Echinacea* have been tolerated well; temperature rises up to 39°C on the day of treatment were only seen in isolated cases. Erythema and localized pain at the injection site were occasionally reported.

Baetgen (1988) obtained similar results in a comparative evaluation of 1280 children suffering from bronchitis. In this retrospective evaluation it was demonstrated that 3 or 4 i.m. injections of *Echinacea purpurea* expressed juice can significantly shorten the duration of the infection in comparison with a group treated with antibiotics alone or with antibiotics plus *Echinacea purpurea* expressed juice. In *Echinacea* group 45.7% of patients improved in 5 days, in *Echinacea* + antibiotics and in antibiotics group the improvement was 25.5% and 16% respectively.

Grimm & Müller (1999) randomly assigned 108 patients with a history of more than 3 colds or respiratory infections in the preceding year to receive 4 ml *Echinacea purpurea* expressed juice (54 patients) or 4 ml placebo juice (54 patients) twice a day in a double-blind, randomised, prospective study. The incidence and severity of colds and respiratory infections were determined during 8 weeks of follow-up, based on patient reported symptoms together with findings on physical examination. The severity of each infection was graded by the investigators. During the 8-week treatment period 35 (65%) of 54 patients in the *Echinacea* group and 40 (74%) of 54 patients in the placebo group had at least one cold or respiratory infection. The average number of colds and respiratory infections per patient was 0.78 in the *Echinacea* group, and 0.93 in the placebo group. Median duration of colds and respiratory infections was 4.5 days in the *Echinacea* group and 6.5 days in the placebo group. Although the incidence, duration and severity of colds and respiratory infections tended to be lower in the *Echinacea* group, none of the results reached statistical significance. Side effects were observed in 11 patients (20%) of the *Echinacea* group and in seven patients (13%) of the placebo group. The majority of adverse events was mild and transient and did not require discontinuation of the allocated treatment. In the *Echinacea* group there were 4 drop-outs because of nausea, constipation, awful taste of the study medication and patient's choice without any specific reaction.

Turner et al. (2000) assessed the effectiveness of *Echinacea* for the prevention of experimental rhinovirus colds in a randomised double–blind placebo-controlled trial. The preparation is not well described in the publication. It contained 0.16% cichoric acid with almost no echinacosides or alkamides. Infection occurred in 44 and 57% and illness occurred in 36 and 43% of the *Echinacea*-

and placebo-treated subjects, respectively. The effect on either the occurrence of infection or the severity of illness was not significant.

Sperber et al. (2004) studied the ability of *Echinacea purpurea* to prevent experimental infection with rhinovirus type 39 (RV-39) in a randomised double–blind placebo-controlled trial. Forty-eight previously healthy adults received pressed juice of the above-ground plant parts of *E. purpurea* placed in a 22% alcohol base (EchinaGuard) or placebo, 2.5 ml 3 times per day, for 7 days before and 7 days after intranasal inoculation with RV-39. Nine symptoms were assessed three times daily for 14 days to evaluate clinical illness. Viral culture and serologic studies were performed to evaluate the presence of rhinovirus infection. A total of 92% of *Echinacea* recipients and 95% of placebo recipients were infected. Colds developed in 58% of *Echinacea* recipients and 82% of placebo recipients ($p = 0.114$ by Fisher's exact test). Seven-day total symptom score was 30.3% higher in placebo group (p 0.317). The most significant difference was in sore throat, which was 135.6% higher in placebo group compared to *Echinacea* ($p = 0.065$). The differences between the *Echinacea* and placebo were not significant; however, because of the small sample size, statistical hypothesis testing had relatively poor power to detect differences in the frequency and severity of illness.

3.1.4 Reviews

An overview and quantitative meta-analysis of the published *Echinacea* trials was performed by Melchart et al. (1994), Dorsch (1996), Barrett et al. (1999), Giles et al. (2000), Bauer (2002), Barrett (2003), Barnes et al. (2005) and Linde et al. (2006). Most of the authors reviewed not only studies with *Echinacea purpurea* expressed juice but also extracts of *E. angustifolia*, *E. pallida* and in combination with other plant extracts or homeopathic dilutions.
In Melcharts review (Melchart et al. 1994), 26 controlled clinical trials were identified, but the methodological quality of most studies was rated low. Nonetheless the authors concluded that *Echinacea* preparations could be efficacious immunomodulators.

Barrett et al. (1999) reviewed 9 treatment trials and 4 prevention trials of sufficient quality. 8 of the treatment trials reported positive results and 3 of the prevention trials reported marginal benefit.
Giles reviewed 41 references and concluded that *Echinacea* appears to be well tolerated with a low frequency of adverse effect, such as mild dyspepsia, headache and dizziness (Giles et al. 2000). Evaluated studies support *Echinacea* in the treatment of URTI, but not to prevent infection.
Bauer reviewed new clinical studies (Bauer 2002). He summarised that corresponding preparations can diminish the severity and the length of common colds significantly, and that they can also be used efficiently for the treatment of children. Stimulation of macrophages and induction of cytokines are major parts of the mode of action and the glycoproteins/polysaccharides and alkamides are part of the activity relevant constituents.
Barrett (2003) concluded in his second review that the treatment of acute upper respiratory infections with *Echinacea purpurea*, is tentatively supported by the available literature. Reduction of symptoms with early treatment has been reported in several moderate quality randomised controlled trials. Benefits appear to be a modest, with a 10 to 40% reduction of symptoms as the most widely reported outcome. Benefit as a cold preventative appears marginal, at best, with an estimated 5 to 15% effect size.

The authors of Cochrane Review concluded (Linde et al. 2006): *Echinacea* preparations tested in clinical trials differ greatly. There is some evidence that preparations based on the aerial parts of *Echinacea purpurea* might be effective for the early treatment of colds in adults but results are not fully consistent. Beneficial effects of other *Echinacea* preparations, and for preventative purposes might exist but have not been shown in independently replicated, rigorous randomised trials.

Since the literature search for the first version of this Assessment report was finished in January 2006, no new clinical trials on *Echinacea purpurea* were published, but 14 new review articles can be found in Pubmed. Some of recent review articles conclude, that the efficacy of *Echinacea purpurea* is not well supported, since the quality of the clinical trials was not adequate, or the results are not fully

consistent. All of the reviews with negative conclusions, that were available to the raporter have major inconsistencies and drawbacks:
Koenig & Roehr (2006) in their one-page review article (plus one page with a Table) state, that: "trials with a larger number of individuals failed to show a significant effect of *Echinacea* treatment, whereas smaller trials did document positive effects." This is in fact not true, it can be seen from the Table 2 of their review (there is no Table 1 in the article), that significant effect was shown by the 1st, 3rd, 6th and 7th largest trials.
In many reviews, different *Echinacea* species and different herbal preparations are not considered separately. As some species and some preparations might not have the same efficacy as the expressed juice of the *Echinacea purpurea* herb, the pooled analysis led to the false conclusion, that the results are inconsistent.

The most thorough review and meta-analysis was made recently by Shah et al. (2007) evaluating the effect of *Echinacea* on the incidence and duration of the common cold. 14 unique studies were included in the meta-analysis. Incidence of the common cold was reported as an odds ratio (OR) with 95% CI, and duration of the common cold was reported as the weighted mean difference (WMD) with 95% CI. Weighted averages and mean differences were calculated by a random-effects model (DerSimonian-Laird methodology). Heterogeneity was assessed by the Q statistic and review of L'Abbé plots, and publication bias was assessed through the Egger weighted regression statistic and visual inspection of funnel plots. *Echinacea* decreased the odds of developing the common cold by 58% (OR 0.42; 95% CI 0.25-0.71; Q statistic $p<0.001$) and the duration of a cold by 1.4 days (WMD -1.44, -2.24 to -0.64; $p = 0.01$). Similarly, significant reductions were maintained in subgroup analyses limited to Echinaguard/Echinacin use, concomitant supplement use, method of cold exposure, Jadad scores less than 3, or use of a fixed-effects model. The authors concluded that published evidence supports *Echinacea*'s benefit in decreasing the incidence and duration of the common cold (Shah et al. 2007).

3.2 Use in special populations

3.2.1 Use during pregnancy and lactation

Pregnancy outcome in women that used *Echinacea* during pregnancy was studied to evaluate the safety of *Echinacea* (Gallo et al. 2000). Since at least half of all pregnancies are unplanned, many women inadvertently use *Echinacea* in their first trimester. The study group consisted of 206 women who were prospectively followed up after contacting the Motherisk Program regarding the gestational use of *Echinacea*, 112 women used the herb in the first trimester. This cohort was disease-matched to women exposed to nonteratogenic agents by maternal age, alcohol, and cigarette use. Rates of major and minor malformations between the groups were compared. There were a total of 195 live births, including 3 sets of twins, 13 spontaneous abortions, and 1 therapeutic abortion in *Echinacea* group. Six major malformations were reported, including 1 chromosomal abnormality, and 4 of these malformations occurred with *Echinacea* exposure in the first trimester. In the control group, there were 206 women with 198 live births, 7 spontaneous abortions, and 1 therapeutic abortion. Seven major malformations were reported. There were no statistical differences between the study and control groups for any of the end points analysed. The authors concluded that gestational use of *Echinacea* during organogenesis is not associated with an increased risk for major malformations.

In a survey among 400 Norwegian women (Nordeng & Havnen, 2004) 36% used herbal drugs during pregnancy with an average of 1.7 products per woman. *Echinacea* was used by 23% of pregnant woman and was by far the mostly used herb.

A review on safety of *Echinacea* during pregnancy and lactation was published recently (Perri et al. 2006). They searched 7 electronic databases and compiled data according to the grade of evidence found. They found a good scientific evidence from a prospective cohort study that oral consumption of *Echinacea* during the first trimester does not increase the risk for major malformations. Low-level evidence based on expert opinion shows that oral consumption of *Echinacea* in recommended doses is

safe for use during pregnancy and lactation. They concluded that *Echinacea* is non-teratogenic when used during pregnancy. Caution with using *Echinacea* during lactation until further high quality human studies can determine its safety.

A study on mice showed that the pregnancy-induced elevation in splenic lymphocytes was reduced by the diet containing *Echinacea purpurea* extract. Such diet also reduced the number of foetuses, although the diet started only after the pregnancy was established. None of the observed changes except for the pregnancy-induced elevation in immune function was significant (Chow et al. 2006).

3.2.2 Use in children

Of the 14 above reviewed clinical trials 4 were performed in children. The youngest children were included in the two studies by Baetgen (Baetgen 1984, Beatgen 1988) where the minimal age was 1 month. The average age was 3.5 years in one study and 2.8 years and 3.1 years for boys and girls, respectively in the other study.
A recent study (Taylor et al. 2003) included 2 years to 11 years old children (mean 5.5 years, standard deviation 2.7 years). In this study adverse events were found in 45.1% of patients receiving *Echinacea* (and in 39.5% of patients receiving placebo). The most frequent adverse events were: stomachache, diarrhea, drowsiness, headache, ”hyper” behaviour, rush and vomiting. Rash was the only side effect that was significantly more frequent in *Echinacea* group compared to placebo.

Information on 4 (unpublished) observational studies regarding the safety of the oral application of *Echinacea purpurea* herba preparations in different dosages for children below the age of 18 was submited by German authority:
415 children with a median age of 15, thereof 198 children below the age of 12, median age 8 suffering from acute respiratory tract infection received tablets containing 100mg dried expressed juice of flowering herb of *Echinacea purpurea* (DER 22-65:1) for 2 weeks. Due to the pharmaceutical form tablets we accepted the following posology for children from 6-12 : 2-3 x l tablet and for children from 12-18 the adult posology. 1 exanthema was observed, 1 x nausea and vomiting otherwise there were no reports concerning adverse events. (scientific investigator: Dr. Hellemann).

359 children thereof 357 in between 1-12 years (290 < 10years; 67 > 11 years) suffering from acute respiratory tract infection received a liquid (100 ml containing 3.75 g dried expressed juice from fresh flowering herb of *Echinacea purpurea* (DER 22-65:1) for 2 weeks. 3 ml of the liquid correspond to 2,1 ml of fresh juice. Children from 6-11 received 3-4 x 2 ml liquid daily (corresponding to 4.2-5.6 ml fresh juice). Reported adverse events were bad taste 1 x and one generalized exanthema, which could be due to the infect as well. (scientific investigator: Dr. Heidi)

140 children (0-1 y n = 26; 1-4y n = 38; 4-12y n = 76) suffering from acute respiratory tract infection received daily doses as followed : 0-1 y 1-2 x 2,5 ml; 1-4y 1-2 x 5 ml; 4-10y 2-3 x 5 ml and 10-12y 1 x 15-2 x l0 ml of expressed juice of flowering herb of *Echinacea purpurea* (DER: 1:0.65–0.85) for 2 weeks. 2 children dropped out due to the bad taste of the preparation adverse events were not reported.

270 children (1-4y n = 140; 4-12y n = 130) suffering from acute respiratory tract infection received a liquid (100g liquid contain 1.0769 dried expressed juice from fresh flowering *Echinacea purpurea* (DER: 22-65:1). 10 ml liquid correspond to 2.5 ml expressed juice). The posology was the following: 14y: 2-3x5 ml liquid (average 14.4 ml liquid corresponding to 3.6 ml expressed juice); 4-12y: 2-3x l0 ml average 27.4 ml liquid corresponding to 6.85 ml expressed juice. The duration of treatment was 10 days. 8 children dropped out due to recovery 10drop outs were due to antibiotic treatment which was defined as elimination criterion, 1 time bad taste. Adverse events reported 1 x nausea, 1 x bad taste.

3.2.3 Use in the elderly

In most clinical studies, the patients up to 65 years old were included (Hoheisel et al. 1997; Brinkeborn et al. 1999; Grimm & Müller 1999; Schulten et al. 2001; Turner et al. 2000; Barrett et al. 2002; Goel et al. 2004; Sperber et al. 2004; Yale & Liu 2004).
No restrictions are known on the use of preparations from *E. purpurea*.

3.2.5 Effects on ability to drive and use machines

None known (ESCOP 2003).

3.3 Dosage

3.3.1 Treatment of common cold

There are no dose-finding studies available.
In a study of **Brinkeborn et al. (1999)** the treatment with 7-fold higher dosage was only slightly more effective than treatment with 36 mg of dry extract.

Table 1 summarizes the doses and formulation of *Echinacea purpurea* products used in clinical trials. The doses and formulation recommended by monographs and reviews are:

WHO 1999	powdered aerial part, pressed juice and galenic preparations thereof	6-9 ml per day
ESCOP 2003	Expressed juice or other equivalent preparations	6-9 ml per day for adults. Proportional dose for children.
Kommission E	Expressed juice	6-9 ml per day
Dorsch et al. 2002	Expressed juice	3 ml per day (1-4 year) 3–5 ml per day (4-10 year) 6–9 ml per day (10-16 years)

3.3.2 Topical use

The posology for topical use (Traditional herbal medicinal product for treatment of small superficial wounds) is not available in the literature. 10 to 20 g of expressed juice per 100 g of preparation is used in most products on European market (see pages 4 to 8)

3.3.3 Duration of use

Most of the monographs (WHO, ESCOP, Kommision E) state the following general precaution: “Not to be used for more than 8 weeks”.
Since only curative but not prophylactic efficacy of *Echinacea purpurea* is demonstrated in clinical trials, this period can be shortened to 2 weeks to minimise the side effects. We therefore recommend the general precaution: “Not to be used for more than 10 days”.

3.4 Traditional use

Medicinal uses of *Echinacea* species among American Indians, were many and varied. *E. angustifolia* was universally used as an antidote for snakebite and other venomous bites and stings and poisonous conditions. *Echinacea* have been used as a remedy for more ailments than any other plant (Foster 1996). *Echinacea purpurea* was first mentioned in 1787. It was used for treating ulcers on a horses's back caused by saddles. Subsequently, the plant was largely neglected until the first edition of the *Eclectic Dispensatory* in 1852. The European history of the introduction and use of *Echinacea*

purpurea in many ways parallels its history in the U.S. By 1895 *Echinacea* products for homeopathic physicians had become available in Germany. Over the next 30 years the demand increased, while shortages were prevalent in Europe. Subsequently, in the late 1930s, commercial cultivation of *E. purpurea* began in Germany, introducing *Echinacea* products to a wide European audience for the first time. The majority of pharmacological and clinical studies conducted since 1939 have involved *E. purpurea* preparations made from the fresh expressed juice of the flowering plant (primarily Echinacin, Madaus AG, Cologne, Germany). Product forms include an ointment, a liquid form for external use, and an extract for internal use, as well as ampoules for intravenous and intramuscular injection.

Information about the use of the plant from traditional healers ranges from external application for wounds, burns and insect bites to the chewing of roots for toothache and throat infections, and internal application for pain, coughs, stomach cramps and snake bites. The interest of white settlers was also drawn to this medicinal plant. The first *Echinacea* preparation, known as Meyers Blood Purifier, arrived on the market around 1880, with rheumatism, neuralgia and rattlesnake bites as indications (Hostettman 2003).

An overview of use in EU member states is presented in the Table on page 4.

4 Safety

4.1 Toxicity

4.1.1 Single-Dose Toxicity

Echinacea purpurea and in particular the expressed juice is toxicologically well examined. Acute intoxications are not reported and on the basis of the animal experimental data are not expected.
After single p.o. or i.v. application of *Echinacea purpurea* expressed juice in dose of p.o. 15,000 mg/kg or i.v. 5,000 mg/kg on rats and p. o. 30,000 mg/kg or i.v. 10,000 mg/kg on mice the animals showed no abnormalities. Since no deaths were observed, the LD50 could only be determined by approximation method. The sections at the end of the experiments did not result in referring to organ changes (Mengs et al. 1991).
A mixture of polysaccharides from the herb of *Echinacea purpurea* and two polysaccharides, obtained from a cell culture medium of *Echinacea*, resulted after i.p. application on mice in LD50-values of >2,500mg/kg and/or >5,000mg/kg. For the two pure polysaccharides (Fucogalactoxyloglucan, acid arabino galactan) no toxicity was stated (Lenk 1989).

4.1.2 Repeat-Dose Toxicity

After 4 weeks of oral administration of *Echinacea purpurea* expressed juice in doses of 800, 2,400 or 8,000 mg/kg daily, the male rats (2,400 and 8,000 mg/kg) showed a statistically significant fall in plasma alkaline phosphatase, while the females (2,400 and 8,000 mg/kg) showed a rise in prothrombin time. Since all the values were still within the range of physiological variation for the used strain of rats, and since there was no dose proportionality, no toxicological significance can be ascribed to these findings. All the other laboratory results, together with the body weights, food consumption, ophthalmoscopy, necropsy findings and histology failed to show any evidence of relevant differences between the groups (Mengs et al. 1991).

4.1.3 Genotoxicity

Echinacea purpurea expressed juice (Echinacin and lyophilised expressed juice) in concentrations from 8 to 5,000 microg/plate were examined. The performance of the tests and the used concentrations were according the „Note for guidance on genotoxicity: Guidance on specific aspects of regulatory genotoxicity tests for pharmaceuticals“ (CPMP/ICH/141/95). In the examined bacterial test systems on

Salmonella typhimurium (TA98, TA100, TA1535, TA1537, TA1538) with and without metabolic activation by the S9-fraction from liver of Arochlor-1254-treated rats no evidence of toxicity was observed. In the mouse lymphoma assay with and without S9-fractions, in the human lymphocyte assay, and in the micronucleus assay no referring to genotoxic effects or to cell transformation effects (Mengs et al. 1991) resulted.
For the neutral polysaccharide NFA 10 from *Echinacea purpurea* tissue culture in human lymphocyte cultur neither in the short time nor in the long-term experiment in concentrations up to 500 microg/ml significant dose-dependent sister chromatid exchance (SCE) inducing activity nor a clastogenic potency was observed (Schimmer et al. 1989).
The fresh plant extract of *Echinacea purpurea* does not show mutagenic effects in the *Salmonella* microsome assay TA98 and TA100. Referring to the mentioned investigations the risk of unwanted mutagenic effects is extremely small in *Echinacea purpurea* preparations.

4.1.4 Carcinogenicity

So far no investigations are present, which permit the evaluation of a cancer risk. In a cell transformation test *Echinacea purpurea* did not produce any morphological transformation of embryonic hamster cells (Mengs et al. 1991). In-vitro model systems of this kind are gaining increasing credibility for the testing of carcinogenic potential, since there is good correlation between the results of such tests and of chronic whole-animal carcinogenicity studies. Because of the negative results of the experimental investigations for genotoxicity it is not necessary to carry out long-term carcinogenicity studies in mammals.

4.1.5 Reproductive and Developmental Toxicity

No preclinical data on reproductive and developmental toxicity of *Echinacea purpurea* is available. There is some data on reproductive and developmental toxicity from clinical studies and this data is described in Clinical overview.

Echinacea purpurea did not have an effect on sperm motility parameters (Ondrizek et al. 1999). Washed sperms were incubated in either saw-palmetto, *Echinacea purpurea*, *Ginkgo biloba*, *Hypericum perforatum* or control medium. Parameters were measured on a Hamilton-Thorn analyzer after 1, 4, 24, and 48 hr at 37°C. Sperm motility was inhibited at the 0.6 mg/ml concentration of St. John's wort. Curvilinear velocities and beat cross frequencies also decreased, but not hyperactivation. High-concentration (8 mg/ml) saw-palmetto, *Echinacea*, or *Ginkgo* inhibited motility at 24 and 48 hr. A potent inhibition of sperm motility was seen in St. John's wort unrelated to changes in pH. Furthermore, sperm viability was compromised in St. John's wort, suggesting a spermicidal effect. Metabolic changes were observed in saw-palmetto-treated sperm. High-concentration *Echinacea purpurea* interfered with sperm enzymes. *Ginkgo* did not have an antioxidant effect on sperm motility.

4.1.6 Local Tolerance

Data on local tolerance of *Echinacea purpurea* are not available.

4.1.7 Immunotoxicity

Echinacea belons to “Pharmacotherapeutic group: ATC-code: L03AW05 immunomodulators of plant origin. *Echinacea purpurea* stimulates nonspecific immune system (phagocytosis by macrophages, natural killer cells activity).
In the “Note for Guidance on immunotoxicity studies for human pharmaceuticals” (EMEA/CHMP/167235/2004) the following statement is given: “Immunotoxicity is, for the purpose of this guideline, defined as **unintended** immunosuppression or enhancement”. Immunotoxicity study of *Echinacea* is therefore not needed.

4.2 Contraindications

Hypersensitivity to plants of the Asteraceae family. As with all immunostimulants, *Echinacea* is not recommended in cases of progressive systemic disorders and autoimmune diseases immunodeficiencies, immunosuppression and diseases of the white blood cell system such as tuberculosis, leukoses, collagenoses, multiple sclerosis, AIDS or HIV-infections
This is a theoretical possibility as until recently; no immunostimulatory herbal supplements have been reported to exacerbate disorders of immune system overactivity (Lee & Werth 2004). Lee reports a case of one 55-year-old patient who was diagnosed as having pemphigus vulgaris in 1995. His medical history was notable for chronic uveitis, which required long-term treatment with systemic steroids, and osteoporosis secondary to long-term systemic steroid use. At diagnosis, the patient had not taken systemic corticosteroids for 4 months. His disease was gradually controlled with prednisone, dapsone, and azathioprine. The patient achieved complete clearance of lesions in October 1997 and continued to be clear of lesions after prednisone and azathioprine therapy was discontinued in May 1998, after which he received low doses of dapsone. In October 1998, he developed an upper respiratory tract infection and began taking an *Echinacea* supplement daily. He had never before taken an herbal supplement. He developed blisters on his trunk, head, and oral mucosa within one week of starting the supplement. He had not had oral mucosa lesions since onset of the disease. After discontinuing the use of the *Echinacea* supplement, partial disease control, but never complete remission, was achieved with prednisone, azathioprine (later changed to mycophenolate mofetil), and dapsone.

Children under 1 year of age should not use *Echinacea*, because their immune system is not fully developed.

4.3 Special warnings/precautions for use

In atopic patients severe immune reactions can occur immediately after the first exposure to *Echinacea*. Five cases were reported in Australia (Mullins & Heddle, 2002).
Regular ingestion of *Echinacea* by up to 5% of surveyed patients with atopy, combined with detection of *Echinacea*-binding IgE in atopic subjects (19% by skin testing; 20% with moderate to strong reactivity by RAST testing), raises the possibility of severe allergic reactions, even with first-time use, due to cross-reactivity with other structurally similar allergens (Mullins 1998).

The association of *Echinacea* with allergic reactions is supported by the evaluation of Huntley et al. (2005). While these reactions are reported to be rare, patients with allergy or asthma are advised to carefully consider their use of *Echinacea*.

The use in children between 1 and 12 years of age is not recommended because efficacy has not been sufficiently documented although specific risk in children over 1 year of age are not documented.

4.4 Undesirable effects

On the basis of the results of the investigations reviewed above, tolerance was well-indicated without exception in the case of oral administration. In parenteral applications, localized symptoms and fevers occasionally occurred.
In a multicentre uncontrolled study, a total of 1,231 patients with relapsing respiratory and urinary infections were treated for 4 to 6 weeks with *Echinacea purpurea* expressed juice. In 5% of patients adverse events were reported (Parnham 1996). The most frequently cited was an unpleasant taste of the study medication in 1.7% of patients, followed by nausea/vomiting (0.5%), recurrent infection (0.4%), sore throat (0.2%), abdominal pain (0.2%), diarrhoea (0.2%), difficulty in swallowing (0.2%), and other single reports (1.5%).

Kemp & Franco (2002) published a case report of leucopoenia associated with long-term use of *Echinacea*. A 51-year-old woman appeared healthy form all aspects with the exception that her white cell count had decreased from 5,800/ microlitre the preceding year to 3,300/ microlitre (normal range 4,000 to 11,000). For the past 8 weeks she had been taking 1,350 mg of *Echinacea* per day. One month after discontinuation of therapy with *Echinacea*, her white cell count had increased to 3,700/ microlitre. Next year she resumed taking *Echinacea* and after two months her white cell count was 2,880/ microlitre. Two months after discontinuing *Echinacea*, her white cell count was 3,440/ microlitre and 7 months later rose to 4,320/ microlitre.
The connection between the *Echinacea purpurea* therapy and the undesirable effect can be estimated as: certain.

Soon & Crawford (2001) reported on a case of a 41 old man with 4 recurrent episodes of erythema nodosum preceded with prodromi like myalgias, arthalgias, fever, headache and malaise, which resolved under prednisolone therapy. Comedication was loratadine as needed, St. John's wort for 6 month; and intermittent *Echinacea* for 18 month. Other reasons were excluded. After dechallenge he was free of erythema nodosum despite persistence of intermittent flulike symptoms for over a year. A rechallenge with *Echinacea* was refused by the patient. The connection between the *Echinacea purpurea* therapy and the undesirable effect can be estimated as: probable.

Logan & Ahmed (2003) reported on a 36 year old woman had taken St.John's wort, *Echinacea* and kava for 2 weeks when she developed a severe general muscle weakness, which resolved under supplementation of $NaHCO_3$ and KCl. Complaints of joint stiffness, fatigue, dry mouth and eyes surfaced 6 weeks later.The serum was negative for double stranded anti DNA, Smith and RNP antibodies. Sjogren Syndrome was diagnosed and Plaquenil treatment begun. The abnormalities renal tubular function resulting in hypokalimea and acidification with muscle weakness are reported because of a Sjogren Syndrome. Problems resolved under therapy with prednisone and cyclophosphamide, which underlines the autoimmunogenesis The connection between the *Echinacea purpurea* therapy and the undesirable effect can be estimated as: possible.

In a clinical study on children (Taylor et al. 2003) there was no difference in the overall rate of adverse events reported in the 2 treatment groups (*Echinacea* and placebo); however, rash occurred during 7.1% of the URIs treated with *Echinacea* and 2.7% of those treated with placebo (P = 0.008).

Data from clinical studies and spontaneous reporting programmes suggest that adverse events with *Echinacea* are not commonly reported (Huntley et al. 2005). Gastrointestinal upsets and rashes occur most frequently. However, in rare cases, *Echinacea* can be associated with allergic reactions that may be severe.

Pharmacovigilance reports from member states:

Ireland	8 case reports including 20 adverse reactions as follows: 1 Joint pain, 3 Abdominal pain, 1 Oedema, 1 Goitre, 1 Pharyngitis, 1 Amenhorrhoea, 1 Abnormal White Blood Cell Count, 1 Fatigue, 1 Hives, 1 Purpura, 1 Dizziness, 2 Headache, 1 Feeling of Drunkenness, 1 Hypoglycaemia, 1 Chest pain, 1 Attention Deficit Hyperactivity Disorder, 1 Bone disorder On case of positive pregnancy test in a patient taking an OCP and *Echinacea* concomitantly.
Germany	127 case reports of adverse events in patients taking *Echinacea* (in most cases in combination with other drugs) were documented in Germany from 1984 to 2006. Many adverse events happened in intravenous application of *Echinacea* extract for treating hypersensitivity, allergic rhinitis, ... In patients with no concomitant therapy besides the oral therapy with *Echinacea* the following adverse events were observed in the period 1991-1996: 6 x : diarhea, nausea 4 x : pain abdominal, rush 3 x: taste disturbance 2 x: itching, rigors, trombocytopenia, urticaria acute, vomiting 1 x: aggraviation of existing disorder, allergic reaction, allergy aggravated, asthmatic attack, cough, cramp abdominal, diarrhoea bloody, dry mouth, dyspepsia, dysphagia, ear ache, erythema, exanthema, face oedema, facial flushing, facial pain, facial swelling, fainting, fever, haematuria, headache, hepatitis A, hives, hypersensitivity, leucopenia, lip oedema, malaise, numbness localized, numbness oral, pemphigus, petechiae, pharingitis, pruritis, pustular rash, Quinckes oedema, sweating attack, swelling non-inflamatory, swelling of knees, swollen eyelids, temperature elevation, therapeutic response decreased, urticaria, vertigo

Some of undesirable events found in pharmacovigilance data and in the uncontrolled study (Parnham 1996) can not be considered as "undesirable effects", since they are the symptoms of the disease which is treated by this medicinal product. The importance of evens connected to GIT can not be estimated without the control group. Many of those undesirable events occurred also in controlled trials, but their frequency was not different to placebo.

4.5 Interactions

See 2.3

4.6 Overdose

No case of overdose has been reported.

5 Overall Conclusion

Preparations of *Echinacea purpurea* are used for many decades in humans.
Pharmacological and toxicological characteristics are specified in this report. All investigations of the acute and subacute toxicity of *Echinacea purpurea* dry expressed juice, even in the case of excessive dosages, did not result in referring to toxic effects. Also the extensive tests for a possible mutagenic potential were negative. Furthermore no malignant transformation of mammalian cells could be induced by *Echinacea purpurea* dry expressed juice in an in-vitro test.
In view of the results of the preclinical toxicological studies, clinical trials and of several decades of experience of its use in human beings, the expressed juice of *Echinacea purpurea* can be classified as a safe and well tolerated drug. Some adverse effects connected to the immunostimulatory action of *Echinacea* were observed in clinical trials and in clinical practice (rush, urticaria).

The pharmacological effects of *Echinacea purpurea* preparations on immune system were proven beyond any doubt. On the other hand the mechanism of action and the active compound are not totally clear yet.

There are many clinical studies published. Despite the fact, that they are not of optimal quality and they did not all prove the efficacy of the drug, herbal drug preparations made of *Echinacea purpurea* can be considered effective in the treatment of respiratory tract infections.

***Echinacea purpurea* has well established use** for the short-term prevention and treatment of common cold. The common cold was in most clinical trials diagnosed by the patients on the basis of first symptoms.
Traditional use of *Echinacea purpurea* in this indication is not possible, due to side effects and interactions, which, although they are very limited, can not be overweighed by the "plausible" therapeutic activity.
Traditional use for topical treatment of small superficial wounds is well documented in the overview of European market.