OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

AZOLE ANTIMYCOTIC RESISTANCE

adopted by the SCCNFP during the 24th Plenary meeting
of 24-25 June 2003

1. Terms of Reference

1.1. Context of the question

Azoles are a large class of compounds characterised by a five-member ring containing an atom of nitrogen and at least one other non-carbon atom (nitrogen, oxygen and sulphur). The pre-fixes furo-, thio-, and pyrro- are used to distinguish three subclasses of azoles. The azoles derivatives can be subdivided into two categories: imidazoles and triazoles. Ketoconazole and clotrimazole are imidazoles, itraconazole and fluoconazole are triazoles.
Azoles are widely used as antifungal agents in agriculture (and in other industries), in clinical practice, and in cosmetic products. In recent years, the identification of azole resistant fungi in clinical practice has stimulated a discussion on the safe use of azole antifungal agents. The SCCNFP was requested to give its opinion on the safe use of azoles, with regard to the adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2. Request to the SCCNFP

The SCCNFP was asked to answer the following questions:

* *Does SCCNFP see any possibility for the development of resistance or cross-resistance by fungi to azole fungicides (including ketoconazole and clotrimazole), if these substances are used in cosmetic products?*

* *Does SCCNFP propose any recommendations on concentration at which azole fungicides (including ketoconazole and clotrimazole) can be used in cosmetic anti-dandruff shampoos?*

1.3 Statements on the SCCNFP evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The SCCNFP has already adopted two opinions on the use of ketoconazole in cosmetics during the plenary meetings of 23 June 1999 and 17 September 2002 respectively. Also the SSC adopted an opinion on "Azole Antimycotic Resistance" at the meeting of 27-28 of June 2002.

In the previous opinions, it was stated that there is at present no scientific evidence of significant resistance or cross-resistance of fungi to ketoconazole, when it is used in cosmetic anti-dandruff shampoos at concentrations up to 2%.

Although the molecular, genetic and cellular mechanisms of azole resistance are poorly understood, it seems that resistance is linked to individual compounds and products and not to azole as a group.

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

- antimicrobial resistance do not only concern the protection of consumer health, since antimicrobial resistance problems affect the strategies and regulations of antimicrobial uses (Recommendation of the Council of the European Union of 15 of November 2001, 2002/77/CE),

- Although, azole-containing products are available on the European market, azoles are widely used in a number of pharmaceuticals including some over-the-counter products. Moreover, azoles are intensively and extensively used as fungicides in agriculture and in other industries. As a consequence of the agricultural and industrial applications, azoles reach sewage treatment plants and the environment.

2. Report of the SCCNFP

2.1. Preamble

Clinically important fungal infections have become more prevalent during the past two decades because therapeutic advances have allowed the survival of an increasing number of immune compromised patients.

It has been suggested, however, that there may be a direct relationship between the development of resistance to azole fungicides used in agricultural practice and/or industry and the development of resistance to antifungals observed in clinical practice.

An exposure of a (micro-) organism, be it fungus or bacteria, to an antibiotic substance involves a risk of resistance developing through the process of selection. The rate and extent of the emergence of resistant organisms, whether this resistance is reversible or not and whether it is an issue relevant to human medicine, depends upon a variety of factors and conditions, including the mechanisms of action of the substance (frequently unknown in the case of antimycotics), the target site(s) in the organism and their number, the risk of transfer of the resistance between individual organisms and species, and the mechanism of that resistance.

Resistance is not transferred between pathogenic fungi in humans and the mechanisms for the development of resistance differ from those seen in bacteria. There is no evidence that the genes that confer resistance on fungi can be transferred experimentally, a key difference with bacteria that has important implications when considering the public health implications of drug resistance amongst fungal pathogens.

2.2. Azoles resistance in clinical practices

Most information on anti-fungal azole resistance relates to clinical observations (e.g., on immune-suppressed patients, on populations of women with vaginal infections and on hospital patients with some specific disorders). These observations have been confirmed by "*in vitro"* laboratory tests, combined, in some cases, with studies to establish the genetic identity of the organisms. The extent of antimycotic resistance resulting from the systemic use of azoles in medicine is affected by the fact that azoles are very quickly metabolised in man to non-active substances. Most of the data on laboratory confirmed antifungal resistance is based on studies in specific patient populations, notably those with AIDS or other serious underlying disease states (Kontoyiannis et al. 2002).

There are three important types of antifungal agents used in clinical practice (with different modes of action). Important problems of resistance are linked to the therapeutic use of two of these, the third is involved only to a limited extent.

a) Flucytosine has been used for over 20 years. Yeasts are known to become resistant largely because the drug action is dependent on a number of stages involving penetration of cells (permease) and conversion to fluorouracil (deaminase), steps which involve specific enzymes which are subject to changes resulting from gene mutation. This has not emerged as a major clinical problem because the drug is used in combination with another antifungal, usually amphotericin B, and its usage is limited to systemic *Candida* infections and cryptococcal meningitis.

b) Azoles. Azole resistance emerged with the use of oral imidazole or triazole drugs for the long term treatment or suppression of fungal infections in patients with different forms of immunodeficiency, notably chronic mucocutaneous candidiasis or oropharyngeal candidiasis in patients with AIDS. The risk of developing resistance varies between different drugs and itraconazole, for instance, appears to be less associated with resistance than fluconazole or ketoconazole. Resistance is seen mainly in *Candida* species although there are a few cases involving *Cryptococcus neoformans*.

There are two forms of resistance, primary (or intrinsic) and secondary resistance. In primary resistance fungi can only be inhibited by high levels of an antifungal drug. For instance most strains of *Aspergillus fumigatus*, a common human pathogen, are intrinsically resistant to fluconazole and do not appear to respond to its clinical use. Similarly, *Candida krusei* and *C. glabrata* strains are often intrinsically resistant to fluconazole. By contrast it appears that fluconazole resistance in *Candida albicans*, can emerge "*de novo"* during prolonged treatment. This is an example of secondary resistance (Perea *et al*, 2001).

There are at least three different mechanisms by which drug resistance might arise. These involve increased efflux of drug, altered target demethylase sites and availability of alternative pathways for the synthesis of cell membrane sterols. *Candida krusei* shows intrinsic resistance to fluconazole largely due to the last mentioned mechanism. *Candida albicans,* a common pathogen in humans, can become resistant to azole antifungals when these are given over a long period to patients with reduced immunity. The increased prevalence of resistant species appears to have followed use of these drugs in predisposed groups (Salonen *et al,* 2001). Resistant *Candida* strains may coexist in the same site as susceptible organisms (Lopez-Ribot et *al*, 1999). There is also evidence that in the clinical setting adoption of different strategies to treat patients over long periods or to prevent infections through antifungal prophylaxis can lead to selection of resistant organisms, either of the same or different species. The emergence of *C. dubliniensis* as a pathogenic organism as with other *Candida* species in some AIDS patients is thought to have followed selection because of its higher MIC values to azoles (Marr *et al*, 1998). Resistance in the setting of AIDS is mainly described with *Candida species* – although there are some cases of resistant *Cryptococcus neoformans* (Xu *et al*, 2001).

The development of resistance is also closely related to the use of antifungal drugs in immunosuppressed patients. In chronic vaginal candidiasis, for instance, where the patients are immunologically normal yet continued or recurrent use of azoles is a common strategy, there has not been an increased frequency of antifungal resistance amongst *Candida* species isolated. A recent study did not establish an association between exposure to OTC antifungals and drug resistant *Candida* species in the vaginal flora, although there were some resistant strains found

(Mathema *et al,* 2001). There have been other studies which have also failed to establish a link between antifungal therapy and drug resistant *Candida* species in the vagina. Studies of dermatophytosis, where long term azole therapy is common, have also not shown a change in the development of resistance in fungi isolated from patients who are usually immunologically normal. The rise in the incidence of resistant fungi has been dominated by resistance occurring in AIDS patients and those with other similar immunodeficiency states. In AIDS patients the use of continuous drug therapy (as described previously), a strategy adopted in some units for suppression of oropharyngeal candidiasis, or the use of long term suppressive therapy, e.g. for cryptococcal meningitis, have both been associated with azole resistance amongst *Candida* strains (Masia Canuto *et al*, 2000). A key feature is that this resistance occurs against a background of immunosuppression either due to disease or to therapeutic interventions. The reasons for this relationship between poor host immunity and resistance is not known, although it is thought to occur because of the high number of colonising or infecting organisms seen with the immunosuppressed, thus allowing a greater chance for the emergence of resistant strains. In addition, some resistant yeasts may be less virulent. Resistance has been described in other severely ill patients but overall the pattern of this problem has been dominated by fungal infection secondary to HIV.

The widespread use of Highly Active Antiretroviral Therapy (HAART) in Europe for patients with AIDS has produced a number of changes in the pattern of this disease. This includes a significant fall in the numbers of opportunistic infections (Skolasky *et al,* 2001; Haddad, 2001) including fungal disease, the numbers of new cases of such secondary infections falling by as much as 60% or more in some cases. There is evidence that the incidence of orophayrngeal candidiasis has also fallen substantially. From a limited number of studies, the incidence of the isolation of azole resistant *Candida* species has also fallen (Martins et *al*, 1998; Ruhnke *et al*, 2000).

Changes in antifungal usage policies, resulting from practical infection control measures (avoidance of long term use of antifungal suppressive therapy, standard dosage regimens etc.), can also produce a fall in the incidence of resistant *Candida albicans* strains (Lopez et al, 2001). The wider use of HAART and the institution of appropriate antifungal usage policies have helped to modify the patterns of antifungal resistance experienced in European centres.

From clinical observations it also appears that moulds, such as *Fusarium spp, Aspergillus spp* and other fungi living free in the environment, are involved as agents of mycoses and many species show a primary resistance to anti-fungal drugs including azoles. This resistance might be due to exposure to fungicides in agriculture, although the extent of primary resistance to certain azoles is unlike that seen with other fungi and suggests that prior exposure to antifungals may not be the cause. However, research would be needed to substantiate this. The main explanation for the rise in aspergillosis and other mould infections in humans is more than likely due to the increased use of immunosuppressive regimens.

c) Other antifungals, apart from those discussed above, are associated with intrinsic resistance. For instance there is a higher prevalence of resistant strains of *Candida lusitaniae* to amphotericin B. However, this is a rare organism and it is unlikely that resistance could be transfered to other fungi.

The foregoing would suggest that there has been an increase in the frequency of isolation of resistant fungi, mainly *Candida* species, to antifungals and specifically to azoles. However, the risk of resistance is correlated with the presence of immunodeficiency in the host population.

Importantly, there is evidence that the acquired resistance is to a large extent reversible because the introduction of a policy to reduce the use of azoles in populations at risk from antifungal resistance is accompanied by a reduction in colonisation or infection by resistant *Candida* species (Lopez et al, 2001). In other words, prudent infection control policies and the use of adjunctive therapies can re-instate the normal pattern of human fungal microflora. The risk of azole resistance is therefore real but associated with a) specific organisms b) specific host conditions and c) is reversible by reducing immunosuppression or introducing policies for limiting exposure to the relevant antifungals.

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.

2.3. Azole resistance in agriculture

Azoles are widely used in agriculture in the European Union and their use has gradually increased from the mid-seventies onwards. It is estimated that currently slightly less than half of the total EU acreage under cereals and grapevine are treated annually with azole fungicides. This compares, for example to less than 5% of the total crop area treated annually in the USA. This difference in azole usage between the two major agricultural areas is important. Despite this difference in usage there is no difference in the prevalence of resistance to treatment in man.

There exists a large variability across fungal species and strains with regard to their sensitivity to anti-fungal molecules and the development of resistance to fungicides. The existence of resistance amongst plant-specific fungi and field fungi, which are not pathogenic to crops has been observed under normal conditions. This problem has been considered to be limited in its extent and to be manageable. It has not been considered to have reached levels that cause widespread concern. Although levels of resistance to azoles have increased over a period of years, there has been no recent evidence of a rapid increase in resistance – due largely to containment as a result of good agricultural practices. Also, there are no indications that the incidence and extent of fungal resistance in the agricultural environment is currently increasing.

In organisms that have demonstrated resistance to azole fungicides, and their prevalence seems to vary from year to year, the resistance observed seems to be reversible in many cases.

Practices including the rotational use of anti-fungal products with different modes of action, the preferential practice of a limited number of interventions with higher doses as opposed to more frequent applications with lower doses, seem to have been effective in controlling the rate of resistance development (Schmid, 2001).

3. Opinion of the SCCNFP

* *Does SCCNFP see any possibility for the development of resistance or cross-resistance by the fungi to azole fungicides (including ketoconazole and clotrimazole), if these substances are used in cosmetic products?*

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.

* *Does SCCNFP propose any recommendation of concentration at which azole fungicides (including ketoconazole and clotrimazole) can be used in cosmetic anti-dandruff shampoos?"*

The SCCNFP refers back to its two previous opinions and does not propose any variation to those opinions.

4. Literature

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