RISK PROFILE

Antifungals of the azole type

This concerns azole type molecules used to eradicate or control fungi. They are used, or may potentially be used, for cosmetic purposes as defined in the European Commission database for cosmetic ingredients currently called CosIng.

Date of reporting 24.11.2014

This risk profile deviates in its format from other risk profiles in the series of pharmacological active substances as presented on the webpages of the Norwegian Food Safety Authority (NFSA). This is because the health risk arising because of use in cosmetics of these molecules mainly has to do with a potential worsening of the serious global problem of antimicrobial/antibiotic resistance/cross-resistance. The other risk profiles in this series concerns the toxicity of different cosmetics ingredients.

The NSFA base this risk profile mainly on concerns expressed by the European Medicinal Agency (EMA) that on two occasions – in 2005 and 2011 - addressed the unfortunate use of azole type antifungals in cosmetics products. Also the NSFA observes that the same concern has been expressed by the Council of Europe in a publication as from 2008 concerning the use of Active ingredients in cosmetics.

Over the years the Norwegian Medicinal Products Agency has strongly encouraged the NFSA forcefully to oppose any use of molecule in cosmetics that might potentially weaken the medicinal anti-fungus armamentarium against life threatening systemic fungal infections in people having a seriously compromised immune system.

Over the years, also the European Commission has addressed this issue asking its scientific committee working in the field of cosmetic products - currently called the Scientific Committee on Consumer Safety (SCCS) - for advice. At the latest the SCCS came out with the following viewpoint (opinion SCCS/1500/13 issued in 2013):

> "The SCCS is of the opinion that the scientific literature should be carefully followed with respect to potential (cross-) resistance of Climbazole and related compounds. When new information with respect to (cross)-resistance development becomes available, re-evaluation of the situation with respect to fungal resistance might be necessary."

The NSFA has carefully followed the scientific literature until present day and remain with the view azole type antifungals should not be used in cosmetic products for reasons explained about in the rest of this risk profile.

Content

Health concern

Overarching viewpoints

In principle, all usage of anti- infective remedies other than in medicinal application is unfortunate as it may worsen the already serious global health problem with antimicrobial/antibiotic resistance. Among all other non-medicinal usages is not least employment of such remedies in topical products like the cosmetic products a concern. These are products purposely put in intimate contact with the human body. In western countries, practically, the whole population apply considerable amounts of cosmetic products on a daily basis for personal hygienic purposes.

Looking for possibilities to curb problematic non-medicinal usage of anti-infective the cosmetic products should be paid due attention. These products stand out in this respect also because none of the ingredients in question is indispensable to the industry. As appears from the CosIng database of the European Commission for each cosmetic functioning there are numerous alternative ingredients. Experience is that when having to discontinue an ingredient for safety reasons industry, spare a few highly exceptional cases, easily could find feasible readily available substitutes.

Fortunately, already many antiinfectives are banned when it comes to cosmetic products. This concerns antibiotics, the "sulpha-drugs", a few other antibacterial chemotherapeutic remedies and four chemotherapeutics used to cure tuberculosis - out of which two are first line remedies.[1] Moreover, even some comparatively much used non-azole antifungals are banned in cosmetics because they meet with an EU definition of an antibiotic.[2] Hence, when it comes to the sector of cosmetic products,

[1] Confer the entries 39 (antibiotics), 307 (sulpha-drugs), 209, 251, 252, 371(antibacterial), 31, 200, 319, 411 (tub.) of the Annex II of the EU Regulation No 1223/2009. With one exception (II/411 – that is for secondary amines) these provisions came about when the cosmetics directive was adopted in 1976. Whether resistance problems played in for these early bans is unknown. Antibiotic resistance was, however, of concern even 40 years ago.

[2] The polyene antifungals *Amphotericin B* and *Nystatin* are derived from a bacterium (*Streptomyces noursei*). The mitotic inhibitor *Grisefulvin* is derived from *Penicillium griseofulvin*. The only definition of an antibiotic there are in

already there are substantial achievement as concerns removal of antibiotic resistant threats. Apparently, a lot more remains to be done, though.

The NFSA observes that in 2009 the EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) came out with an opinion concerning the possibility use of biocides for many different purposes in the society may possibly endanger the health saving effect of antibiotics. SCENIHR ended its conclusion on that occasion stating that:

> "Therefore, in order to preserve the role of biocides in infection control and hygiene, it is paramount to prevent the emergence of bacterial resistance and cross-resistance through their appropriate and prudent use."

The SCENIHR assessment did not include antifungal remedies. The concentration was on antimicrobials capable of killing bacteria. The NFSA believes, however, that this conclusive statement could well be applied also to the case about the use of azole antifungal remedies. It would indeed be paramount to prevent resistant and cross-resistance that could jeopardize the role of azole antifungals as life-saving medicines via infection control in people sick of serious invasive mycoses.

Some of the biocides play an important role in the health sector in relation to infections. This concerns surgical scrubs, other antiseptics etc. As said by the SCENIHR such biocide remedies should be preserved.

Another important group of chemical products meant to work against microbes is the pesticides that help secure the supplies of plant-based food. Among the different types of pesticides are not least the azole pesticides that work against fungal pests *via* a fungicide/fungi static mechanism identical to the mechanism by which the medicinal azoles kill/control pathogenic fungi. Further, their molecular structure resembles closely that of the medicinal azoles. Lager parts of the crops within the EU are secured using azole pesticides so, truly, they too are indeed important antimicrobials[3].

A decade ago or so, because of suspicions that the azole resistance in medicine has something to do with the use of azoles in agriculture, the European Commission asked its former Scientific Steering Committee (SSC) look into this question. In the year 2002, the SSC concluded evidencing such a link is impossible. Lately, however, new data generated makes it somewhat more probable that, actually, there is a link. Annex 1 gives more information on this subject. It remains to be proved for a fact that the use of pesticide azoles contribute to resistance problems within human medicine. If eventually proved definitively, agricultural practices[4] and regulations will most probably have to be changed with the aim to eliminate this threat to continued life saving clinical use of azoles – preserving at the same time the role of azole pesticides as important crop securing remedies.

Self-evidently, contrasting the mentioned biocides and pesticides, cosmetic products containing azole antifungal ingredients are not necessities. Preservation would not be necessary for any health reason.

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the EU legislation is the one laid down in the Regulation (EC) No 1831/3003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition, Art 2(2)(j):

> "antibiotic" means antimicrobial produced by or derived from a micro-organism, which destroys or inhibits the growth of other micro-organisms;

WHO consider all the polyene antifungals antibiotics – but not the azoles (http://en.wikipedia.org/wiki/ATC_code_D01)

[3] Slightly less than half of the total EU acreage under cereals and grapevine are treated annually with azole fungicides (SSC 2002). Azole fungicides are broadly used to control mildews and rusts of grains, fruits, vegetables, and ornamentals; powdery mildew in cereals, berry fruits, vines, and tomatoes; and several other plant pathogenic fungi. Azole residues have been detected in various food items such as strawberries, grapes, peppermint, carrots and apples with a maximum load from 0.5 mg/kg to 2.2 mg/kg (EMA 2005).

[4] Favourable practices apparently were in place 15 years ago (SCC 2002).

On a principal note, the NFSA is of the view that unless it can be proved (restricted) long-lasting non-medicinal usage is safe, inherently toxic pharmaceutically active ingredients, like the antiinfectives, should be reserved for the sector of medicinal products. Use causing resistant/cross-resistant problems is not safe.

Earlier work of the Council of Europe related to use of azole antifungals in cosmetics

In a Council of Europe publication issued in 2008 on the theme of "Active principles in cosmetics", the council's expert committee for cosmetic products concluded that due to risk for serious resistance /cross-resistance problems, azole antifungal molecules have no place in cosmetics. Separate monographs on the azoles *Ketoconazole*, *Elubiol*, *Miconazole* and *Econazole* all contained that conclusion.

The driving forces behind enhancement of life-threatening mycoses prevalence

In the western world, there is a continuous rise in the frequency of cancer sicknesses involving intensive chemotherapy. Further, more and more people get stem cell or organ transplants. Consequently, also the number of severely immunocompromised people under medicinal care increases. Important in this connection also is the expanding aging population. Fungi may cause local and even systemic infective diseases - and then especially in people with an impaired immune system. Therefore, increasing cancers and transplants prevalence cause enhancement of the prevalence of invasive potentially life-threatening fungal infections. Further, the occurrence in the general population of the less serious local mycoses is poised to increase with the spread of diabetes mellitus and the older the population gets[5].

Medicinal courts thought the prevalence of the serious mycoses increased dramatically in the period 1985 - 2005 (White TC *et al* 1998, EMA 2005, Chowta MN 2007). In recent years, however, the mycosis problems due to HIV/AIDS have largely been contained thanks to the introduction of antiretroviral therapy /new HIV medications. These mycosis problems have not vanished completely, though.[6] Irrespective of this medicinal progress, EMA in 2011 expressed that the incidence of invasive fungal infections increased slightly over the past 20 years. Data reported April 2013 by "The Fungal Infection Trust" indicate that the prevalence of mycoses still is at a comparatively high level. This concerns both the invasive serious mycoses and the *vulvo vaginitis* variant. Hence, the graveness of the situation persists.

Aspergillus fumigatus now is the main culprit – increasing resistance everywhere

Writing in 2010 the authors Heeres J *et al* conveyed that the fungus *Candida albicans* continue to be responsible for the majority of fungal infections in man, but that in later years there is a trend indicating a shift toward infections by *Aspergillus* species, and previously uncommon opportunistic fungi.[7] EMA in 2011 expressed that due to increased use of antifungal prophylaxis the prevalence of *aspergillosis* is already greater than the prevalence of *candidiasis*.

[5] EMA (2005): Most of the patients having *candidemia* have an impaired immune system, i.e. elderly, those having gone through surgical interventions, cancer and transplant patients, HIV-positive/AIDS-patients, etc. Hence, the immune system may weaken also because of ageing.

[6] "The Fungal Infection Trust report" April 2013 also confer that *Candida* infection of the oesophagus (gullet) affects ca 20% of HIV/AIDS-patients not on anti-retroviral therapy, and ca 0.5% if on antiretroviral therapy develop it. All HIV/AIDS infected patients who should be receiving anti-retroviral therapy are at risk of Pneumocystis pneumonia (PCP), as well as many other immunocompromised patients, unless taking oral antifungal prophylaxis with cotrimoxazole.

[7] In 2004 the author Maertens JA wrote that "Although *Candida* and *Aspergillus* species still represent the vast majority of fungal isolates encountered in human pathology, a battery of new species—both yeasts and filamentous fungi—is increasingly recognized as opportunistic pathogens. Of particular concern is the fact that many of these so-called 'emerging' pathogens are not covered by *Fluconazole*."

Apparently, these days *Aspergillus fumigatus* infections cause one of the highest numbers of deaths among patients with fungal infections. Azoles are the mainstay of oral therapy for *aspergillosis* and azole-resistance in isolates of this fungus is increasingly reported all over the world. In the UK, for example, the frequency of *Aspergillus fumigatus* strains resistant to *Itraconazole* increased from 0 %–5 % during 2002–2004 to 17 %–20 % in 2007–2009 (ref in Chowdhary A *et al* 2013)[8].

The overall situation still looks grave

The following figures illustrate the graveness of the situation:

- Incidence: 35% in lung and heart transplant recipients and up to 40% after liver transplantation. Up to 30% of patients with acute leukaemia experience invasive fungal infections (EMA 2005).

- The crude mortality of hospital acquired *candidemia* is approximately 60% with a mortality of 49% (EMA 2005)[9].

- Incidence only as concerns *aspergillosis*: 5-25 % in lung and hart transplant recipients; 10-20 % of patients who are receiving intensive chemotherapy for leukemia; 5 – 13 % of recipients of bone marrow transplants (Harman EM 2014).

- Reported mortality from *candidiasis* or *aspergillosis* ranges from 40 to 50%, and mortality from *fusariosis* or *zygomycosis* is 70% or more (Oliver A *et al* 2007). For *aspergillosis* after transplantation Baddley JW *et al* in 2010 report a mortality rate of 34 – 58 %.

So the invasive fungal infections are potentially life threatening diseases. Survival outcome is strongly influenced by adequate antifungal therapy at an early stage (Glöckner *et al* 2010).

The less serious local mycoses also need to be cured

Fungal infection occurs even in *immune competent* people like, for example, the majority of women plagued with vaginal mycoses[10]. They mostly self medicate themselves with OTC-azole-antifungal products. At the age of 25 years ca. 50% of all women have experienced at least one episode of *Candida vaginitis*. Approximately 5 % have a chronic infection with several episodes of clinical symptoms each year (EMA 2005).

Reporting in 2013 an online omnibus survey including 6000 adult women in five European countries and the USA was carried out as to the prevalence of *vulvovaginal candidiasis*. Depending on the country between 29 % and 49% of the participant reported having a health care provider-diagnosed such fungal infection during their lifetime. The researchers reporting thought that recurring *vulvovaginal candidiasis* is a significant health problem in western countries and that the probability is high the disease will progress to a recurrent disease (Foxman B *et al* 2013).

Even comparatively innocent local ailments like athlete's foot, nail mycosis, oral thrush, ringworm, etc. needs to be cured. Even seborrhoea should be counted in among these local illnesses since overgrowth of the fungus *Malassezia furfur* is considered the main causing factor[11]. Curing is necessary not only because of the plague/pain these ailments may cause but also because they could possibly evolve into worse medicinal conditions. Some fungal infections can sometimes open up for

[8] When getting into use in 1992 *Itraconazole* represented a major step forward in the treatment of *aspergillosis* (ref in Zirngible 1998).

[9] 17 years ago systemic candidiasis had a very high mortality rate especially in new born – up to 65 % (Pacheco-Rios et al 1997) and among cardiac surgery patients – up to 30 % (Michalopoulos *et al* 1997). The mortality rates in systemic candidiasis were up at 30 – 40 % also in 2007 (2nd European Conference on Infection in Leukemia).

[10] *Vulvo vaginal* candidiasis is often associated with conditions such as diabetes mellitus, antibiotic therapy, and pregnancy, but many women have no predisposing factors (ref. in Sheehan DJ *et al* 1999).

[11] *Malassezia* species are commensal inhabitants of the skin of human and animals, but are also capable of causing septic infections in human.

bacterial infections. Foot fungal infections, for example, can develop into more serious disabling secondary Gram-negative bacterial infection. Besides, the common superficial fungal infections in man affect large proportions of the population, prevalence ranging from 2 % to 15 % in western countries.

Need for efficient remedies is as strong as ever – need for measures securing efficiency

Hence, obviously on this background, the need for effective anti-fungal remedies persists and is as strong as ever. Consequently, fungal resistance that might cause even deaths ought to be prevented as much as possible implementing adequate measures forcefully.

The opinions of the European Medicines Agency (EMA)

These days EMA once again has expressed concern about the development of antimicrobial resistance in general. [12] Induction of resistance threatens the workability of medicines and thereby the public health. Reporting 17 February 2005 (EMA 2005) and 25 October 2011 (EMA 2011) EMA expressed views on the deliberate use in cosmetics products of anti-fungal remedies of the azoles type. It is the understanding of the NFSA that EMA is concerned this non-medicinal use may contribute to the already worrisome extensive development of resistance against these important remedies. The European Commission commissioned these EMA reports. The cosmetics industry is well aware of these reports.

Further efforts is needed

Despite EMA' worries and despite the recommendations of the Council of Europe back in 2008, the NFSA regrets observing that azole antifungals continue to be employed in cosmetic products. Because of the seriousness of this matter, the NFSA think it important once more to address this unnecessary potentially health detrimental non-medicinal usage. The NFSA now wishes to highlight more explicitly about the problem more in general and to suggest an adequate reduction of employment in cosmetic products – namely a ban on all of them.

Antifungal armamentarium changes – constant need for new remedies – new ones slow to come

EMA in 2005 evaluating the risk for resistance because of use of *Ketoconazole* - and other azoles - in cosmetics composed a list of the different authorized azole-antifungals in Europe. This list contained 26 different remedies. At the time, these generic medicines constituted the anti-fungal armamentarium in the EU as concerns azoles. The individual drugs are shown in Annex 2 - and the five ones used to fight serious systemic infection are shown in the below figure. Among the few important remedies are *Ketoconazole*, *Fluconazole* and *Itraconazole* used extensively for 20 – 30 years.

Because of its many shortcomings *Ketoconazole* is nearing the obsolete stage as concerns serious systemic mycoses (Maertens JA 2004 and Gubbins PO 2007). Further, EMA the other year recommended banning the use of *Ketoconazole* for systemic use in humans throughout the European Union, after concluding that the risk of serious liver injury from systemic *Ketoconazole* outweighs its benefits[13]. The NFSA, therefore, expects that within short *Ketoconazole* will no longer be available for treatment of serious fungal infections.

Presently, because of build up of considerable resistance over these many years, the usability of *Fluconazole* and *Itraconazole* are much less useful than before. Sometimes lifesaving treatment even fails.

In the beginning of the 90s two new azoles being developed were foreseen as successors to *Fluconazole* and *Itraconnazole*, namely *Genaconazole* and *Saproconazole*. The Phase I and II trials

[12] http://www.ema.europa.eu/ema/index.jsp?curl=pages/special_topics/general/general_content_000439.jsp

[13] *EMA press Release*: 2013-07-26., In spite of its shortcomings for nearly a decade after been introduced in 1979 -1981 *Ketoconazole* was the only oral agent available for the treatment of systemic fungal infections (Gubbins PO 2007)

looked very promising clinicians expecting real breakthroughs in finally getting really powerful remedies against the life threatening invasive mycoses. Later it became apparent, however, that lifelong dosing of rodents produced malignant tumours/ hepatocellular cancer in the animals. According to the source *"Progress in drug research 1997"* this discovery led to withdrawal of the two drugs from the market. However, EMA included them in the armamentarium thereby indicating *Genaconazole* and *Saproconazole* continue to be available to clinicians in at least some EU countries.

Their outstanding curative benefits may possibly continue outweighing the risk for cancer in the countries concerned. If this is not the case, it seems highly probable *Genaconazole* and *Saproconazole* are soon to be deprived of their authorisation everywhere in the European community. Such a development would represent a non-negligible setback in the efforts to have new efficient remedies that could be employed for a few years without any substantial loss in efficiency.

It was not until 2002 and 2006 that the pharmaceutical industry managed to place two new azoles on the market that could be used to fight the serious systemic mycoses; *Voriconazole* and *Posaconazole,* respectively. Both have increased activity against *Fluconazole*-resistant *Candida* spp. and filamentous moulds and are still not severely affected by resistance (ref. in Sampinato C *et al* 2013).

After the launch of *Posacoazole,* 8 years have now elapsed without any new azole remedy emerging on the market in Europe. [14]

Characterising the field of anti-fungal remedies is the markedly slow development of new remedies. This is true not only as concerns the azole remedies. It concerns medicinal antifungals as a whole. Actually, antiinfectives in general has become an area of lesser interest to the pharmaceutical industry. For many years, already, concerned parties have addressed this regrettable situation repeatedly. One of many examples on that is a special issue 3 October 2011 of the journal *"Current Opinion in Pharmacology"* devoted to antiinfectives wherein the following statement were put:

"Nearly dry antibacterial and antifungal R&D pipelines will fall short of addressing currently untreatable infections caused by MDR bacteria and fungi."[15]

EU considers this situation is a major health problem and has introduced multifaceted measures. These will take years to take effect.[16]

Even Climbazole needs to be taken into account

The NFSA thinks it only prudent also including the azole *Climbazole* in this context. *Climbazole* acts against the mycoses by way of the same mechanisms as do the other azole antifungals (inhibits lanosterol demethylase) – and its molecular structure is typical to azole antifungals (Annex 2). Currently, for the most part *Climbazole* find use as antidandruff in cosmetics products. It is regulated as a cosmetic' preservative since 1986, though. However, even though no EU country considers *Climbazole* a medicinal product (EMA 2011) it is for a fact that to some extent, this azole also is used to remedy with the disease *seborrheic dermatitis* (EMA 2011) – and even some other local fungal infections as well.[17]

[14] In August 2014 EMA started an approval process as concerns the molecule *Isavuconazole*

[15] http://theuretzbacher.wordpress.com/tag/resistance/

[16] Confer the Joint Program initiatives on Antimicrobial Resistance (http://www.jpiamr.eu/)

[17] Actually, in 2005, EMA included the compound among the 26 authorized anti-fungal generic medicines. This stands to reason because it for years has been used to remedy with the disease *seborrheic dermatitis* (EMA 2011) - and even the mycosis *pityriasis versicolor* (Meisel C 1991). In later years, *Climbazole* also was employed in topical medicinal products meant for treatment of *onychomycosis* (nail fungus infections) (Frangi A *et al* 2005). WHO considers *seborrheic dermatitis* a disease – confer the WHO coding: ICD-10-CM index entry L21.9. *Seborrhoea* may evolve into a worse condition because of the itching going with it that causes scratching and that, in the worst case, may inflict an additional bacterial infection.

The extent to which azoles find use in cosmetics

In 2010 the Council of Europe Member countries carried out an investigation as to whether "medicinal azoles" find use as ingredients in cosmetic products being marketed in their respective countries. Norway by the NFSA participated in that work. The NFSA additionally in April 2012 and then again in august 2014, collected usage information from other sources. The results are assembled in Annex 3. It appears that out of the 26 "medicinal azoles" 6 find use as active ingredients in cosmetic products. This concerns *Ketoconazole*[18], *Miconazole*, *Econazole*, *Bifonazole*, *Clotrimazole* and *Climabazole*. Besides the compounds *Itraconazole* and *Tioconazole* are – or have been[19] - mentioned in the CosIng database of the European Commission this indicating that also these azoles may be employed in cosmetic products somewhere in the European community. The azoles in question are used in different kinds of products; anti-dandruffs, foot-care products, nail-products, body-protection sprays and anti-itching products.[20] According to CosIng they are in the products as antimicrobial, antidandruff or preservative. These are all acknowledged cosmetic functioning.

CosIng also contains an unregulated antimicrobial of the azole-type called *Elubiol* that carries the INCI name *Dichlorophenyl imidazoldioxolan*. Structurally, it differs from *Ketoconazole* in minute details only.[21] *Elubiol* seems to be utilized medicinally only very sparingly.[22] However, because the Council of Europe concluded in 2008 that also *Elubiol* should not be used in cosmetics due to risk for cross-resistance problems, the NFSA think it only prudent to include it in the present context. According to the authors Pierard GE *et al* (1996) *Elubiol* is used for skin and hair care in subjects with oily skin or dandruff. From the Internet it is apparent that one big international antidandruff brand also currently marketed in Europe, contains *Elubiol* as an ingredient.

Detection of outlet to the environment

Normal use of cosmetics involves outlet of considerable amounts of the formulations to the environment. None of the azoles mentioned serve agricultural purposes. Therefore, the amounts ending up in rivers, lakes and effluent water from wastewater treatment plants occur because of medicinal usage – and/or due to the use for cosmetic purposes. *Clotrimazole* is frequently detected in rivers in the UK (OSPAR 2013). Recently, *Climbazole* was detected for the first time in a German wastewater treatment plant effluent the authors ascribing the occurrence to the antidandruff usage (Richter E *et al* 2013)[23].

EMA (2005) pointed out that

> "Due to selective pressure the occurrence of inherently ketoconazole-resistant and other azole cross-resistant fungi might increase in the environment; "multidrug-resistant" human pathogenic

[18] As from 1 December 2010 Ketoconazole is no longer allowed in cosmetics because of being a CMR chemical of the category 1b.

[19] Lately, the European Commission suppressed in CosIng those azoles for which the industry did not indicate an acknowledged cosmetic function (concerns *Tioconazole*).

[20] Particularly, the use of *Climbazole* has been on the rise in later years now finding use as the active ingredient in about 80 antidandruff shampoos on the market.

[21] A terminal –C(O)O-CH2-CH_3 group instead of a –CHO group – See Annex 1

[22] Recently, a company announced that *Elubiol* under the brand name ECOGARD™ Novazole is effective against microorganisms associated with dandruff and *seborrhea*. (http://www.ulprospector.com/en/eu/PersonalCare/Detail/5101/190857/ECOGARD-Novazole--Dichlorophenyl-Imidazoldioxolan)

[23] In the year 2010, effluents from 90 European wastewater treatment plants were analysed for 156 polar organic chemical contaminants. Among the many pharmaceutical compounds detected also was the medicinally most used antifungal *Fluconazol*. Seemingly, *Fluconazol* is still not used for cosmetic purposes or any other non-medicinal purpose *Clotrimazole* and *Miconazole* also was identified – but in smaller concentrations than for *Fluconazole* (Loos R *et al* 2013).

mould has been isolated from the environment and an increased number of infections due to those fungi during the last decade have been demonstrated".

Fear for more use of azoles in cosmetics

An important driving force azoles being employed as new active ingredients in cosmetics, is the superior efficiency of the azoles compared to traditional non-medicinal antifungals finding use in anti-dandruffs, foot-care products, intimate products etc.

When medicinal products authorities consider whether to downgrade drugs from the status of being a prescription drug they usually evaluate whether the eventual downgrading will cause resistance problems. This is because downgrading means a wider use of the drug. Unfortunately, a similar preventive mechanism do not exist as concerns eventual "downgrading" from the legislative area of medicines to the cosmetic products area.

The NFSA worries that the observed employment of drugs in cosmetics – even therapeutically important ones – continue and may eventually become more frequent. Currently, European law contain no provision preventing that probable development.

Possibly, even azoles for treatment of the serious systemic infections may eventually find use in cosmetics. For example, this concerns the important drugs *Itraconazole* and *Fluconazole*. [24]

The medicinal importance of the azoles

The use of azoles clinically is of high priority, since there are only a few available alternatives in medicine for prophylactic and therapeutic treatment of yeast and other fungal infections.
EMA (2005) pointed out that the azoles stand out as particularly important medicines in relation to infection diseases in man:

> *"the medicinal use of azoles is of high importance, since there are currently only a few alternatives.", "azoles are still of major importance in the treatment of various, including life threatening, fungal infections"*

The importance of the azoles shines through clinicians expressing worries that non-medicinal use will ultimately render them useless. The authors Müller F-M, C *et al* (2007) expressed that:

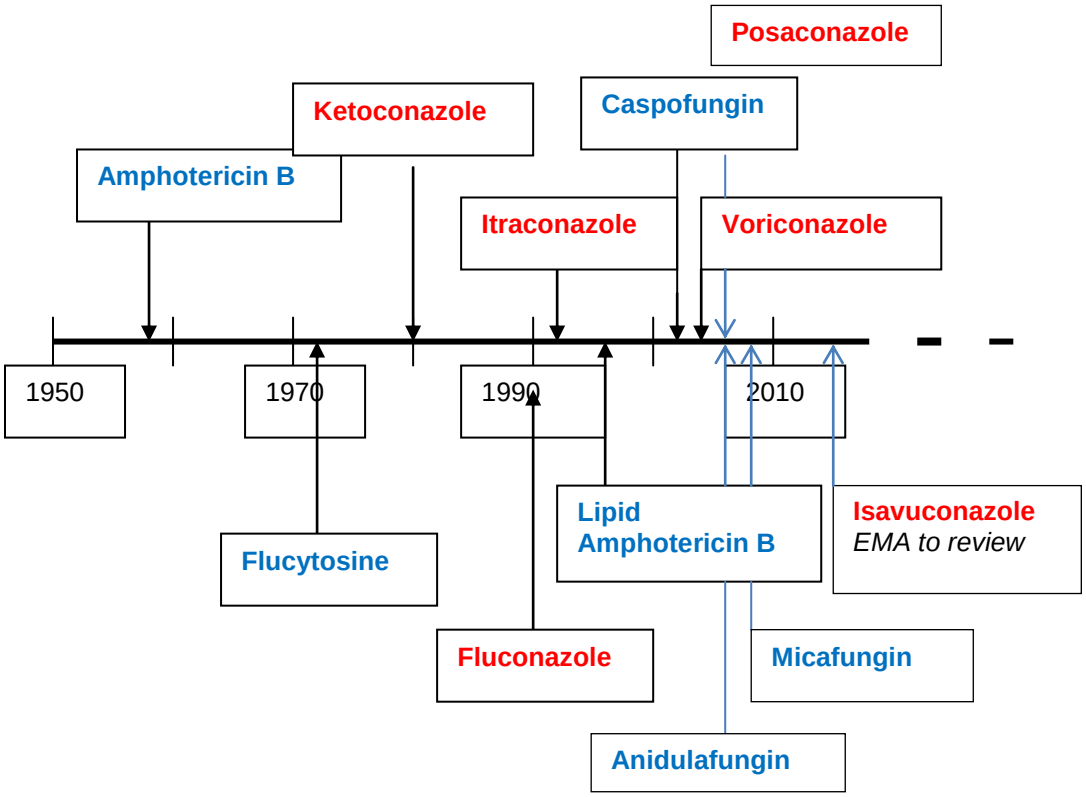
> *"Precautions against the unnecessary widespread use of azoles in the environment and human medicine are strongly recommended to prevent patients from acquiring azole-resistant yeasts"*

Since 2005 two non-azole remedies of the echinocandins type, i.e. *amidulafungin* and *micafungin*, have strengthened the anti-fungus armamentarium in regard to serious systemic fungal infections, in 2007 and 2008 respectively. The echinocandines can, however, solely be administrated *in venous* (IV) and they are unable to completely kill or inhibit *Aspergillus.* The azoles, on the other hand, are broad-spectrum agents in relation to the different pathogenic fungi in question and are often preferred because of their favourable oral bio-availability and safety profile. Therefore, the recent introduction of

[24] Apparently, the longer a remedy has been utilized for medicinal purposes the greater the chance is the cosmetics industry starts making use of it. For example, in 2005 industry tried (in vain) to have the European Commission authorise the oldest of the important azoles, *Ketoconazole*, as a cosmetics ingredient. *Ketoconazole* also is nearing the obsolete stage as an anti-fungal drug. The next azole in line may be *Itraconazole* which now have been in use for 22 years and the use of which is quite hampered because of resistance build up. The mentioning of it in the CosIng database may signal that the cosmetics industry plans to make use of it. With one exception all the other azoles now fining use in cosmetics have been used medicinally for a longer time than *Ketoconazole* and are probably no longer of great interest to the pharmaceutical industry economically – confer Annex 2.

the two echinocandins has not made the azole remedies less important. Clinicians continuous wish for new azole remedies also bear witness of how important these drugs still are[25].

Currently, the more important antifungal remedies at disposal for treatments of serious systemic infections counts in only 12 different drugs - out of which as few as 6 are azoles:[26]



Resistance mechanisms cause cross-resistance between most if not all the azoles

All the involved azoles execute their antimicrobial effect by the same biological mechanism (Annex 4). The fungal pathogens acquire azole resistance by way of a few specific mechanisms (EMA 2011, Espinel-Ingroff A 2008 – confer Annex 5 for details).

White TC *et al* (2002) detected that there exist strains of *Candida albican* overexpressing efflux pumps being resistant to *all* azoles. According to EMA (2005) up-regulation of multidrug efflux transporter genes is one of the important mechanisms of resistance in *Candida*. Some workers think overexpression of efflux-encoding genes *the* most frequently documented mechanism of azole resistance in *Candida albicans* isolates (Perea S *et al* 2001). Often involved is the ATP-binding cassette (ABC) transporter genes. The ABC-transporters accept as substrates almost the entire spectrum of azole antifungals used in medicine. Therefore, says EMA, it is no surprise that cross-resistance between azoles have been reported to occur quite frequently. Cools HJ *et al* (2008) held the view that there exists a biological potential for cross-resistance to *all* azoles. Hence, seemingly,

[25] In 2013 Spampinato C *et al* expressed that antifungal resistance based on different mechanisms continue to grow and evolve and exacerbate the need for new treatment against *Candida* infections.

[26] Data collected from the following sources http://www.cshp-bc.com/events/2008/springtherapeutics/IDSA_Guidelines.pdf *and Gubbins PO et al 2007.*

because they may induce resistance by the same mechanism and resembles each other that closely as to the molecular structure, cross-resistance may occur between most – if not *all* the azoles[27].

EMA (2005) gave a broad overview as concerns the cross-resistance topic. From the susceptibility pattern analysis performed for *Ketoconazole* in relation to *Fluconazolee*, *Itraconazole* and *Voriconazole* 65 different patterns of cross-resistance were found ranging from no cross-resistance (predominant) to complete cross- resistance.[28]

Later, Falici DR *et al* in 2013 expressed that cross-resistance still is an important concern in the class azole antifungals as a whole.

Annex 6 presents some supplementary scientific contributions devoted the cross-resistance issue. Also shown are data collected as part of a global antifungal surveillance program. These other extra data only seem to confirm the cross-resistant picture drawn up by EMA in 2005.

Predominantly, cross-resistance investigations involve the few azoles used to fight the life-threatening invasive mycoses. This is because susceptibility testing is *normally* performed as concerns these particular azoles. Not so as concerns the azoles primarily used for treatment of local fungal infections. EMA (2005) assumed, however, that also these, i.e. *Miconazole*, *Tioconazole*, *Sertaconazole*, *Clotrimazole* and the alike, demonstrate the same type and amount of cross-resistance with other azoles. For example, the following concrete studies revealed cross-resistance between azoles primarily meant for local infections:

- *Fluconazole*-resistant blood stream isolates of *Candida albicans* and *Candida glabrata* obtained from cancer patients with *mycotic vaginitis* were cross-resistant to ***Miconazole***, ***Clotrimazole***, and ***Tioconazole*** (ref. No 12 in EMA 2005).
- *Fluconazole* and *Itraconazole* resistant *Candida albicans* clinical isolates collected from immune competent *mycotic vaginitis* patients were cross-resistant mainly to *Ketoconazole* and *Miconazole* but also to *Clotrimazole* (Sojakova M *et al* 2004)[29].
- A total of 88 *Candida albicans* strains were isolated from infants and young children with dermatocandidiasis. The isolates were resistant to *Fluconazole*, *Clotrimazole*, ***Econazole***, *Miconazole* and ***Bifonazole*** in 1,4,3,5 and 9 strains respectively. Resistance was observed along with cross-resistance (Wang Xue-jun *et al* 2008).
- *In vitro* testing of 18 isolates of *Malassezia furfur* (involved in dandruff/ Seborrheic dermatitis) showed cross-resistance between *Econazole* and ***Oxiconazole*** on part of several of the isolates (Smith S *et al*, 1988).

There is a pronounced scarcity of reports providing data on cross-resistance involving the "topical azoles". This is because susceptibility studies are not normally performed when it comes to the more innocent fungal infections like seborrhoea, athlete's foot, nail mycosis, oral thrush, ringworm etc.

Some of the azoles in question may be used comparatively little. Particularly, this concerns *Climbazole* that is primarily used for cosmetic purposes. Annex 8 provides some further information as concerns this particular antifungal. Absence of data should not be construed to mean that the use of *Climbazole* poses no problem as concerns resistance/cross-resistance.

There is no reason to believe that the development *of Climbazole* resistance is different from development of resistance to other azole drugs. Increased, prolonged usage of *Climbazole* will inevitably lead to resistance (EMA).

[27] Hence, the situation is quite similar to what has been observed as concerns cross-resistance between the different molecules within a particular class of antibiotics. On the latter the EU scientific committee SCENIHR (2009) expressed that: *"Within a class of antibiotics the individual molecules have similar structures and mode of action. Within that class, the target in the bacterial cell and the mode of action of the antibiotics is the same or similar. Therefore, some mechanisms of the resistance will confer resistance to most or all members of a class, i.e. cross resistance".*

[28] EMA emphasized that there may still be large "dark figures" because of the inadequacies of the pharmacovigiliance systems in providing data on the frequency of cross-resistance. EMA pointed out that due to the slow development of standardised methods, available data on antifungal susceptibility is limited.

[29] Almost 13 % of the strains were resistant to *Fluconazole* and 18 % to *Itraconazole*

Pronounced cross-resistance threatens the medicinal value of the entire azole group

Concluding EMA (2005) stated that:

> "There is sufficient evidence from published scientific literature to conclude that a widespread use of ketoconazole including non-medicinal products will lead to resistance to this drug and *cross-resistance* to other azole drugs. This could ultimately reduce the medical value of the azole group of antifungals and potentially jeopardise the treatment of fungal infections, including life-threatening *Candida* infections."

Dealing with the *Climbazole* case EMA in 2011 concluded:

> … in view of its mechanism of action, the use of climbazole in cosmetic products may increase the risk of *cross-resistanc*e to other azole antifungals used as medicinal products, the greatest concern being the possible effect of climbazole on microbiota on the human skin and the possibility for development of cross-resistance for other azole antifungals, especially in immune compromised individuals".

Other expert's opinion

Annex 9 provide information about views expressed by others about the risk for cross-reference azoles finding use in cosmetics. Therein we comments the specific arguments put by one expert consulted by the SCCS. Views expressed by only one expert in the field of microbiology, which is not an exact science, has not made the us put less weight to the opinions delivered by EMA. Annex 9 provides further explanations.

Information gaps

It might be argued that conclusive studies are missing as concerns the resistance development and transmission of potential human pathogen fungi as a result of the use of azole antifungals in cosmetic products. Also missing are studies on long-term incubation or repeated inoculation of cutaneous fungi with azole antifungals followed by appropriate control studies on potential resistance development *in vitro* and *in vivo*.

NFSA recognizes that even though these data are missing EMA in 2005 thought that there is sufficient evidence from published scientific literature to conclude that a widespread use of *Ketoconazole* including non-medicinal products would lead to resistance to this drug and *cross-resistance* to other azole drugs. The new data generated after EMA drew this conclusion casts no doubt as to its correctness – rather the opposite.

It would be the responsibility of the cosmetics industry to generate the missing data. Most probable, the ingredients in question are not indispensable to this industry – very few cosmetic ingredients are. Already, there are feasible substitutes. Therefore, having in mind the expenditure required in generating these data NFSA would think it unrealistic expecting industry to invest in obtaining them.

Experience is there will always be a need for even more data when it goes about assessment of health risks. More often than not appearing risks are managed by instalment of appropriate measures before 100 % certainty has been achieved as concerns the risk assessment. In view of the increasing prevalence as concerns the potentially life threatening fungal mycoses involving an unacceptably high mortality, NFSA thinks it inappropriate to wait for new data – that will not be generated anyway.

Conclusion

Azole antifungals are of critical importance therapeutically. They are critically few and markedly slow to come. Among antiinfectives needing “protection” in the sense they should not be used for other purposes than for medicinal purposes, the azole antifungals stand out in particular. The prevalence is still increasing as concerns the potentially life threatening fungal mycoses involving an unacceptably high mortality in people undergoing cancer treatment and transplantations.

On this background NFSA is of the opinion that azole antifungals should not be used in cosmetic products. This concerns the particular azole antifungals mentioned in this document and all other such molecules, unless it can be demonstrated beyond any doubt that a particular azole antifungal ingredient can be used safely in the sense it would not cause resistance / cross-resistance problems.

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Annex 1

Environmentally mediated development of azole resistance

The EU Scientific Steering Committee in 2002 concluded that there was not enough evidence to support the hypothesis that azole resistance in medicine is linked to the use of azoles in agriculture (SSC 2002).

EMA (2005) briefly mentioned that resistant strains also developed in the environment and gain access to humans or animals. It had been reported that a certain extent of airborne *Cryptococcus* taken up by AIDS patients and other immunocompromised patients were resistant to drug treatment. In addition, azole-resistant *Candida* strains had been found in patients not previously exposed to antifungal agents (ref. in EMA' opinion).

EMA (2005) informed that azole residues (stemming from azole pesticides) had been detected in various food items such as strawberries, grapes, peppermint, carrots and apples with a maximum load from 0.5 ppm to 2.2 ppm. This implies, EMA thought, that on plant surfaces and certain food items the concentration of azole-residues could reach minimum inhibitory concentrations in the low range of medically important azoles, or at least subinhibitory concentrations which, due to the long half live of azoles, could persist for several months. EMA also thought, though, that the dietary intake of azole residues generally does not reach a level high enough to cause any harmful effects to consumers.

Lately, Chowdhary A *et al* (2013) devoted larger parts of their latest contribution to the topic of environmentally mediated development of azole resistance. Focus is on the *Aspergillus fumigatus* fungus a human pathogenic fungus that also has a natural habitat in the environment, including soil and plants. Observing certain recent finds Chowdary A *et al* hypothesis that azole-resistant *Aspergillus fumigatus* (ARAF) strains in patients with invasive *aspergillosis* were more likely to be acquired from environmental sources rather than from de novo mutation and selection within patients during azole therapy.

ARAF strains have, namely, been found in patients who had never been treated with azole antifungal drugs. And further, ARAF has been found in many environmental niches and even in aerial samples of hospitals. Apparently, the majority of the environmental ARAF isolates collected in the environment harbor the TR_{34}/L98H mutations at the *CYP* 51A gene.

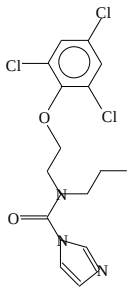
Some ARAF strains from patients harbouring this gene mutation showed cross-resistance to *Voriconazole*, *Posaconazole*, *Itraconazole*, but also to six triazole fungicides used extensively in agriculture.

Chowdhary *et al* thought that most patients acquire *Aspergillus fumigatus* from the environment.

EMA in 2011 informed that a maize fungus (*Colletotrichum graminicola*) exposed to the pesticide *Tebuconazole* developed cross-resistance to *Itraconazole* and *Voriconazole* (ref. in EMA 2011).

Faria-Ramos I *et al* (2014) informs about development of cross-resistance by *Aspergillus fumigatus* to clinical azoles following exposure to Prochloraz, a much used agricultural azole. The test involved daily incubation of susceptible *A. fumigatus* isolates. Cross-resistance to all the tested clinical azoles was observed. This concerned the much used *Posaconazole*, *Itraconazole* and *Voriconazole* azoles that remedy with invasive fungal infections. *Prochloraz* is one of the main azoles used within EU for crop protection. The authors conveyed:

> "Meanwhile, our study suggests that the abuse of azole antifungals in nature may cause serious health problems since azole-resistance and cross-resistance has the potential to further compromise the efficacy of clinical azoles in the future. Furthermore, we can speculate that the exposure of clinically relevant moulds other than *A. fumigatus* to agricultural azoles may also be associated with the emergence of cross-resistance to clinical azoles. Several compounds are being tested in order to find new antifungal alternatives, anticipating the possible loss of efficacy of clinical azoles. On the other hand, efforts should be made to find safer compounds to use in agriculture."



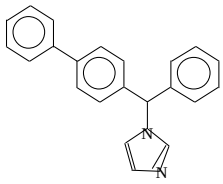
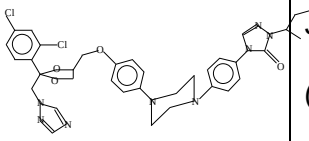
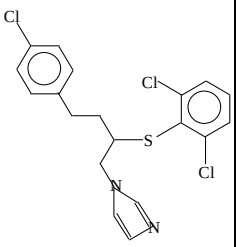
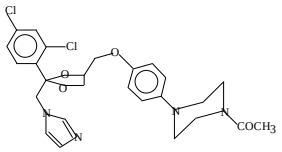
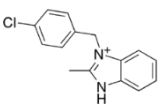
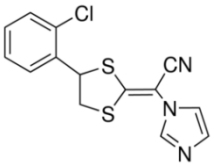
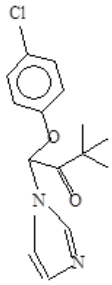
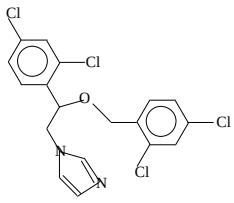
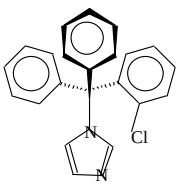
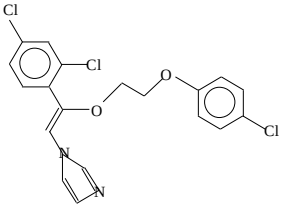
Prochloraz (CAS No: 67747-09-5)

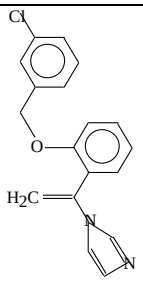
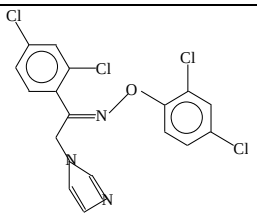
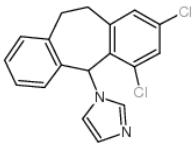
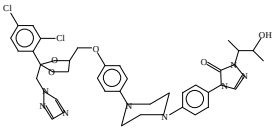
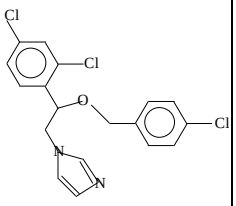
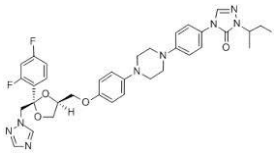
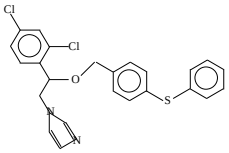
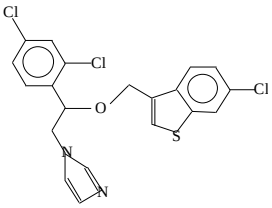
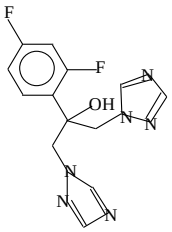
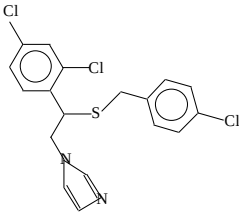
Annex 2 / *The different azoles*

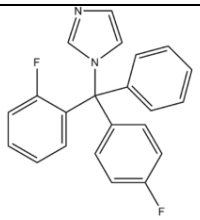
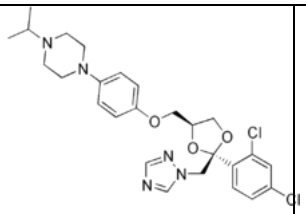
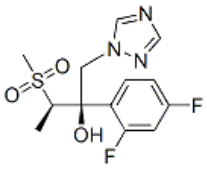
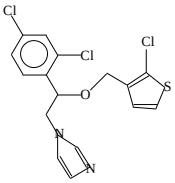
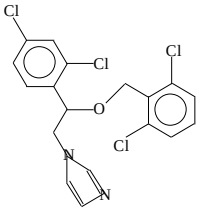
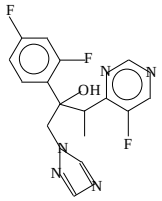
The azoles mentioned by EMA (2005):

No	INCI name /CAS No	*INN*	No	INCI name /CAS No	*INN*
1	60628-96-8	Bifonazole	14	84625-61-6	Itraconazole
2	64872-76-0	Butoconazole	15	65277-42-1	Ketoconazole
3	3689-76-7	Chlormidazole	16	101530-10-3	Lanoconazole
4	38083-17-9	Climbazole	17	22916-47-8	Miconazole
5	23593-75-1	Clotrimazole	18	74512-12-2	Omoconazole
6	77175-51-0	Croconazole	19	64211-45-6	Oxiconazole
7	128326-82-9	Eberconazole	20	171228-49-2	Posaconazole
8	27220-47-9	Econazole	21	110588-57-3	Saperconazole
9	72479-26-6	Fenticonazole	22	99592-32-2	Sertaconazole
10	86386-73-4	Fluconazole	23	61318-90-9	Sulconazole
11	119006-77-8	Flutrimazole	24	67915-31-5	Terconazole
12	121650-83-7	Genaconazole	25	65899-73-2	Tioconazole
13	24168-96-8	Isoconazole	26	137234-62-9	Voriconazole

Below is shown the drugs molecular structure as well as their ATC code as provided by the WHO.

Name INN / INCI	Molecular structure .	ATC code *(route of admini-stration)*		*Name INN /INCI*	Molecular structure	ATC code *(route of admini-stration)*
Bifonazole INCI: Bifonazole		D01AC10 *(Topical)*		Itraconazole INCI : Itraconazole		J02AC02 *(Systemic)*
Butoconazole		G01AF15 *(Topical)*		Ketoconazole INCI: Ketoconazole		D01AC08 G01AF11 J02AB02 *(Topical and systemic)*
Chlormidazole		D01AC04		Lanoconazole		Not provided *(Topical)*
Climbazole INCI: Climbazole		Not provided *(Topical)*		Miconazole INCI : Miconazole		D01AC02 A01AB09 A07AC01 G01AF04 J02AB01 S02AA13 *(Topical)*
Clotrimazole INCI: Clotrimazole		A01AB18, D01AC01, G01AF02, QJ02AB90 *(Topical)*		Omoconazole		D01AC13 G01AF16 *(Topical)*

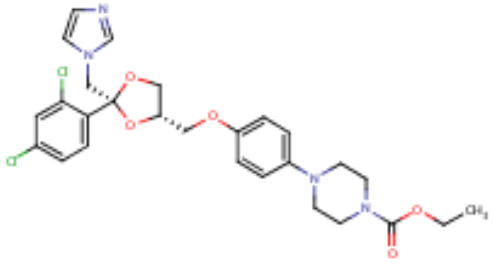
Croconazole		Not provided *(Topical)*		Oxiconazole		D01AC11 G01AF17 *(Topical)*
Eberconazole		D01AC17 *(Topical)*		Posaconazole		J02AC04 *(Systemic)*
Econazole		D01AC03 G01AF05 *(Topical)*		Saperconazole		Not provided *(Systemic)* Withdrawn ? Carcino-genic in animal long term use (Chu 1997)
Fenticonazole		D01AC12 G01AF12 *(Topical)*		Sertaconazole		D01AC14 *(Topical)*
Fluconazole		D01AC15 J02AC01 *(Systemic)*		Sulconazole		D01AC09 *(Topical)*
Flutrimazole		D01AC16 G01AF18 *(Topical)*		Terconazole		G01AG02 *(Topical)*

						
Genaconazole		Not provided *(Systemic)* Withdrawn? Carinogenic in animal long term use (Chu 1997)		Tioconazole INCI: tioconazole		D01AC07 G01AF08 *(Topical)*
Isoconazole		D01AC05 G01AF07 *(Topical)*		Voriconazole		J02AC03 *(Systemic)*

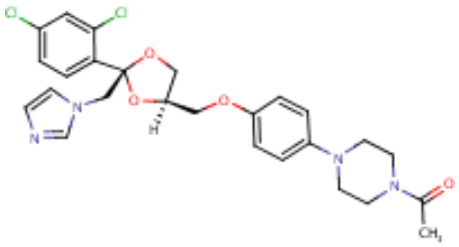
(1) Sources: Fromtling RA 1988, Zirngibl L 1998, International Application Published under the patent co-opertaion treaty (PCT), 10 May 2002, Gubbins PO et al 2007, Internet search.

See Annex 7 about the different ATC codes

Elubiol compared to *Ketoconazole* structurally:



Elubiol (CAS No 85058-43-1)



Ketoconazole

In the following table the different drugs are ranged as to the point in time they were introduced – also showing how the use in cosmetic products correlates with that timing.

Name *INN / INCI*	Year launched to the market. (1) .	Find use in cosmetics		*Name* *INN /INCI*	Year launched to the market (1)	Find use in cosmetics
Chlormidazole (D01AC04)	Beginning of the 60s			Fenticonazole (D01AC12) (G01AF12)	1986	
Clotrimazole (D01AC01) (G01AF02)	1969	Yes		Terconazole (G01AG02)	In the 80s	
Miconazole (D01AC02) (J02AB01) (G01AF04)	1969	Yes		Omoconazole (D01AC13) (G01AF16)	1991	
Econazole (D01AC03) (G01AF05)	1974	Yes		Fluconazole (D01AC15) (J02AC01)	1990	
Isoconazole (D01AC05) (G01AF07)	2nd part of the 70s			Itraconazole (J02AC02)	1992	May be
Tioconazole (D01AC07) (G01AF08)	1975	May be		Sertaconazole (D01AC14)	1992-6	
Climbazole	Around 1980	Yes		Flutrimazole (D01AC16) (G01AF18)	1995	
Ketoconazole (D01AC08) (J02AB02) (G01AF11)	1979-1980	In use up till 2010 (*Elubiol is used)*		Genaconazole	Withdrawn 1997?	

(*Elubiol*)						
Sulconazole (D01AC09)	Beginning of the 80s			Saperconazole	Withdrawn 1997?	
Bifonazole (D01AC10)	1983	Yes		Voriconazole (J02AC03)	2002	
Butoconazole (G01AF15)	1985			Eberconazole (D01AC17)	2003	
Croconazole	Around 1985			Lanoconazole	2006	
Oxiconazole (D01AC11) (G01AF17)	Around 1985			Posaconazole (J02AC04)	2006	

Other azoles

Probably soon to be approved by EMA is *Isavuconazole* (CAS No: 241479-67-4)
A swish company announced 21 August 2014 that (EMA) has accepted its *Isavuconazole* Marketing Authorization Application for review.

Not yet approved in Europe but in the US and Canada is *Efinaconazole* (CAS No 164650-44-6) June 9, 2014 a US company announced FDA had approved its nail-fungus product based upon *Efinaconazole* (10% topical solution), which would be the first topical triazole approved for the treatment of onychomycosis in the toenails. The product got its first global approval in Canada October 2013 and is now under regulatory review in Japan.

Not yet approved in Europe but in the Americas and Japan is *Luliconazole* (CAS No 187164-19-8) 15. November 2013 the FDA approved a *Luliconazole* 1% cream indicated for the topical treatment of interdigital tinea pedis (athlete's foot), tinea cruris (jock itch), and tinea corporis (ringworm). In Japan, where it was developed, the authorities approved it in 2005 already.

Other conazoles for human medicinal purposes that the WHO has allocated an ATC code until the present (D01 and J02) are:

Name (INN)	CAS No	Comments
Albaconazole	187949-02-6	In Phase II (Medicines in development 2013)
Neticonazole	111788-99-9	Approved in Japan only (in 1993). More cases reports on allergic reactions.

Over the years rather many conazoles have been synthesized without ever reaching the developmental stage of applying for admittance to the market or even being moved to the phase III stage. This concerns among other compounds the following ones; *Democonazole*, CAS No 70161-09-0, *Lombazole*, CAS No 60628-98-0, *Paraconazole*, CAS No 61400-59-7 (marketed as a veterinary

drug), *Ravuconazole*[30]; CAS No 182760-06-1, *Vibraconazole*; CAS No 80456-55-9 and *Vibunazole* , CAS No 80456-55-9.

The reasons why these molecules failed in reaching the marketplace have not been explained about in the public domain.[31] Seemingly, none have been introduced to the market of cosmetic products. Several are offered for sales on the internet, though. Hence, they are produced and probably find use in some non-medicinal product sector – if not the sector of cosmetics this far.[32] One of the azoles currently finding medicinal usage in Europe, *Clotrimazole*, may possibly find use also in shoe insoles or sandals for cellulose or synthetic fibres and for finishing underwear and socks (Zirngibl L 1998).

The difference between the medicinal and the agricultural azoles

The molecular structure of the mentioned azoles resembles closely the ones finding much use as pesticides. However, not one of those mentioned find use as a pesticide as well. This is apparent from the following sources:

- "The Pesticide Manual" 12th edition, Editor: CDS Tomlin"
- "Pesticide Properties Data Base" (PPDB) of the University of Hertfordshire in the UK[33]-

Most probably, this has to do with inefficacy against fungal problems pertaining to crops. Importantly, except for *Aspergillus fumigatus* and a few other moulds, the many different fungi causing mycoses in humans are different from the fungi species posing a problem in crop production. Besides, and not least important, therapeutic use involves requirements to safety and efficiency that are different from those necessary to meet with within agricultural practice. Still further, the environmental aspect is clearly secondary to the treatment aspect within human medicine – whereas the pesticide azoles must be as environmentally friendly as possible.

Because of these important differences it is necessary to "tailor make" molecules for their specific purposes. Hence, azoles meant for treatment of mycoses in humans are specifically designed for this application, whereas those meant for plant protection are "tailor made" for that purpose. Therefore, apart from sharing the common trait of containing either an imidazole or a triazole ring moiety, all these different molecules deviate from each other as to their chemical structure. Even minutely small structural deviations are of crucial importance as to the fungistatic /fungicidal ability.

The below table shows the 27 pesticide azoles that the committee is aware of (Name and CAS No):

Azaconazole 60207-31-0	Bitertanol 70585-36-3	Bromuconazole 116255-48-2
Cyproconazole 94361-06-5	Difenoconazole 119446-68-3	Diniconazole 83657-24-3
Epoxiconazole 106325-08-0	Fenbuconazole 114369-43-6	Fluquinconazole 136426-54-5
Flusilazole 85509-19-9	Flutriafol 76674-21-0	Hexaconazole 79983-71-4
Imibenconazole 86598-92-7	Ipconazole 125225-28-7	Metconazole 125116-23-6

[30] *Ravuconazole* may have passed the phase II and in 2005 EMA expected it soon to reach the marketing stage. The development seems to have come to a halt.

[31] Sources: Ref. Fromtling RA 1988, International Application Published under the patent Cooperation Treaty (PCT), 10 May 2002; Annual Reports in Medicinal Chemistry 2013 and 2012, Medicines in development

[32] For example a German and a UK producer/marketer deliver of *Lombazole* http://www.buyersguidechem.com/chemical_supplier/Lombazole.php, http://www.molbase.com/en/60628-98-0_supplier-21856_product-14110649.html

[33] Online at: http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm#D

Myclobutanil 88671-89-0	Penconazole 66246-88-6	Propiconazole 60207-90-1
Triadimefon 107534-96-3	Tebuconazole 112281-77-3	Tetraconazole 43121-43-3
Triadimenol 55219-65-3	Triticonazole 131983-72-7	
Imazalil 3554-44-0	Pefurazoate 101903-30-4	Prochloraz 67747-09-5
Triazoxide 72459-58-6	Triflumizole 99387-89-0	

Annex 3 / Result of mapping out of the use in cosmetic products

The below table shows the result of an investigation into which "medicinal azoles" are used in cosmetic products. In addition to the investigations conducted by the CoE member states in the years 2010 the activity rapporteur (Norway) also April 2012 and then again August 2014 looked up on the extensive German data base the Codecheck database (http://www.codecheck.info).

Name (INN)	Type of product seen in market-place Code-check 2012, 2014 Info from member states 2010 CoE mono-graphs 2008	Type of topical medicinal containing azole	Function in cosmetics according to CosIng
Bifonazole	Dandruff (Code-check) Different products NL 2010	Various infections including athelet's foot	Antidanfruff Antimicrobial
Butoconazole		*(vulvo-vaginal infection* *Intra-vaginal admini-stration)*	

Name (INN)	Type of product seen in market-place Code-check 2012 Info from member states 2010 CoE mono-graphs 2008	Type of topical medicinal containing azole	Function in cosmetics according to CosIng
I**traconazole**		Different nail infections Scalp infections (Also: *(Severe systemic infections)*	Anti-microbial
Ketoconazole	Dandruff EU 2005 Different products F and NL	Se-borrhoea Different nail infections Oral trush "Ringworm" Skin trush	Delisted Forbidden

						Tinea versicolor Trush in external genital organs, the groin and around anus (Also: *(Severe systemic infections)*	
Chlormidazole		Skin and nail infections		**Lanoconazole**		Skin fungal infections	
Climbazole	See explanation in the below	Se-borrhoea Fungal skin and nail infections	Preservative Antimicrobial	**Miconazole**	*Foot care B 2010* *Different products F 2010* *Different products NL 2010* *Dandruff (Code-check)* *(CoE mono-graph: dandruff, foot care*)	Athlete's Foot" Skin trush "Ringworm" Tinea versicolor Trush in external genital organs, the groin and around anus Jock itch" other than trush in the groins caused by candidatis	Anti-microbial
Clotrimazole	Code – check; Foot care Nail and body protect ion spray Foot powder Dandruff (2014) All in all 10 products in the	"Athlete's Foot" Oral trush "Ringworm" Tinea versicol Skin trush Trush in external genital organs, the groin and around	Antidanfruff Antimicrobial	**Omoconazole**		Vaginal candi-diasis	

	database 2014 NL 2010: Different products A 2011: anti-itching product	anus Jock itch" other than trush in the groins caused by candidatis					
Croconazole		tinea pedis inter-digital space on foot		**Oxiconazole**		Skin thrush Thrush in external genital organs, the groin and around anus	
Eberconazole		Derma-tophy-toses		**Posaconazole**		*(Severe systemic infections)*	
Econazole	*Different products (dandruff NL 2010)* *(CoE mono-graph: dandruff, foot care*) Dandruff Adamski Z et al (2006)	Skin thrush Thrush in external genital organs, the groin and around anus		**Saperconazole**		*(Severe systemic infections)*	
Fenticonazole		vaginal and skin infection		**Sertaconazole**		Tinea pedis	
Fluconazole		Different fungal nail infections including Oral thrush (Also: *(Severe systemic infections)*		**Sulconazole**		athlete's foot, ringworm, jock itch, and sun fungus	
Flutrimazole		Skin infections		**Terconazole**		*(vulvo-vaginal infection* *Intra-vaginal admini-*	

Genaconazole		*(Severe systemic infections)*	
Isoconazole		foot and vaginal infections	

		station)	
Tioconazole		Different fungal nail infections *Intra-vaginal admini-station)*	Not mentioned Delisted
Voriconazole		*(Severe systemic infections)*	

Information collected from the Codecheck database reveals that the situation looks pretty much the same in August 2014 as in April 2012 except for *Climbazole**.***

The use of Climbazole

Climbazole is regulated specifically in the EU cosmetics regulation currently being allowed as a preservative in Annex V/32 at 0.5%. Based on toxicity safety assessments by the scientific committee of the European Commission for consumer products (SCCS) this regulation is currently up for a revision. A stricter regulation as a preservative is foreseen. To the knowledge of the NFSA *Climbazole* is only sparingly employed as a preservative. The main use is as anti-dandruff.

Scanning through the Internet in 2012 it also was observed that rather many *Climbazole*-antidandruff products were advertised. In addition to only 9 being on the market in 2004, 30 apparent newcomers were observed. Hence, at least 39 antidandruff shampoos containing *Climbazole* as an active ingredient seemed to be on sale at that time in Europe. Looking up the Codecheck database again August 2014 it turned out that the number of such products had increased to 80.

The impression is that during the last 10 years *Climbazole* has come much more in use. Apparently, anti-dandruff products relying on *Climbazole* for the claimed effect are about to capture larger market shares on the expense of the traditional anti-dandruff products based mostly on *Zinc pyrithione* for the claimed effect. NFSA observes that the use of *Climbazole* started rising markedly about the time industry' wish to have *Ketoconazole* authorized as an anti-dandruff was rejected. It has been demonstrated that *Climabzole* is about as efficient against dandruff as are *Ketoconazole*. Both are used against the disease *seborrhoea*.

Climbazol also find use in some foot caring products (6) and nail lacquers (2) that claim they prevent up-coming of foul smell, cleans or protect from attracting a fungus infection – that is it keeps the body parts in good condition (see Codecheck).

The use of Elubiol

At least one well-known antidandruff brand is on the market in Europe as well. [34]

The use of azoles in cosmetics sold in the USA

Climbazole and the other azoles, except for *Elubiol* are used less in the US - as can be appreciated looking up a freely open database on cosmetic products established and managed by the American

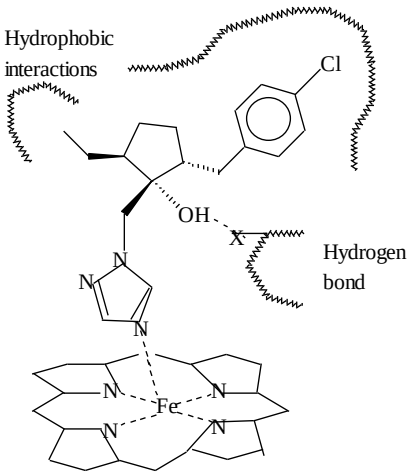
[34] http://www.starmarket.com/pd/Neutrogena/TGel-Dandruff-Shampoo-Plus-Conditioner-Daily/8-50-fl-oz/070501090008/.
http://www.directionsforme.org/index.php/directions/product/HAIRPL/00050428142769

interest group the “Environmental Working Group”[35] (EWG). Per August 2014 this database embraced the following tiny numbers of products containing one or the other of the azoles in question.

Azole	Type of products						
	Anti-perspirant/ deodorant	Anti itch	Anti dandruff	Nail treatment	Body oil	Foot cleansing /odour control/ moisturising	Other
Miconazole	1	1					
Clotrimazole		3		3	1	1	
Ketoconazole			4				
Climbazole			2			3	
Elubiol			1	2			9

Annex 4 / *Mechanism by which the azoles work against the fungi*

Azole antifungal drugs inhibit the enzyme lanosterol 14 alpha – demethylase (CYP51A1) which is a cytochrome P450 enzyme. This enzyme is necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth (Sheehan DJ *et a*l 1999). Some studies suggest that the antifungal activities of many of the azoles can be clearly attributed the strong inhibition of CYP51 enzymatic activities in *M. globosa* sterol biosynthesis through tight binding to the penta-coordinated heme iron atom of the P450 enzyme. (Donghak K *et al* 2010).



CYP51A1 with conazole substrate

Annex 5 / *Resistant mechanisms*

There are several known molecular mechanisms of resistance to azoles. This goes about point mutations in the gene encoding lanosterol demethylase (*ERG11*). Overexpression of this gene leadis to higher intracellular concentrations of ERG11p, which overwhelms the antifungal, up-regulation of

[35] For a presentation see following address: http://www.ewg.org/about-us

the efflux pump genes (in particular the CDR genes of the ATP binding cassette transporters (*ABCT*), conferring cross-resistance to all azoles, but also the MDR genes of the major facilitators class, specific for fluconazole resistance) (EMA 2011).

The efflux pumps or ABC-transporters are membrane proteins that mediate multidrug resistance through an ATP-binding drug efflux mechanism. *Candida spp.* express a number of different ABC-transporters, and two of the characterised pumps appear to be involved in the development of antifungal resistance. One of these has been reported to have a wide substrate spectrum, including azole antifungals drugs (EMA 2005).

The authors Heeres J *et al* in their review article about the conazoles in 2010 also present resistance mechanistic details. Among others information it is conveyed that the clinical isolates ABC transporters like CDR1 and CDR2 are predominantly involved and that *Ketoconazole*, *Fluconazole*, *Itraconazole*, and *Voriconazole* are good substrates to be transported.

Cross-resistance between azole drugs depends on specific mutations in *cyp51A*. Thus, a substitution of glycine in position 54 of Cyp51A confers cross-resistance between *Itraconazole* and *Posaconazole*. A substitution of methionine at position 220 or a duplication in tandem of a 34-bp fragment in the *cyp51A* promoter combined with a substitution of leucine at position 98 for histidine, confers cross-resistance to all azole drugs tested (Rodriguez-Tudela JL *et al* 2008).

Annex 6 / *Further data on cross-resistance*

Some more recent scientific contributions wholly or partly devoted to the cross-resistance phenomenon in fungi.

Excerpts from article

- "These observations suggest that *C. glabrata* exhibits considerable clinically significant cross-resistance between older azole drugs (fluconazole and itraconazole) and voriconazole. Caution is advised when considering voriconazole therapy for *C. glabrata* candidemia that occurs in patients with extensive prior azole drug exposure." (Panackal AA *et al* 2006)

- "Few data exist to describe *in vitro* patterns of cross-resistance among large collections of clinical *Aspergillus* isolates, including those of species other than *Aspergillus fumigatus*. We examined 771 *Aspergillus* spp. clinical isolates collected from 2000 to 2006 as part of a global antifungal surveillance program ……. Antifungal susceptibility testing was performed by the Clinical and Laboratory Standards Institute (CLSI) M38-A broth dilution method with itraconazole (ITR), posaconazole (POS), ravuconazole (RAV), and voriconazole (VOR). We examined the potential for cross-resistance by using measures of correlation overall and by species. For most *Aspergillus* isolates (….,) MICs of each triazole were ≤1 µg/ml. When all 771 isolates were examined, there were statistically significant correlations for all six triazole-triazole pairs. For *A. fumigatus*, the strongest correlations seen were those between VOR and RAV MICs (r = 0.7) and ITR and POS MICs (r = 0.4). Similarly, for *A. flavus*, only VOR and RAV MICs and ITR and POS MICs demonstrated statistically significant positive correlations. We have demonstrated correlations among triazole MICs for *Aspergillus*, which for the most common species (*A. fumigatus* and *A. flavus*) were strongest between VOR and RAV MICs and ITR and POS MICs. However, *Aspergillus* species for which MICs of VOR or POS were >2 µg/ml remain extremely rare (<1% of isolates)", (Pfaller MA *et al* 2008).

- "…. Azole resistance in *Aspergillus* has been reported infrequently. … Of the 34 itraconazole-resistant isolates we studied, 65% (22) were cross-resistant to voriconazole and 74% (25) were cross-resistant to posaconazole. Thirteen of 14 evaluable patients in our study had prior azole exposure; 8 infections failed therapy (progressed), and 5 failed to improve (remained stable). .." (Howard SJ et al 2009).

- The medical doctors Rex JH *et al* in an always up-dated monograph published on-line in the source "Antimicrobe" presents a few data on cross-resistance between the four most important remedies for treatment of invasive candidiasis. Referring to 4 case-reports they state that *Fluconazole* resistance is often (but not always) associated with cross-resistance to the other azole antifungal agents (http://www.antimicrobe.org/new/f14.asp

- "Almost half (48.9%) of the Azole-resistant A. fumigatus isolates from the SCARE network in European countries were resistant to multiple azoles and harbored the TR_{34}/L98H mutation in the *cyp*51A gene" (Chowdary *et al* 2013)

Annex 7

The ATC codes of the involved medicinal azoles

- D01A — ANTIFUNGALS FOR TOPICAL USE

- G01A — ANTIINFECTIVES AND ANTISEPTICS, EXCL. COMBINATIONS WITH CORTICOSTEROIDS
- G01AF — Imidazole derivatives

- J02A — ANTIMYCOTICS FOR SYSTEMIC USE
- J02AB — Imidazole derivatives

- A01A — STOMATOLOGICAL PREPARATIONS
- A01AB — Antiinfectives and antiseptics for local oral treatment

- S02A — ANTIINFECTIVES
- S02AA — Antiinfectives

- A01A — STOMATOLOGICAL PREPARATIONS
- A01AB — Antiinfectives and antiseptics for local oral treatment

- A07A — INTESTINAL ANTIINFECTIVES
- A07AC — Imidazole derivatives

Annex 8 / *Climbazole data*

Climbazole is primarily used for cosmetic purposes – and then for the most part as an anti-dandruff product (particularly in later years). Dandruff is not a disease. Understandably, therefore, commercial interests behind this usage have not tried to find out whether extensive usage might produce resistant *Malassezia furfur* strains. *Malassezia furfur* plays a decisive role for the upcoming of dandruff. Besides, investigations into the susceptibility of the lipophilic fungi *Malassezia furfur* against azole antifungals pose considerable challenges (Robson D 2007). Most probably, this explains the apparent paucity in the scientific literature as concerns reports about resistance/ cross-resistance involving *Climbazole*.

Climbazole compares *to Ketoconazole* as concern the efficiency against *Malassezia furfur*. MICs values determined by Schmidt A (1997) are (microgram/ml):

Climbazole: < 0.06 to 0.5 (median < 0.06)

Ketoconazole: < 0.06 to 0.12 (median < 0.06)

Applying in 1996 for approval of *Climbazole* as OTC antidandruff in the USA the European producer forwarded an efficacy-report for *Climbazole* being used at a concentration of no more than 0.5 %.[36]

According to EMA (2011) it is still unclear if *Climbazole* is efficacious against *Malassezia* at all concentrations, and in particular at lower concentrations (under 0.5%, as currently approved for use in cosmetics). One study (Mayser *et al* 2003) suggests that a 1% concentration is particularly effective; other studies indicate even lower values (0.65%, Wigger-Alberti *et al* 2001). Further, EMA thought the available data scars with regard to the *in vitro* activity of *Climbazole* against *Malassezia* isolates. EMA pointed out that the MIC data produced by Schmidt was published in the 1990's and that it is perfectly possible that they are not actual anymore.

Concluding EMA said that due to the extreme scarcity of the published data on fungal resistance to *Climbazole* and to the lack of susceptibility breakpoints set for this compound, it is currently not possible to quantify the levels of *Malassezia* resistance to *Climbazole*; however, it can be assumed, says EMA, that resistance of *Malassezia* to *Climbazole* is probably low.

Annex 9 / *Other expert's opinion*

As an integral part of the law-making work of the European Commission[37], single scientists in the field of microbiology were consulted the SCCNFP and the SCCS assessing the safety of use of the azoles *Ketoconazole* and *Climbazole*. The reason for drawing on the competence of these non-member scientists has been explained as follows in SCCNFP opinion 24-25 June 2003 (SCCNFP/0706/03, final) for the *Ketoconazole* case:

> *"Antibiotic, antimycotic and antimicrobial resistance issues are a set of topics that exceed the competence of the SCCNFP"*

Importantly, the scientific body EMA conducts its work within the health sector possessing unique competence as concerns medicinal remedies. It has strong ties to the "clinical world" and is, therefore, equipped with thorough insight as concerns the antibiotic resistance issue so many-faceted. EMA, more than other EU scientific bodies, is occupied with the resistance/cross-resistance threat towards medicine's workability.

The working mode of EMA is so that EMA circulate draft opinions to experts in member states around the continent thereby collecting viewpoints on the different evaluations made and the proposed conclusion. As is commonly known, scientists working within disciplines that are not exact sciences may often disagree considerably among one another on health risk issues. The good thing with whole panels of scientists is, therefore, that the opinion they as a group finally agree on is well balanced in relation to available data and the general updated disciplinal knowledge. Self-evidently, authorities should, therefore, principally not base risk management on views expressed by one, or a few, scientist only.

- ***The Ketoconazole case***

[36] (Report No 45/72/94) : http://www.fda.gov/OHRMS/dockets/dailys/04/oct04/101904/04n-0050-rpt0001-E-26-von-Octopyrox-vol8.pdf

[37] Important in this connection is the fact that the European Commission had to refer the *Climbazole* case back to the SCCS subsequent to EMA' assessment. Presumably, this procedural course was followed because already *Climbazole* is an authorized preservative. Because of this procedure, then, EMA was not given the chance to respond to the viewpoints put by the single microbiology expert hired in by the SCCS. *Ketoconazol* on the other hand was initially not specifically regulated in the Cosmetics Directive. Probably, therefore, the case was not referred back to the scientific committee subsequent to EMA' assessment. So in the end of the day the European Commission decided to shelve further plans to authorize *Ketoconazole* due to EMA' assessment and the support it got from EU member states.

(*This information appears from minutes from the meetings of the Commission's working party for cosmetic products. These minutes are accessible to the NFSA. Distribution to other parties requires the consent of the European Commission*).

As concerns the *Ketocoazole* case the viewpoints of other scientist is presented in the SCCNFP onion 24-25 June 2003 (SCCNFP/0706/03, final). Here the conclusion drawn is that

> *"The SCCNFP refers back to the opinion adopted by the Scientific Steering Committee on azole antimycotic resistance (27-28 June 2002) and SCCNFP opinions on ketoconazole adopted on 17 September 2002 and 23 June 1999 meetings, that there is at present no scientific evidence of development of resistance or cross-resistance of fungi to azole fungicides used in cosmetics."*

Hence, because SCCNFP lacked own competence as mentioned it based its view on the SCC opinion as from 2002 about the possibility that the agricultural use of azole antifungals might possibly cause resistance against azole antifungals used clinically.

The SCC thought that the rise in the incidence of resistant fungi had been dominated by resistance occurring in AIDS patients and those with other similar immunodeficiency state. So the occurrence of resistance in these patient groups was associated with their immune defence deficiency. Particularly, the severely immune compromised AIDS-patients sick also with candidiasis were in the focus. SCC thought it appropriate to concentrate on the immunodeficiency aspect also because in chronic vaginal candidiasis, and other local candidiasis, there had not been an increased frequency of antifungal resistance amongst *Candida* species isolated. People suffering from local candidiasis are for the most part immunologically normal. Besides, continued or recurrent use of azoles is a common therapeutic strategy in these ailments.

Further, the SCC thought that the manner in which the medicinal treatment had been conducted had played in heavily for the treatment failures observed. SCC then referred to the success of the introduction, at that time, of the "Highly Active Antiretroviral Therapy" (HAART) in Europe. To a very large degree introduction of HAART had brought down the numbers of new cases of secondary infections.

SCC also observed that moulds, such as *Fusarium spp, Aspergillus spp,* that live free in the environment, were involved as agents of mycoses and that many species of these fungi showed a primary resistance to anti-fungal drugs including azoles. SCC hypothesised that this resistance might possibly have been due to exposure to fungicides in agriculture, but thought it more likely being due to the increased use of immunosuppressive regimens.

Concluding the chapter about azole resistance SCC stated:

> *In conclusion, these observations suggest that there has been a rise in the incidence of drug resistant Candida infections but that this has now reached a stable level and in some units has actually fallen. This is associated with changes in the management of HIV infection and the implementation of appropriate control measures. This situation could change if HAART therapy fails to control HIV viral replication in AIDS patients.*

NFSA comments

We, firstly, observes that apparently the SCCNFP (SCCS) were not aware that very few antifungals were at the disposal of the clinicians, that the azole ones in particular were few (only 4 at the time), that they were of crucial clinical importance (also then) and particularly susceptible to resistance build up. Also at the time, the antifungal armamentarium was renewed at a critically slow pace. Today, EU thinks this a big health problem in a wider context. Besides, SCCNFP (SCCS) did not take any note of the fact that cross-resistance is that widespread it threatens the medicinal value of the entire azole group.

As concerns the HAART-argument EMA subsequently in 2005 remarked that:

> *It has been claimed that introduction of HAART (Highly Active Antiretroviral Therapy) will give rise to fewer yeast infections in patients with HIV/AIDS. This may be true in Europe, but certainly not in many other countries (Especially in Africa).*

Rightly, because of HAART the mycosis problems occurring because of HIV/AIDS have largely been contained. However, some of these problems persist since, apparently, *Candida* infection of the

oesophagus (gullet) still affects ca 20% of HIV/AIDS-patients not on anti-retroviral therapy, and ca 0.5% of those on it (The Fungal Infection Trust report" April 2013). The increase of the prevalence of the life-threatening mycoses, apparently, no longer rises steeply – but the prevalence seems still to be on the rise. Now the enhancement – as correctly remarked by SCCNFP(SCC) – is probably mostly due to (necessary) immunosuppressive regimens in connection with aggressive cancer treatment and transplants. The cancer sicknesses are nearing epidemiological proportions in the western world. So SCCNFP (SCC) was not right, the problem with serious invasive mycoses – now mostly *aspergillosis* – are not largely solved. The problem remains and the mortalities remain at a very high level. The need for efficient antifungals is as strong as ever.

- ***The Climbazole case***

The following text is collected from the SCCP opinion SCCS/1500/13:

> "As the limited number of *in vitro* and *in vivo* data on Climbazole, available in the public domain with respect to fungal resistance, are of a rather poor quality and susceptibility breakpoints for Climbazole are lacking, the SCCS felt that it was not in a position to exclude a potential relationship between the use of Climbazole and the development of (cross)-resistance. Therefore the opinion of an external internationally recognised authority in this field was asked. The following arguments were provided by the expert:
>
> (i) The mechanisms of resistance of fungi to azole antifungals vary across different species and with route of infection and administration.
>
> (ii) There is no precedent described in the peer reviewed literature where a topically applied azole has been shown to induce cross resistance to either skin organisms or internal pathogenic fungi.
>
> (iii) Fungi differ from bacteria in that exchange of genetic material responsible for drug resistance between different organisms is not known to occur, reducing the facility to spread resistance between microflora, including commensal organisms.
>
> (iv) Resistance to azoles amongst Malassezia species is rare although differences in treatment responses may be due to the documented variations in "*in vitro*" drug sensitivities, but these values only rarely reach break point levels accepted under laboratory standards as indicative of microbiological resistance.
>
> (v) Resistance to Climbazole has not been reported in the scientific literature.
>
> In the expert' opinion it was concluded that the mechanism described in the analysis by the EMA is rather a theoretical possibility, not backed up by scientific observations and therefore that from the view point of resistance, Climbazole remains a safe product to apply to the skin. It was further emphasized that from the point of antimicrobial resistance, there is no difference between its use as a leave-on and rinse-off application versus a rinse-off application only.
> …
> The SCCS is of the opinion that the scientific literature should be carefully followed with respect to potential (cross-) resistance of Climbazole and related compounds. When new information with respect to (cross)-resistance development becomes available, re-evaluation of the situation with respect to fungal resistance might be necessary.

NFSA comments to expert's viewpoints

Expert points	Comments
i and iii	The information put is only textbook knowledge that EMA , of course, has been ware of producing opinions as asked for by COM

ii	One example of the opposite is*:* EMA (2005) referring (ref 12) to the article: *Cross, E. W., S. Park and D. S. Perlin. 2000. Cross-Resistance of clinical isolates of Candida albicans and Candida glabrata to over-the-counter azoles used in the treatment of vaginitis. Microb. Drug Resist. 6:155-161* According to this article it has also been shown that *Fluconazole*-resistant blood stream isolates of *Candida albicans* and *Candida glabrata* obtained from cancer patients were cross-resistant to *Miconazole*, *Clotrimazole*, and *Tioconazole* According to WHO *Miconazole*, *Clotrimazol* and *Tioconazole* are topical azole antifungals. Articles abstract also conveys that : ….. Our findings demonstrate cross-resistance of Candida strains to fluconazole and OTC azole antifungals, and support the notion that OTC drugs can promote azole resistance in Candida spp. PMID: 10990271 [PubMed - indexed for MEDLINE] The contribution of Cross EWS *et al* has been peer-reviewed before publication in *Microb. Drug Resist*- a well renowned scientific journal. The article has been referred to by other authors confer the link: http://www.ncbi.nlm.nih.gov/pubmed/10990271 Another example is: Sojakova M *et al*, Fluconazole and Intraconazole susceptibility of vaginal yeast isolated from Slovakia, Mycophathologia 157: 163-169, 2004 Abstract: confer the link http://www.ncbi.nlm.nih.gov/pubmed/15119851 Hence, according to these peer reviewed scientific articles topically applied azole has been shown to induce cross-resistance.
iv	Investigations into the susceptibility of the lipophilic fungi *Malassezia furfur* against azole antifungals pose considerable challenges (Robson D 2007). Inaccurate measures because of this circumstance may explain the observation by some researchers that resistance to azoles amongst Malassezia species occurred rarely.
v	Most probably resistance *to Climbazole* has not been reported because susceptibility studies are not normally performed when it coms to the more innocent fungal infections like seborrhoea, athlete's foot, nail mycosis, oral thrush, ringworm etc. Besides, *Climbazole* is only sparingly used medicinally. The main use is as a cosmetic ingredient (antidandruff, foot-care). Industry saw no point in carrying out studies with the purpose of finding out whether antidandruff or foot care use might cause resistance in the fungi concerned. Absence of data should not be construed to mean that the use of *Climbazole* poses no problem as concerns resistance/cross-resistance.

In lack of own competence in the field of microbiology the SCCS seemingly try to balance the views of the EMA against that of the consultant. Because microbiology is not an exact science the view of a whole panel of experts (EMA) should weigh in more than the view of one single expert. Therefor, the NFSA consider the SCCS opinion unbalanced. More weight should have been put on the views of EMA.

We also mention that in its opinion the SCCS refers to allegations put by the Cosmetics Europe (CE), one of which is:

- The environmental exposure to Climbazole caused by cosmetic use is very small in comparison with its use in agriculture and therefore has no significance in contributing to environmental resistance.

According to the Pesticide department of the NFSA no agricultural use of Climbazole is known. The Pesticide department also looked up an EU register for registered pesticide in the EU. Climbazole is not mentioned in that register. We also recognize that the substance is not mentioned in "The Pesticide Manual" (12th edition, Editor: CDS Tomlin). Further, when looking up the extensive Pesticide Properties Database (PPDB) at the University of Hertfordshire in the UK[38] we saw the following message as concerns Climbazole:

> "A topically applied antifungal agent used to treat human fungal skin infections and as a preservative in some personal care products. No known agricultural uses."

Whether the mentioned, by all probability, erroneous allegation has in any way influenced on the conclusion drawn the SCCS seems uncertain. Only the suspicion it might have influenced calls for a new SCCS safety assessment – which the NFSA has pointed out to the European Commission in an e-mail 26 October 2014.

[38] The PPDB is a comprehensive relational database of pesticide physicochemical and ecotoxicological data. It has been developed by the Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire, from the database that originally accompanied the EMA (Environmental Management for Agriculture) software (also developed by AERU). (http://sitem.herts.ac.uk/aeru/footprint/index2.htm