28 January 2014
EMA/HMPC/551899/2013
Committee on Herbal Medicinal Products (HMPC)

Overview of comments received on Public statement on *Sambucus nigra* L., fructus (EMA/HMPC/44208/2012)

Table 1: Organisations and/or individuals that commented on the draft Public statement *Sambucus nigra* L., fructus as released for public consultation on 15 April 2013 until 15 July 2013.

	Organisations and/or individuals
1	AESGP
2	PALM Research, Bergen, Norway
3	SURO, Quebec, Canada

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Table 2: Discussion of comments

GENERAL COMMENTS		
Interested party	**Comment and Rationale**	**Outcome**
SURO	I am writing on the fact that it is said that: *The requirement to show 30 years of medicinal use for an herbal preparation with a defined posology for a traditional indication is not fulfilled for elderberry.* Our company has submitted applications for Natural Products in Canada to government body "Health Canada", based on *Sambucus nigra*,L, Fructus. All our products have been granted a Natural Product Number because we have been able to prove the traditional use of elderberry with a defined posology. *Sambucus nigra* L., fructus should be included, with no doubt, in the EMA monographies because it *DOES* have a long standing use, much more than 30 years and *DOES* have posology given in the traditional references as you will see below. We thank you for the opportunity to send in comments. I cannot go thru the five years of work and research I had to do to prove this to Health Canada because I would divulgate to everyone all of my sources. I will however share some of them so you can see there is definitly traditional references that include posologies. Also, it should be noted that Health Canada has some of the highest standards in terms of Natural Products and has started this process before many other countries. The following references should be considered for the 30 years criteria for a traditional indication and they do include a posology:	Not endorsed. The available information does not fulfil the criteria for inclusion in a Community herbal monograph as outlined in the regulation, guidelines and the assessment report. *http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003644.pdf*

GENERAL COMMENTS		
SURO	GRIEVE (original 1931, this version 1959): promoting perspiration "diaphoretic" and demulcent: **Grieve, Maud Mrs, A Modern Herbal, Hafner publishing Co., New York, 1959.** "To make Elerberry Rob, 5 lb. of fresh ripe, crushed berries are simmered with 1 lb of loaf sugar and the juice evaporated to the thickness of honey. It is cordial, aperient and diuretic. One or two tablespoonsful mixed with a tumblerful of hot water, taken at night, promotes perspiration and is demulcent to the chest".	Posology, indication and daily dosage are provided, but the herbal preparation is not described in a reproducible manner. This information was already assessed and not found acceptable for inclusion in a monograph (see assessment report) for this reason. This syrup has not an exact ratio between berries, water and sugar. There are also many similarities between the syrup described in Grieve 1931 and the description of Succus Sambuci inspissatus in Pharmacopoeia Helvetica V. edition 1953. This version of Grieve from 1959 is not available, but the same information is already included in the assessed version of Grieve 1931. A syrup can be prepared as described in Grieve 1931 from elderberry and a density can be calculated and checked against the viscosity of honey, and the product can in theory be compared with a 10 ml of syrup at night as a posology for use in common cold. However, we don't have this information available, and the herbal preparation can not be recommended for inclusion in a monograph. Now included in the assessment report: *This syrup is also used according to Grieve 1931 to promote perspiration and to be demulcent to the chest. For this use the defined posology is one or two tablespoonsful mixed with a tumblerful of hot water, taken at night. It is described that the syrup should have a thickness as honey according to Grieve 1931. The description on how the syrup is prepared from berries does not provide the necessary information needed in order to calculate a defined strength that can be used to prepare a herbal preparation with an acceptable level of reproducibility from batch to batch.*

GENERAL COMMENTS		
SURO	BRITISH FLORA MEDICA (1837): for promoting diaphoretic action, sore throats and acute rheumatic affections.: **Barton B. Castle T. 1837. The British Flora Medica, London : E.Cox, St. Thomas's Street, Southwark.** "Elder Rob. Take of ripe Elder-berries five parts, sugar one part. Boil with a gentle heat to the consistency of a thick honey. This is prescribed as a diaphoretic, in the dose of an ounce and a half to two ounces, in febrile disease, and in acute rheumatic affections; likewise made into a gargle for sore throats."	Reference available at: http://archive.org/stream/britishfloramedi02bartuoft/britishfloramedi02bartuoft_djvu.txt This reference is searched for the available information, but the information was not found. No information on daily dosage. Included in the assessment report.
SURO	LA PHARMACOPÉE UNIVERSELLE OU CONSPECTUS DES PHARMACOPÉES (1828): promoting diaphoretic action: **Jourdan A.-J.-L.,1828. Pharmacopée universelle ou Conspectus des pharmacopées. J._B. Baillere, Libraire de l'Académie Royale de Médecine. Bruxelles. Belgique.** "Suc exprimé de baies de sureau. Faites dépurer par le repos, puis évaporez convenablement, sur un feu modéré. Diaphorétique: Dose, une once et demie à deux onces."	Reference available at: http://archive.org/stream/pharmacopeunive00jourgoog#page/n6/mode/2up Not included as the information is very similar to the information from the British Flora Medica already included. No information on daily dosage is provided.
AESGP	AESGP is aware of the problem that only limited information on the traditional use of elderberries is available, particularly on the posology. However, we have had a closer look into some (older) textbooks and we would like to submit the following comments. According to Hagers Handbuch 1979 (quoted in the HMPC reference list) [1], a traditional use of elderberries can in general be proven. It relates to the pressed juice from fresh berries which have been used against neuralgia and to “Roob Sambuci” as well as to the dried berries used as a laxative, diuretic and diaphoretic agent [Hager 1979 page 259, so far not mentioned in the HMPC reference list).	Not endorsed. The provided references are included and discussed in the assessment report. The provided reference to Karl 1970 with a specified posology for use in neuralgia will be included in the LoR and discussed in the assessment report. Hagers Handbuch 1979 will be included in the assessment report. For inclusion in the assessment report under 2.3 Specified

GENERAL COMMENTS		
	[1], a traditional use can be assumed. Moreover, Karl (1970) [3] describes a use in neuralgia with a posology of 2 times daily 20 g of the freshly prepared juice, each dose together with 15 g of port wine for 14 days. For these reasons we cannot support the conclusion of the Assessment Report stating that the requirement to show 30 years of medicinal use for an herbal preparation with a defined posology for a traditional indication is not fulfilled for elderberries. **References (attached)** [1] List PH, Hörhammer L (eds.) Hagers Handbuch der Pharmazeutischen Praxis, Vol. 6. Berlin-Heidelberg-New York 1979:254-264. [2] Teuscher E, Willuhn G, Loew D. Sambuci fructus. In: Wichtl M (ed.). Teedrogen und Phytopharmaka. 5th ed. Stuttgart: Wissenschaftliche Verlagsgesellschaft 2009: 601-602. [3] Karl J. Phytotherapie - Ein Lehr- und Verordnungsbuch. Puchheim: T. Marczell 1970: 312.	strength/posology/route of administration/duration of use for relevant preparations and indications Assessor's comment: *The pharmaceutical dosage form, freshly prepared juice in combination with port wine as described by Karl 1970 for use in neuralgia, is not defined as required for a herbal preparation for inclusion in a Community monograph.* *Hagers Handbuch 1979 refers to juice pressed from the fresh berries used by Epstein as Spezificum against genuine neuralgia in folk medicine ("Volksmedicine"), and it is written that addition of 20 % alcohol improves the effects.* *Neuralgia is associated with a variety of pain conditions, and is therfore considered to be an indication not suitable to prevent treatment of more serious pathologies. * Traditional herbal medicinal products are intended and designed for use without the supervision of a medical practitioner for diagnostic purposes or for prescription or monitoring of treatment.*** "Roob Sambuci" and the dried berries are included in the assessment report and listed for use as laxatives, diuretics and diaphoretic remedies in handbooks. No posology is reported. The German edition of Teedrogen Und Phytopharmaka. 5th. Ed. By Teuscher et al. 2009 is not added as other versions are included. *Public statement on the interpretation of therapeutic indications appropriate to traditional herbal medicinal products in Community herbal monographs. EMA/HMPC/473587/2011. European Medicines Agency.

GENERAL COMMENTS		
		www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2011/09/WC500115281.pdf **Directive 2004/24/EC of the European Parliament and of the Council of 31 March 2004 amending, as regards traditional herbal medicinal products, Directive 2001/83/EC on the community code relating to medicinal products for human use

SPECIFIC COMMENTS ON TEXT

<table>
<tr><th>Section number and heading</th><th>Interested party</th><th>Comment and Rationale</th><th>Outcome</th></tr>
<tr><td>Section 2.2</td><td>PALM Research</td><td>Comment: The material presented in this section clearly demonstrates an extensive use of elderberry preparations in traditional medicine in Europe. In many cases posology and specified strength is identifiable.

Proposed change (if any):</td><td>Not endorsed.

The available information does not fulfil the criteria for inclusion in a Community herbal monograph as explained in the regulation, guidelines and the assessment report.

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003644.pdf</td></tr>
<tr><td>Section 2.3

(Para. 1, lines 2-6)</td><td>PALM Research</td><td>Comment. Regarding “Specified strength/posology” a search in the literature shows that the amounts of elderberry fruit employed in a series of scientific studies range from mg quantities up to 250g/day. There is thus adequate demonstration of “Specified strength” in the literature regarding use of elderberry.

Specified strength

Table providing an overview of the various doses of elderberry preparations taken by humans as reported in the literature.
<table>
<tr><th>Author</th><th>Source of elderberry</th><th>Dose / day</th></tr>
<tr><td>Cao & Prior (1999)</td><td>Commercially available elderberry extract</td><td>100g*</td></tr>
<tr><td>Cao et al (2000)**</td><td>Commercially available elderberry extract</td><td>50g*</td></tr>
<tr><td>Milbury et al (2002)**</td><td>Commercially available elderberry extract</td><td>50g*</td></tr>
</table>
</td><td>Not endorsed.

Specified strength

Comment to table providing an overview of the various doses of elderberry preparations taken by humans as reported in the literature.

Overall the existing data mentioned in this table cannot be considered to meet the criteria for "well-established medicinal use" or “traditional use” in accordance with Directive 2001/83/EC as amended Three controlled clinical studies have been conducted to determine the effectiveness of herbal preparations of elderberry, with very small numbers of patients. In two of the clinical trials Zakay-Rones et al. (1995, 2004) studied the effectiveness of elderberry on flu treatment and the third one by Murkowic (2004) on blood lipids reduction. The other studies mentioned in this overview are not</td></tr>
</table>

Wu et al (2002)**	Commercially available elderberry extract	50g*
Murkovic et al (2004)	Iprona, Lana, Italy	3 – 30g
Bitsch et al (2004a)	Wild Heidelberg, Germany	10g*
Bitsch et al (2004b)**	Wild Heidelberg, Germany	250g*
Frank et al (2005)**	Wild Heidelberg, Germany	250g*
Netzel et al (2005)**	Institute of Enology and Beverage Research at Geisenheim, Germany	25-50g*
Franck et al (2007)**	Wild Heidelberg, Germany	20 -130g*
Chrubasik et al (2008)	GmbH Immensee	100g***
Kong (2009)	HerbalScience Singapore Pte. Ltd. (Lozenges)	0.7g
Vlachojannis et al (2010)	Handpicked berries (German traditional medicine)	50 – 100g
Zakay-Rones et al 1995; 2004	**Sambucol®** Europe and worldwide. Made commercially available in 1998.	7.6-20g

*Calculated from data provided by Wu et al (2006)

**Studies approved by local ethical committees

***Based on data in Akbulut et al (2009)

References

Akbulut, M., Ercisli, S. & Tosun, M. (2009). Physico-chemical characteristics of some wild grown European elderberry (*Sambucus nigra* L.) genotypes. *Pharmacognosy Magazine.* **5**, 320-323.itsch, I., Janssen, M., Netzel, M., Strass, G. & Frank, T. (2004a). Bioavailability of anthocyanidin-3-glycosides following consumption of elderberry extract and blackcurrant juice. *International Journal of Clinical Pharmacology and Therapeutics*. **42**, 293-300.

fulfilling the criteria for assessment for well-established or traditional use. Results from two clinical studies indicate possible effectiveness of elderberry aqueous extract for treatment of influenza suggesting a faster recovery. More studies are needed to confirm this effect as required. The provided references on bioavailability will be assessed for possible inclusion in the assessment report and list of references. A posology listed in a clinical study is only valuable as evidence for traditional use when 30 years of use with this posology is documented, and 10 years is needed to document well-established use as described.

Annex I of Directive 2001/83/EC lays down specific rules for the demonstration of a well-established medicinal use, with recognized efficacy and an acceptable level of safety.

Posology

(a) Traditional German Medicine.

These posologies are assessed. They are not complying with the established requirements for inclusion in Community monographs. See assessment report.

(b) Sambucol® Black Elderberry Immune System Support Dietary Supplement and posology

The requirements are not fulfilled. Posologies listed in clinical studies are only valuable as evidence for traditional use when 30 years of use with a posology is

		Bitsch, R., Netzel, M., Sonntag, S., Strass, G., Frank, T. & Bitsch, I. (2004b). Urinary excretion of cyanidin glucosides and glucuronides in healthy humans after elderberry juice ingestion. *Journal of Biomedicine and Biotechnology.* Issue **5**, 343-345. Cao, G. & Prior, R.L. (1999). Anthocyanins are detected in human plasma after oral administration of an elderberry extract. *Clinical Chemistry*. **45**, 574-576. Cao, G., Muccitelli, H.U., Sanchez-Moreno, C. & Prior, R.L. (2000). Anthocyanins are absorbed in glycated forms in elder women: a pharmacokinetic study. *American Journal of Clinical Nutrition*. **73,** 920–926. Chrubasik, C., Maier, T., Dawid, C., Torda, T., Schieber, A., Hofmann, T. & Chrubasik, S. (2008). An observational study and quantification of the actives in a supplement with *Sambucus nigra* and *Asparagus officinalis* used for weight reduction. *Phytotherapy Research.* **22**, 913–918. Frank, T., Sonntag, S., Strass, G., Bitsch, I., Bitsch, R. & Netzel, M. (2005). Urinary pharmacokinetics of cyanidin glycosides in healthy young men following consumption of elderberry juice *International Journal of Clinical Pharmacology Research.* **25**, 47-56. Frank, T., Janssen, M., Netzel, G., Christian, B., Bitsch, I. & Netzel, M. (2007). Absorption and excretion of elderberry (*Sambucus nigra L.*) anthocyanins in healthy humans. *Methods and Findings in Experimental Clinical Pharmacol*ogy. **29**, 525.	documented, and 10 years is needed to document well-established use as outlined in annex I of Directive 2001/83/EC. **(c) Posology for commercially available elderberry preparations.** The requirements for inclusion of these herbal preparations are not fulfilled. Posologies for commercially available elderberry preparations are only valuable as evidence for traditional use when 30 years of use with this posology is documented, 10 years is needed to document well-established use as outlined in annex I of Directive 2001/83/EC. Suggested for inclusion in the assessment report : *Information on period of medicinal use in the community* *Elderberry is still commonly used for various purposes according to published studies and from what is seen in health food stores as food supplements and marketed on various Internet webshops within EU.*

		Milbury, P.E., Cao, G., Prior, R.L. & Blumberg, J. (2002). Bioavailablility of elderberry anthocyanins. *Mechanisms of Ageing and Development*. **123**, 997–1006. Murkovic, M., Abuja, P.M., Bergmann, A.R., Zirngast, A., Adam, U., Winklhofer-Roob, B.M. & Toplak, H. (2004). Effects of elderberry juice on fasting and postprandial serum lipids and low-density lipoprotein oxidation in healthy volunteers: a randomized, double-blind, placebo-controlled study. *European Journal of Clinical Nutrition*. **58**, 244–249. Netzel, M., Strass, G., Herbst, M., Dietrich, H., Bitsch, R., Bitsch, I & Frank, T. (2005). The excretion and biological antioxidant activity of elderberry antioxidants in healthy humans. *Food Research International*. **38**, 905–910. USDA (U.S. Department of Agriculture, Agricultural Research Service). (2010). National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page. http://www.ars.usda.gov/ Vlachojannis, J.E, Cameron, M. & Chrubasik, S. (2010). A systematic review on the Sambuci fructus effect and efficacy profiles. *Phytotherapy Research*. **24**, 1-8. Wu, X., Cao, G. &. Prior, R.L. (2002). Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. *Journal of Nutrition*. **132,** 1865-1871. Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B. et al. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal Agricultural Food Chemistry*. **54**, 4069–4075.	

		Zakay-Rones, Z., Varsano, N., Zlotnik, M. et al (1995). Inhibition of several strains of influenza virus *in vitro* and reduction of symptoms by an elderberry extract (*Sambucus nigra* L.) during an outbreak of influenza B Panama. *Journal of Alternative and Complementary Medicine*. **1**, 361-369. Comment. Before being carried out many of the studies referred to in the table above received approval from a local Ethics Committee, including a study where a dose equivalent to a given strength of 250g elderberries was used. One must assume that the doses used were known not to cause adverse events before permission for use in human studies was granted. It is important to note that the amount used (i.e. a daily dose of approximately 100g of elderberries) is in line with that adopted in traditional German medicine (in common use for many centuries, as reported by Vlachojannis et al, 2010). It would seem highly likely that the decisions of the various Ethics Committees, regarding the awards of approval, were based on such knowledge. One can therefore conclude that elderberry doses of at least 250g are acceptable. In the study performed by Chrubasik et al (2008) it was evident that a daily intake of about 100g elderberries over a period of 15 days had in fact health promoting effects since the participants (80 individuals) at the end of the study reported that both their physical and emotional well-being, and hence the quality of life, were significantly improved. The suppliers of the commercially available elderberry product Sambucol®) recommend a dose equivalent to a standard strength of 7.6 g elderberries per day to maintain a healthy immune system, or 20g/day for intensive use to alleviate the symptoms associated with a cold or influenza. This dose has	

		received worldwide acceptance following its use for almost 20 years, and “No adverse events” have been recorded. For more detail see b) below. One can thus conclude that a daily intake of 7.6 - 20g elderberries can be considered as safe and thus represent recommendable doses (of specified strength) for human use. Sambucol® has been in use in the EU for approximately 15 years. It must be pointed out that 10 fold higher doses have been used in human studies recommended by different Ethical Committees in the USA and Europe, such that amounts far in excess of 20g elderberries per day are clearly acceptable for human use. **2. Posology** **(a) Traditional German Medicine.** Vlachojannis et al (2010) have written a systematic review on the “Sambuci fructus, effect and efficacy profiles”. In the Introduction p.1 we read : “*The dried ripe or fresh berries of Sambucus nigra L. (European elder, Fam. Caprifoliaceae) are used in traditional German medicine for the treatment of constipation, to increase diuresis, as a diaphoretic in upper respiratory tract infections, for the alleviation of low back and/or neuropathic pain, headache and toothache. For treatment of these complaints, patients consume elderberry juice or they drink a cup of tea (aqueous extract) several times per day. The infusion is prepared from 10 g dried berries standing in cold water for several minutes, then slowly heated up, and briefly boiled. Before filtering, a drawing-time of 5 to*	

		10 min is recommended." Comment. A tea was prepared from 10g dried berries, and drunk several times a day. From 5 to10 cups a day would be equivalent to an intake of up to 100g elderberries per day in the form of an aqueous extract. Since this amount was adapted for use in traditional German medicine (Anonymous 1994) it must therefore represent an acceptable level of intake i.e. specified strength. This is taken as substantial evidence for the early establishment of a posology at a specified strength in traditional medicine in Europe. **References.** Anonymous (1994). *Sambucus nigra* L. In : Hagers Handbuch der Pharmazeutischen Praxis Bd. 6, eds. Hänsel, R., Keller, K., Rimpler, H. and Schneider G. Springer Press, Berlin, Heidelberg, New York, pp. 579-586. Vlachojannis, J.E, Cameron, M. & Chrubasik, S. (2010). A systematic review on the Sambuci fructus effect and efficacy profiles. Phytotherapy Research. 24, 1-8. **(b) Sambucol® Black Elderberry Immune System Support Dietary Supplement and posology** According to the product information available at the site : (http://www.sambucolusa.com/store/sambucolstory) Sambucol® was developed in Europe in 1991 as a result of 20 years of research, and, as a natural product, has been marketed in Europe since 1998 for the treatment of colds and flu. The efficacy of the preparation was tested in a placebo-controlled, double-blind clinical study during an outbreak of	

		influenza B/Panama (Zakay-Rones et al 1995). It is regarded as a safe and well-trusted product. Directions for use **:** Adults: Take 2 teaspoons daily Children: Take 1 teaspoon daily For Intensive Use Adults: Take 1 tablespoon four times daily. Children: Take 1 tablespoon two times daily For daily maintenance of the immune system the suppliers recommend that adults should take 2 teaspoons of **Sambucol®** per day. From the product specification it is evident that 1 teaspoon consists of an extract of 3.8g elderberries. Thus a recommended daily dose (posology) for adults would be equivalent to an intake of 7.6g /day. For intensive use we read that the recommended posology is "1 tablespoon four times daily" i.e. a daily intake of approximately 20g elderberries. Thus two different doses are recommended, the lower dose being to maintain an active immune system (i.e. for daily use) and the higher dose to combat the symptoms associated with an outbreak of a cold or influenza. The amounts suggested for intake are judged to be safe by the suppliers of the product, who recommend the following: "*Take **Sambucol®** every day for continuous immune system support*". Comment. **Sambucol®** has been used in the EU for approximately 15 years and the daily posology is well defined for both adults and children for a traditional indication i.e. colds and flu. Posology is stipulated at two different doses (specified strengths) for either "Daily Maintenance" or for "Intensive use".	

		(c) Posology for commercially available elderberry preparations. There are a large number of elderberry preparations available in the EU where specific strength and posology is indicated: Sambucol - Original Formula (chewing tablets) «Immune system support» «boosts immunity» 1 tablet contains 130 mg dried exstract Posology 1 tablet twice daily. 2 tablets 3 times daily for intensive use (equivalent to 780 mg dry extract) A.Vogel – Anti-Ageing Complex (gelatin capsules) 1 capsule contains 100 mg fruit concentrate - Posology : 2 capsules daily Solray – Sambuactin (chewing tablets) «Guaranteed potency» 1 tablet contains 200 mg elderberry extract Also contains 100 mg vitamin C. - Posology 2 tablets daily Solray – Sambuactin (120 ml liquid extract) «Guaranteed potency» 10 ml contains 4.67g elderberry extract Posology: 2 tea spoons (10 ml) daily Frøy – Elderberry (200 ml concentrated elderberry juice) Active against flu and influenza Posology = 10 ml daily Intensiv use, posology = 40 ml Natures Answer – Elderberry (118 ml extract) 1 ml is equivalent to 1 g dried berries Posology: 1 tea spoon (10 ml) 4 times daily when the first	

		symptoms have become evident Waldholler – Kraftrunk (180 g powder) - 15g powder mixed with 200 ml water Posology = equivalent to 5.25 g powder/day ViaBiona – Schwarzer Holunder (60 capsules = 37 g) Berry and flower extract NeutraCeutical Posology: 2 capsules Each capsule contains an equivalent of 500 mg powder of elderberry flowers + 500 mg powder of berries Soria Natural – Holunder Complex (250 ml concentrate) To stregthen the immune system Aqueous extract of elderberry flowers + 13% berry extract + vitamin C Posology: 10 ml/day Dr. Hall vital-control (250 g powder) Posology: 25 g powder mixed with 250 ml varm (but not boiling) water Daily dose: 125 mg elderberry powder Rubini – (30 capsules) Strengthens the immune system Posology: 1 capsule daily (465 mg Rubini ProFlavon Complex) Posology for intensive use: 3-5 capsules daily Comment. That such a large number of elderberry preparations are available for medicinal use in Europe, where specific strength and posology is stipulated, indicates the wide use of elderberry in the EU. From the data available for products on the market it is evident that daily doses between 1g and 10g are recommended. The actual dose depends on the	

		manner of preparation of the individual product (tea, extract, concentrate, dried berries, freeze dried powder etc). Proposed change (if any):	
Section 3.1 Page 16/26, line 10	PALM Research	Comment. The Rapporteur states : "There is not enough scientific information available to conclude on an immunomodulatory effect or on an antibacterial effect of any of these elderberry extracts" More substantiating information has now appeared in the scientific literature. These two issues are addressed below : 1. Immunomodulatory effect. (a) In a recent paper the immunomodulatory effects of elderberry (both flower and berry) are documented : Title : "Comparison of Carbohydrate Structures and Immunomodulating Properties of Extracts from Berries and Flowers of *Sambucus nigra* L." Authors : Hilde Barsett, Torun H. Aslaksen, Parakastha Gildhyal, Terje E. Michaelsen and Berit Smestad Paulsen. European Journal of Medicinal Plants 2 (2012) 216-229. Quoting from this article : "Abstract Aims: To investigate if the immunomodulating activity of compounds present in berries and flowers of S. nigra were of the same order, or different, and also if the most active components were of high or low molecular weight nature. Methodology: The immunomodulating effects were investigated using a complement fixing assay as well as a system for measuring the production of NO after stimulation of	Partly accepted. Both Barsett *et al.* 2012 and Krawitz *et al.* 2011 is now included in the assessment report and list of references (LoR). *Barsett H; Aslaksen, TH; Gildhyal P; Michaelsen TE, Paulsen. BS Comparison of carbohydrate structures and immunomodulating properties of extracts from berries and flowers of Sambucus nigra L. European Journal of Medicinal Plants, 2012, 216- 229* Suggestion for alteration of the Assessors comment included in the assessment report: This comment is added in Assessor's comment in 3.1 in the assessment report. *Information on immunomodulatory and antibacterial effects are available, but the tested herbal preparations are not fulfilling the requirements for inclusion in a monograph.* And the following phrase is suggested deleted as it is inconsistent to stress results from one study when there are more than one: *An elderberry extract has shown inhibition of hemagglutinin and replication of common human and animal influenza A and B in vitro.* This abstract of what was studied and found is also included: *Barsett et al. (2012) studied the immunomodulatory*

		macrophages with the different fractions. Page 222, lines 3–5: "One of the objects for the present study was to investigate if there were compounds present in the extracts of berries and flowers of S. nigra* that would have an immunomodulating effect." *Source of commercially available plant material (see page 218) : "Dried berries (prod. no. 100308) and flowers (prod. no. 100309) were purchased from Odin´s Marked, Norway (org. no. 876905892)." The authors tested the immunomodulatory effect of elderberry in two ways : 1. We therefore tested the effect in the complement assay, a test system that indicates immunomodulating activity. The complement fixing test is based on inhibition of hemolysis of antibody sensitized sheep erythrocytes (SRBC) (Michaelsen et al., 2000). The activity for all fractions was concentration dependent at the range of concentrations." The results are presented in Fig. 2 on page 222. The authors state (page 223, lines 1-3) "Interestingly, all the crude extracts apart from 50WSnBe, had an effect in the complement assay equal to or higher than that of the very active polysaccharide standard compound PMII, isolated form Plantago major (Samuelsen et al., 1996)." 2. Page 225, (para. 2, lines 1-2) The ability to activate mouse macrophages was also determined for the high molecular weight fractions from both berries and flowers. The production of nitric oxide (NO) was measured after treatment of the macrophages with the extracts for 24 hrs. LPS, a constituent of the outer membrane of gram negative bacteria, was utilised as	*effects in order to clarify the pharmacology of substances from Sambucus nigra L. They wanted to investigate if the immunomodulating activity of compounds present in berries and flowers of S. nigra were of the same order, or different, and also if the most active components were of high or low molecular weight nature. Defatted material of berries and flowers of S. nigra were extracted with 50% ethanol and with water of 50 C and 100 C. High molecular weight fractions were obtained after gel filtration on BioGelP6DG. The different fractions were investigated for their monosaccharide contents and carbohydrate structures. The immunomodulating effects were investigated using a complement fixing assay as well as a system for measuring the production of NO after stimulation of macrophages with the different fractions. All fractions contained substantial amounts of carbohydrates. Removal of low molecular weight material revealed polysaccharide fractions containing monosaccharides typical for pectins and showed enhanced bioactivity. High molecular weight fractions from elderflowers showed higher bioactivity than the equally extracted fractions from elderberries. The 100 C water flower fraction gave after gel filtration the fraction with the highest activity and with the longest backbone of rhamnogalacturonan I.* Included in LoR: *Krawitz C, Mraheil MA, Stein M, Imirzalioglu C, Domann E, Pleschka S, Hain T. Inhibitory activity of a standardized elderberry liquid extract against clinically-relevant human respiratory bacterial pathogens and*

		a positive control, as it is a potent stimulator of cells of the monocytic lineage (Sweet and Hume, 1996). From Fig. 4 it is interesting to note that the fraction 100WSnFl-1 was the most active fraction in this assay while only the 50%ethanol-water extract form the berries (SNBe50-1) showed to induce production of NO. This effect was comparable to that of fraction 50WSnFl-1. The authors conclude : Page 225 (para. 3, lines 1-2) – "The extracts from berries and flowers of S. nigra all contained polysaccharides and they had varying effects in immunomodulating test systems." Additionally, Barsett et al identified the presence of xyloglucans, arabinogalactans, homogalacturonan and rhamnogalacturonan in elderberry extracts (see 3rd paragraph, p. 223). All these are well known in the literature and have been extensively documented as being immunomodulatory molecules [for example see "Immunomodulatory dietary polysaccharides: a systematic review of the literature". Authors : Jane E Ramberg, Erika D Nelson, Robert A Sinnott. Nutrition Journal 9 (2010) 54-75]. Comment. From the work of Barsett et al (2012) it is evident that specific fractions isolated from elderberry fruit exhibit immunomodulatory effects. Furthermore, individual molecules characterized in the elderberry preparation were identified by others as having immunomodulatory properties. (b) It is well established that lectins have an immune function [see for example chapter 13.8.1 in S.A. Brooks, M.V. Dwek and U. Schumacher, "Functional & Molecular Glycobiology", BIOS Scientific Publishers Ltd. 2002, or E.J.M. Van Damme, W.J.	*influenza A and B viruses. BMC Complementary and Alternative Medicine 2011, 11:16.* http://www.biomedcentral.com/1472-6882/11/16 Included in assessment report: *Krawitz et al. (2011) analyzed a standardized elderberry extract (Rubini, BerryPharma AG) for its antimicrobial and antiviral activity using the microtitre broth micro-dilution assay against three Gram-positive bacteria and one Gram-negative bacteria responsible for infections of the upper respiratory tract, as well as cell culture experiments for two different strains of influenza virus. The antimicrobial activity of the elderberry extract was determined by bacterial growth experiments in liquid cultures using the extract at concentrations of 5%, 10%, 15% and 20%. The inhibitory effects were determined by plating the bacteria on agar plates. In addition, the inhibitory potential of the extract on the propagation of human pathogenic H5N1-type influenza A virus isolated from a patient and an influenza B virus strain was investigated using MTT and focus assays. It was shown that a standardized elderberry liquid extract possesses antimicrobial activity against both Gram-positive bacteria of Streptococcus pyogenes and group C and G Streptococci, and the Gram-negative bacterium Branhamella catarrhalis in liquid cultures. The liquid extract also displays an inhibitory effect on the propagation of human pathogenic influenza viruses. Rubini elderberry liquid extract showed activity against human pathogenic bacteria as well as influenza viruses.*

		Peumans, A. Pusztai, and S. Bardocz, in "Handbook of Plant Lectins: Properties and Biomedical Applications", John Wiley and Sons, 1997]. As indicated in the EMA assessment report on *Sambucus nigra* L., fructus (page 14/26 “Lectin activities” there are a number of lectins found in elderberry. Of these the binding of SNA-II to receptors on the surfaces of different types of cells found in Peyer´s patches has been studied by Sharma et al. (1996), where human biopsy material from the small intestine was used. The lectin was shown to bind to M-cells, enterocytes and Goblet cells of the follicle-associated epithelium, goblet cells of the villus epithelium and macrophages found in the gut-associated lymphoid tissue (GALT). The binding of the lectin to GALT will inevitably result in the initiation of an immune response. An interaction between GALT and mistletoe lectins (share similar biological properties with elderberry lectins) has been demonstrated in a pilot experiment performed by Winge et al (2010) where circulating antibodies to the lectins were observed in human subjects following oral intake of a preparation enriched in mistletoe lectins. Since Sharma et al (1996) showed that mistletoe lectin (ML-II) demonstrates similar binding in GALT to that exhibited by elderberry lectin SNA-II then it is almost certain that they will share the same immunomodulatory properties. Pusztai (1993) states : “In addition, lectins which are transported across the gut wall into the systemic circulation can modulate the body's hormone balance, metabolism and health. Although these physiological effects are mediated or reinforced by immune responses, they are primarily the result of the specific chemical reactivity of lectins with cell surface	The articles with data on lectins from other plants are not included as they give general information on lectins not directly useful for the assessment of Sambuci fructus. This is a general policy practised in the assessment of general effects of constituents of herbal material by the HMPC. The remaining studies are considered to be of minor importance for the assessment of Sambuci fructus preparations at this point. These studies will be included and assessed when herbal preparations fulfilling the criteria for inclusion in a monograph are found for Sambuci fructus.

		receptors of the gut". Comment. It is well known that elderberry fruit contains lectins and it has been clearly established that lectins exhibit immunostimulatory properties. The elderberry lectins will thus contribute to an immunomodulatory effect. **References.** Pusztai, A. (1993). Dietary lectins are metabolic signals for the gut and modulate immune and hormone functions, European Journal of Clinical Nutrition, 47, 691-699. Sharma, R., van Damme, E.J., Peumans, W.J., Sarsfield, P., and Schumacher, U. (1996). Lectin binding reveals divergent carbohydrate expression in human and mouse Peyer's patches. Histochemistry and Cell Biology, 105, 459-465. Winge, I., Dale, T.M. Tilrem, P. and Pryme, I.F. (2010). A mistletoe lectin-containing preparation for oral use provokes an immune response and induces an increase in the population of activated natural killer cells. In: Comprehensive Bioactive Natural Products vol. 5, Immune-modulation & Vaccine adjuvants Ed. V.K. Gupta. pp. 279-295. Studium Press LLC, USA. ISBN 1-933699-55-8. (c) In a recent review article on *Sambucus nigra* (Lim, 2012) the author termed a section "Immunomodulatory Activity" (page 36). Quoting from this article : "Immunomodulatory Activity In addition to its antiviral properties, Sambucol Elderberry Extract and its formulations were found to stimulate the	

		healthy immune system by increasing inflammatory cytokine production (Barak et al, 2001). Production of inflammatory cytokines (interleukin IL-1 beta,IL-6, IL-8, tumour necrosis factor – TNF □) we Sambucol Black Elderberry Extract (2-45 fold), as compared to LPS, a known monocyte activator (3.6-10.7 fold). The most notable increase was observed in TNF □ In a follow-on study, the Sambucol preparations increased the production of five cytokines that included four inflammatory cytokines (interleukin IL-1 beta, tumour necrosis factor – TNF and IL-6 and IL-8), and one anti-inflammatory cytokine (IL-10) by 1.3-6.2 fold compared to the control (Barak et al, 2002). The three Sambucol formulations activated the healthy immune system by increasing inflammatory and anti-inflammatory cytokine production. Sambucol may therefore be beneficial to the immune system activation and in the inflammatory process in healthy individuals or in patients with various diseases. Sambucol could also have an immuno-protective or immunostimulatory effect when administered to cancer or AIDS patients, in conjunction with chemotherapeutic or other treatments." **References.** Barak, V., Halperin, T. & Kalickman, I. (2001). The effect of Sambucol, a black elderberry-based, natural product, on the production of human cytokines. 1. Inflammatory cytokines. Eur. Cytokine Netw. 12, 290-296. Barak, V., Birkenfeld, S., Halperin, T. & Kalickman, I. (2002). The effect of herbal remedies on the production of human inflammatory and anti-inflammatory cytokines. Isr. Med. Assoc.	

		J. 4, S919-S922. Lim, T.K. (2012). *Sambucus nigra*: In "Edible Medicinal and Non-Medicinal Plants", Vol. 1, pp. 30-44. Springer-Science. Comment. Convincing evidence from numerous studies has demonstrated an immunomodulatory effect of elderberry. **Antibacterial effect.** Antibacterial effects of elderberry preparations have been reported by Chatterjee et al (2004) and Hearst et al (2010). Both were cited in the EMA Assessment report on *Sambucus nigra* L., fructus. Additional evidence describing the antibacterial effect of a standardized elderberry liquid extract : Krawitz et al (2011) have published new relevant data in their paper entitled "Inhibitory activity of a standardized elderberry liquid extract against clinically-relevant human respiratory bacterial pathogens and influenza A and B viruses" authors Christian Krawitz, Mobarak A Mraheil, Michael Stein, Can Imirzalioglu, Eugen Domann, Stephan Pleschka and Torsten Hain. BMC Complementary and Alternative Medicine 2011, 11:16. Quoting from their paper : Krawitz C, Mraheil MA, Stein M, Imirzalioglu C, Domann E, Pleschka S, Hain T. Inhibitory activity of a standardized elderberry liquid extract against clinically-relevant human respiratory bacterial pathogens and influenza A and B viruses. BMC Complementary and Alternative Medicine 2011, 11:16. http://www.biomedcentral.com/1472-6882/11/16	

		"Results For the first time, it was shown that a standardized elderberry liquid extract (Rubini, BerryPharma AG) possesses antimicrobial activity against both Gram-positive bacteria of Streptococcus pyogenes and group C and G Streptococci, and the Gram-negative bacterium Branhamella catarrhalis in liquid cultures. The liquid extract also displays an inhibitory effect on the propagation of human pathogenic influenza viruses." Conclusion Rubini elderberry liquid extract is active against human pathogenic bacteria as well as influenza viruses. The activities shown suggest that additional and alternative approaches to combat infections might be provided by this natural product. Comment. Taking into account the above discussion it is evident that an antibacterial effect of a commercially available elderberry extract is now well documented. Proposed change (if any): There is now sufficient scientific evidence available for one to conclude that elderberry extracts re immunomodulatory and demonstrate antibacterial effects.	
p.25	SURO	Comment: Same as above. Proposed change (if any):	This is the overall conclusion. See answer to general comment.