Microbiologists, toxicologists and chemists have created a list of preservatives of known efficacy and toxicity and these have been listed in Annex VI of Commission Directive 1976/768/EEC, to be replaced by Annex V of Regulation (EC) No 1223/2009 of The European Parliament and of the Council in July 2013. This later document defines preservatives as substances which are exclusively or mainly intended to inhibit the development of micro-organisms in the cosmetic product. Article 14 states cosmetic products shall not contain preservatives other than those listed in Annex V.

Despite these clearly stated regulations a significant proportion of consumers have been persuaded that the acknowledged preservatives are unsuitable for their needs and seek alternative systems. Ingredient suppliers have been looking to supply this niche market and a variety of possible systems have been developed.

For all formulators interested in reducing reliance on established preservatives I can recommend the book by Kabara and Orth, Preservative-free and Self-preserving Cosmetics and Drugs [Ref 1]. It was published in 1997 so the search for ways of reducing or eliminating established preservatives in cosmetic products is not a new science. Kabara and Orth proposed the use of hurdle technology, which involves reducing water activity and availability, lowering pH, chelating metal ions, and disruption of cell membranes with surface active ingredients. The book includes chapters on the effects of pH, water activity, surfactants, fatty acids and esters, biomimetic phospholipids, antioxidants, fragrance components and chelating agents on micro-organisms.

An introduction to hurdle technology is available from **Innolex** as Alternative Preservation – a safe and sensible solution for cosmetic formulation. It describes the many ways that cosmetic products may be preserved while avoiding those in Annex V and also briefly describes the mechanism that makes alternative systems work. For information about the constituents of essential oils and plant extracts and their antimicrobial properties refer to dweckdata.com. Tony Dweck is the acknowledged expert on all things natural and his web site includes copies of his many lectures and papers on the subject plus details of his remarkable books on cosmetic ingredients [Ref 2].

Surprisingly, although the general trend has been towards a reduction in the use of established preservatives in cosmetics a look at the INCI listing of well-known brands reveals the presence of preservatives in anhydrous products; in products with a high proportion of ethanol; in antiperspirants with 25% aluminium chlorhydrate and in products at extremes of pH. Other products reveal an excessive number of preservatives where the formulator has lacked attention to detail and additional preservatives have found their way into the formula via preserved additives. The first step in avoiding Annex V preservatives is to determine whether preservatives are actually necessary.

There are extra difficulties for companies wishing to claim natural or organic certification. The various certification bodies have their own ideas of what constitutes good and bad preservatives. Thus parabens are bad, benzyl alcohol is good; phenoxyethanol is bad, dehydroacetic acid is good. In fact organic acids such as

sorbic acid and benzoic acid are generally approved despite the need for acidic pH and their poor spectrum of activity. Maybe someone did not understand the term organic! Throughout this article Ecocert has been selected as representative of all certification bodies but there may be some differences.

Reviewing alternative preservative systems they fall into four main groups: those based on preservatives listed in Annex V that are allowed as preservatives in cosmetic products where approval is required by Ecocert to claim natural or organic certification. There are synthetic materials that are not in Annex V but have an antimicrobial effect: these include caprylyl glycol, certain fatty acids and some fatty acid esters. The third group relies on extracts of plants and essential oils. The fourth group are materials that occur in nature but are produced in commercial quantities by synthesis or fermentation procedures; this includes gluconic acid and its esters such as gluconolactone and zinc gluconate.

Referring to the list of preservatives allowed by Ecocert I fail to understand why benzyl alcohol, a named fragrance allergen, can be considered suitable. If it is present in a fragrance such that its concentration exceeds 0.001% in a leave-on product it must be declared but it is allowed at up to 1% as a preservative. It is reported to be most active against Gram-positive bacterial, to have some activity against Gram-negative bacteria and yeast but to be weak against mould. It is inactivated by non-ionic emulsifiers and surfactants and its activity is poor at pH>7. Its prime function would seem to be as a solvent for other antimicrobial ingredients.

The organic acids permitted by Ecocert with their maximum concentration allowed in the finished product under Annex V of the recast EU regulations are benzoic acid (0.50%), dehydroacetic acid (0.60%), sorbic acid (0.60%) and salicylic acid (0.50%). Salicylic acid may be used at higher levels if its primary purpose is not as a preservative. The salts of these acids may also be used, which improves their water solubility. The activity of these acids and their salts is very pH dependent and the final compositions must be at acid pH; at pH \leq 2 for salicylic acid and its salts; pH \leq 3.0 for benzoic acid and benzoates; pH \leq 4.5 for sorbic acid and sorbates and pH \leq 6.0 for dehydroacetic acid and its salts. They are most effective against fungi, have limited activity against bacteria and lack activity against pseudomonads [Ref 3]

There are many sources of organic acids but some suppliers have produced mixtures that include them with other materials to enhance their activity. For example **Lonza** supply Geogard ECT [INCI: benzyl alcohol, salicylic acid, sorbic acid and glycerin] and Geogard 221 [INCI: Dehydroacetic acid and benzyl alcohol]. Both may be used in cosmetics seeking Ecocert approval. They are more frequently found in mixtures with phenoxyethanol, which improves their solubility and spectrum of activity but it is not an Ecocert approved ingredient.

Higher molecular weight glycols have antimicrobial activity and the longer the chain length the lower the level needed to be effective. Thus glycerin needs to represent at least 40% of the formulation; butylene glycol 10% and caprylyl glycol is effective above 1%. They are more effective against bacteria than fungi and also make good solvents for other antimicrobial ingredients. Caprylyl glycol is one of the more

Cosmetic Preservation 1st Published in SPC - 2012 John Woodruff popular multifunctional ingredients. It is added as a humectant and emollient to emulsions but also has antimicrobial activity.

Fatty acids like lauric acid and fatty acid esters such as glyceryl laurate have an established antibacterial action. Sucrose esters may also be useful and **Sisterna** has published data and suggested applications that demonstrate their anti-microbial properties. Those with a short chain length and a high degree of substitution are the most effective and these are marketed as Sisterna L-70C and Sisterna SP-70C. The L-70C is a solution of sucrose laurate in aqueous/ethanol and SP-70C is sucrose stearate. They are not completely effective as product preservatives but can be used in association with other ingredients for this purpose. Sisterna suggests their prime use is in products to treat body malodour, athlete's foot and dental caries and also nappy rash and dandruff.

Dr Straetmans is a well-established supplier of multi-functional ingredients and has a wide range of materials that have antimicrobial properties as well as being useful as emulsifiers, emollients or humectants. They are sold under the Dermosoft trade name and are created using natural or nature-identical materials. The active content of Dermosoft materials includes p-anisic acid and levulinic acid and their sodium salts; glyceryl caprylate and sodium lauroyl lactylate.

Levulinic acid is derived from sugar and is much used in preserving foodstuffs. Anisic acid is found in anise and is prepared by the oxidation of anethole, which occurs naturally in a wide range essential oils. A paper presented before the Hellenic⁻ Society of Dermatology and Venerology, Athens, Greece in 2009 [Ref 4] described an investigation into the antimicrobial efficacy of extracts of *Lonicera raprifidmm* and *Lonicera japonica* in combination with glyceryl caprylate and/or levulinic acid, p-anisic acid, and ethanol. All the tested antimicrobial systems exerted excellent activity against Gram-positive and Gram-negative bacteria in an acidic (pH 5.5) environment. The conclusion was that the addition of multifunctional ingredients such as glyceryl caprylate, levulinic acid, p-anisic acid and ethanol were beneficial as preservatives and that ethanol at a concentration of 5% w/w improved the performance of the materials under test.

Usnic acid is extracted from lichens, particularly from Usnea barbata, which is widely distributed in Eastern Europe. **Variati** supplies the sodium salt under its trade name Evonsina and shows that sodium usinate has the same antibacterial properties as the pure acid. It is most effective at neutral or slightly alkaline against Gram +ve bacteria and finds application in deodorant products but it is weak against Gram –ve organisms.

Long before the publication of his book [Ref 1] Kabara presented a paper entitled GRAS Antimicrobial Agents for Cosmetic Products before the Society of Cosmetic Chemists in Dallas Texas in 1979 [Ref 5]. Kabara found that materials used as antioxidants and chelating agents were shown to enhance antimicrobial activity of established preservatives. Combinations of glyceryl laurate, BHA and EDTA were shown to have a wide spectrum of antimicrobial activity, which was enhanced in the presence of alcohol. Kabara also reported that the properties that determine the antimicrobial action of lipids are related to their Cosmetic Preservation 1st Published in SPC - 2012 John Woodruff structure. The monoglycerides are active; diglycerides and triglycerides are inactive and lauric acid has greater activity than caprylic acid, capric acid or myristic acid.

Glyceryl laurate is also known as monolaurin and much work has been done to investigate its antimicrobial properties. H.G. Preuss looked at the bactericidal effects of monolaurin and of oregano oil, alone and in combination. [Ref 6].

Hurdle technology is the science behind three new alternative preservative systems from **Inolex** under its Spectrastat trade name. They are based on a chelating agent, caprylhydroxamic acid (CHA), which is effective at pH 7; it chelates Fe++ and Fe+++, which reduces mould growth, and because of its short chain length it disrupts bacterial membranes. Spectrastat is CHA, caprylyl glycol and glycerin; Spectrastat E is CHA with ethylhexylycerin and methylpropanediol and Spectrastat G is CHA with glyceryl caprylate and methylpropanediol. All are water-soluble and each is targeted to be effective at approximately 1% in the finished com position.

Ethylhexylglycerin is also a major constituent of Sensiva PA 20 from **Shulke & Mayr GmbH**. It is mixed with phenethyl alcohol to combine the skin care and deodorising properties of ethylhexylglycerin with the antimicrobial properties of phenethyl alcohol. Phenethyl alcohol is a nature-identical fragrance ingredient with a mild flowery rose-like scent which can mask possible unpleasant odours of other ingredients. Test results available from Shulke show that although ethylhexylglycerin has no antimicrobial properties it significantly reduces kill times of phenethyl alcohol across a wide spectrum of bacteria and moulds. Sensiva PA 20 also appears to be particularly effective against the Gram-positive bacteria responsible for body malodour, making it of interest in wet-wipe applications.

Phenethyl alcohol may be found in numerous plants and may be extracted from their essential oils. It is a favoured ingredient in cosmetic products striving to avoid recognised preservatives and is mixed with caprylyl glycol to create BlagGuard NPP from **Blagden Chemicals**. A similar mixture is available from **Sinerga** as Feniol and from **Akema** as Stabil. It is also an essential ingredient of Conarom P, [INCI: Phenethyl alcohol, caprylyl glycol, trideceth-8] from the ISP Division of **Ashland Speciality Ingredients.** It is most effective at pH \leq 5 but is not destroyed by temperatures up to 80°C.

Ashland provides a variety of preservatives under its Rockensal and Optiphen trade names including Optiphen BSB-N; a mixture of benzyl alcohol, glycerin, benzoic acid and sorbic acid; Optiphen BD [INCI: Benzyl alcohol, benzoic acid, dehydroacetic acid] and Optiphen BSB-W [INCI: Benzyl alcohol, aqua, sodium benzoate, potassium sorbate], which are described as nature identical. Other Ashland preservatives contain generally accepted alternative preservative systems that include phenoxyethanol.

Phenoxyethanol is not accepted by Ecocert but finds wide application in systems not reliant on the more established preservatives. Thus **Ashland** supplies Optiphen as a mixture of phenoxyethanol and caprylyl glycol and Optiphen Plus, which also contains sorbic acid. Optiphen BSP is phenoxyethanol with propylene glycol, benzoic acid and sorbic acid while Optiphen ND is phenoxyethanol with benzoic acid and Cosmetic Preservation 1st Published in SPC - 2012 John Woodruff dehydroacetic acid. As with all preservatives based on organic acids the pH of the product is critical.

Returning to the purely natural, Batterikal from **Kalichem** is a mixture of Copaifera officinalis resin, Olea europaea fruit oil, Andiroba oil and Lectospermum scoparium oil. While not sufficiently broad spectrum to be used as the sole preservative it is recommended for deodorants and for the treatment of bacterial infections caused by streptococcus, propionibacterium acnes and staphylococcus.

Naticide from **Sinerga** is a fragrance said to have a light and pleasant note of almond and vanilla that inhibits bacterial growth in cosmetic formulations. It has the INCI name Parfum and is claimed to be a blend of natural extracts and to conform to IFFRA recommendations. It can be used at about 0.60% to adequately preserve most cosmetic compositions within a pH range of 4 to 9.

Obtained from natural sources is a range of preservative mixtures from **Salicylates and Chemicals** sold under the trade name Salinaturals by **Surfachem.** Salinaturals CCL is based on curry leaf and cinnamon leaf oils. Salinaturals OLG is based on orange, lemongrass and sesame oils. Salinaturals BCLS is based on basil extract and clove bud, lemongrass and sesame oils and Salinaturals TCLS is based on turmeric, clove bud, lemongrass and sesame oils. The same company also provide preservatives based on undecylenic acid and one using octanohydroxamic acid, which is effective at $pH \le 7.5$.

Jeecide NAS-CC from **Jeen International Corporation** contains Murraya koenigii (curry) leaf oil, Cinnamomum zeylanicum (cinnamon) leaf oil, Sesamum indicum (sesame) oil. Jeen also supplies Jeeplex NAS, [INCI: Glycerin, citric acid, lactic acid, ascorbic acid] and Jeeplex NAS-CG, [INCI: Calcium gluconate, glyceryl stearate, glycerin, citric acid, lactic acid, ascorbic acid]. The Jeeplexes are described as natural antioxidants and preservatives that are mild, yet effective, and impart little odour and colour to a wide range of leave-on and rinse-off personal care products.

Many essential oils are known to have antimicrobial properties and there has been much investigative work into their activity. It is mainly due to their content of phenolic terpenes, phenylpropanoids and alcohols and these can be extracted and concentrated to provide ingredients of natural origin or the whole oil may be used, if its odour is acceptable.

A review paper with 273 references discusses the antibacterial and antifungal properties of essential oils [Ref 7] and describes in detail the methods used to determine their efficacy. It concludes that among the many tested, essential oils of thyme, origanum, mint, cinnamon, salvia and clove possess the strongest antimicrobial properties. Work has also been done on finding synergy between mixtures or on broadening their antimicrobial spectrum. A review of this activity with over 100 references is given in a paper that first appeared in Molecules published in April 2012 [Ref 8].

Gluconic acid is a mild organic acid occurring abundantly in plants, fruits and dairy products and it is also produced in the human body. Commercially it is obtained by various chemical or fermentation processes, the most favoured on which is by fermentation of glucose using *Aspergillus niger*. Gluconic acid and gluconolactone are useful chelating agents with some antimicrobial activity. Zinc gluconate is effective against the bacterial responsible for human body malodours but is subject to a maximum permitted use in Europe of 1% zinc content in the final composition.

Utilising the properties of gluconolactone **Lonza** provides Geogard Ultra, [INCI Gluconolactone, sodium benzoate, calcium gluconate]. **PCCA** working with **Univar** supplies NataPres comprising gluconolactone with glycerin, radish root ferment filtrate, Lonicera japonica (honeysuckle) flower extract, Lonicera caprifolium (honeysuckle) flower extract and Populus tremuloides bark extract. It is a water-soluble liquid that has a broad spectrum of activity between pH 2 - 8. It is a little weak against moulds and fungi and PCCA suggest the addition of sodium benzoate and potassium sorbate, with which NataPres exhibits a synergistic activity.

Avoiding the more popular ingredients of alternative preservative systems **Global Seven** has introduced Hest G-18-0; a mixture of glycereth-18 ethylhexanoate and glycereth-18. It is an effective emollient and moisturising agent and a good solvent with anti-microbial properties. It is water-soluble and its efficacy is not pH dependent. Ajidew Zn-100 is zinc PCA from **Ajinomoto**, which has an anti-microbial effect and can be used to assist in product preservation.

Obtaining complete solution of the preservative in the aqueous phase of any cosmetic composition is as important when using alternative technology as for the more established preservatives. **Clariant** suggest Velsan SC for both types of preservative. It is sorbitan caprylate synthesised from renewable resources and is approved by Ecocert. It is not a preservative but enhances the activity of organic acids, anisic acid, phenoxyethanol and benzyl alcohol. **Clariant** also supplies anisic acid as Velsan AS and publishes data that demonstrates its efficacy when mixed with Velsan SC.

A product that relies on a naturally occurring antimicrobial-antioxidant protection system is Biovert, formally from **Arch Personal Care** but now from **Lonza**. When the two-part system is combined, a cascade of linked reactions takes place to generate antimicrobial products *in situ*. The cascade is initiated by the action of the glucose oxidase enzyme in the presence of its substrate (glucose) and oxygen. This generates hydrogen peroxide, which is used by the lactoperoxidase to catalyze the oxidation of I- and SCN- anions, forming hypoiodite and hypothiocyanate which have antimicrobial activity, resulting in rapid microbial cell death. Biovert exhibits broadspectrum antibacterial action, contributes to fungal control and reduces the risk of oxidative degradation.

JM ActiCare from Clariant is a suspension of particles of a silver chloride/titanium dioxide composite in a water/sulfosuccinate gel which improves its activity against yeasts and moulds. Although recommended for most types of leave-on and rinse-off products, JM ActiCare is especially useful for preserving products containing finely dispersed particulates such as sunscreen preparations based on microfine inorganic oxides and for make-up preparations.

Patents can be a rich source of information when formulating cosmetic products and several have focused on alternative preservative systems. GB 2437149, Synergistic preservative systems and their use in cosmetic compositions, relates to mixtures based on one or more oil-miscible glycols selected from the group consisting of pentylene glycol, neopentyl glycol, caprylyl glycol, benzyl glycol, hexanediol, ethyl hexanediol, and any combinations thereof; and a preservative comprising gluconolactone and one or more additional preservative components.

USP 7,758,851 describes preservative systems and their use in cosmetic preparations and concerns the use of the ethyl ester of lauric argininate, also known as lauramide arginine ethyl ester. It is a cationic material and while insufficient in itself to offer complete preservation it works in synergy with many established systems to reduce the amount of preservative required.

Sorbic acid and sorbates are approved by Ecocert and appear in Annex V, which make them an attractive ingredient in alternative preservative systems but they are prone to produce brown discolorations through oxidation. USP 6,592,880 describes a method for inhibition of sorbate-induced brown discolorations in cosmetic compositions and foodstuffs, which comprises adding allantoin and citrates to the formulations.

USP 6,267,996 relates to antimicrobial formulations comprising a hydrophilic extract of Krameria obtained by extracting one or more of the roots, barks or leaves of Krameria with a protic solvent such as acetone in combination with a lipophilic extract of Mesua ferrea obtained by extracting one or more of buds or flowers of Mesua ferrea with an aprotic solvent such as hexane.

USP 4,966,754 describes the preservation of cosmetic compositions using essential oils and their extracts. The effectiveness of many oils and mixtures is discussed and a blend shown to be effective against *Aspergillus niger, Candida albicans, Staphylococcus aureus* and *Pseudomonas aeruginosa* is given.

USP 5,527,492 describes cosmetic and detergent product comprising hinokitiol and a mixture of anionic surfactant and amphoteric surfactant. It is primarily aimed at treating atopic dermatitis with a very mild surfactant system but interestingly names hinokitiol as the preservative of choice. Natural hinokitiol obtained from the wood of trees of the *Cupressaceae* family is available from **A&E Connock Ltd**. as a yellowish-white crystalline powder. It is a natural monoterpinoid that exhibits strong antibacterial activity [Ref 9].

In researching this article it became apparent that there are many opportunities to avoid preservatives in Annex V and yet still be able to protect cosmetic compositions from microbial contamination. Whether this is a legal avoidance of legislative requirements remains to be seen. It is also apparent that the activity of many of the ingredients used is very pH dependent so stability testing to ensure the product is not susceptible to pH drift is essential. Also a cocktail of active ingredients will most likely be required; they must all perform within the same pH range and be mutually compatible.

Finally, a comment from "Jay" Jayesekara of Panspermia Microbiology: added preservatives must not be used as a substitute for good manufacturing practice.

Preservatives are added to product formulations which do not have adequate antimicrobial activity. Adequately preserved products will prevent multiplication of micro-organisms during use and storage but its container and closure may also enhance or diminish antimicrobial activity, therefore wherever possible in addition to carrying out an initial challenge test at the product development stage it is advisable to carry out challenge testing after a period of storage of the filled product.

Ref 1 Kabara and Orth, Preservative-free and Self-preserving Cosmetics and Drugs; Marcel Dekker1997

Ref 2 Dweck, A., Handbook of Natural Ingredients; 3rd edition, 2012; available through Amazon Books

Ref 3 Steinberg, D.C., Preservatives for Cosmetics; Allured 2006. Note: This has recently been updated

Ref 4 Papageorgiou, S *et al*, New alternatives to cosmetics preservation; J, Comet. Sci., 61, 107-123 (March/April)

Ref 5 Kabara, J., GRAS Antimicrobial agents for cosmetic products, J. Soc. Cosmet. Chem., 31, 1-10 (January/February 1980)

Ref 6 Preuss HG., *et al.* Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria; Mol Cell Biochem 2005 Apr;272(1-2):29-34.

Ref 7 Kalemba D., Kunicka A., Antibacterial and antifungal properties of essential oils; Current Medicinal Chemistry, 2003, 10, 813-829

Ref 8 Bassolé, I.H.N., Juliani, R., Essential oils in combination and their antimicrobial properties; Molecules **2012**, 17, 3989-4006. ISSN 1420-3049

Ref 9 Monita, Y., *et al*, The mechanism of the bactericidal activity of hinokitiol, Biocontrol Sci. 2007 Sep; 12(3):101-10.

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