Zinc Pyrithione a Comprehensive Review of Antimicrobial Agent for Personal Care and Industrial Application

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Abstract:
Zinc Pyrithione ($ZnPT_2$), the derivative of PT that is produced prevalently is a well-known chemical. It has many names and was first reported in 1930. Zinc Pyrithione (ZPTO) is used as an antifungal, and antibacterial agent. It is employed as a preservative in various commercial products such as cosmetics or industrial fluids. Apart from these, ZPTO is best known for its use as the active ingredient in several anti-dandruff shampoos. This paper presents a concise review about zinc Pyrithione, its applications, and mode of action. Zinc pyrithione (ZPTO) is a versatile compound with significant medical and industrial applications. In the medical field, ZPTO acts as an antifungal and antibacterial agent, making it a key ingredient in anti-dandruff shampoos, lotions, and creams. Clinical tests have demonstrated its unique ability to reduce surfactant irritation, offering relief from irritant contact dermatitis. Beyond dermatological use, ZPTO has proven effective against various pathogens and is utilized in treatments for conditions like psoriasis, eczema, and athlete's foot. In industrial applications, ZPTO is used as an algaecide in outdoor paints, offering protection against marine fouling organisms. Due to its low solubility, it serves as a substitute biocide in anti-fouling paints following the ban on organotin anti-foulants. However, its limited water solubility necessitates specific usage restrictions and considerations in formulations, with its maximum concentration regulated in cosmetic products. There are multifaceted mode of action of Pyrithione, an antimicrobial and fungistatic agent, specifically focusing on its use in antidandruff shampoos. While its precise mechanisms remain unclear, hypotheses suggest its entry into cells as a chelate, potentially disrupting metal cofactors of enzymes or interfering with thymidine uptake. Moreover, Pyrithione may induce membrane depolarization and inhibit the primary proton pump, affecting membrane transport. Studies explore its impact on cytosolic pH, external pH dependence, and electrical conductance. Investigations with zinc pyrithione shampoo indicate that its effectiveness against dandruff is more likely antifungal than cytostatic. This research raises questions about squame cohesion and microbial theories of dandruff, prompting further examination.

Keywords: Zinc Pyrithione, Anti-dandruff, Anti-microbial, Personal Care, Paint and Coating.

1 Summary

1.1 Background

Zinc pyrithione (ZPTO) is used as an antifungal and antibacterial agent. It is employed as a preservative in various commercial products such as cosmetics or industrial fluids. Apart from these, ZPTO is best known for its use as the active ingredient in several anti-dandruff shampoos. This report presents a concise review about the zinc pyrithione, its applications, mode of action and various toxicological studies.

2 Review of Literature

Pyrithione (PT, Omadine®):
1-Hydroxy-2-pyridinethione **1** (Fig. **1**) known as Pyrithione (PT) or Omadine® (CAS# 1121-31-9), is an aromatic heterocycle related to pyridine. It is the conjugate base derived from 2-mercaptopyridine-N-oxide, a derivative of pyridine-*N*-oxide. For fifty years PT has been noted for its high bactericidal and fungicidal action [1-3]

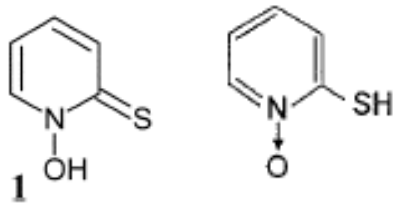


Fig. 1: 1-Hydroxy-2-pyridinethione (PT)

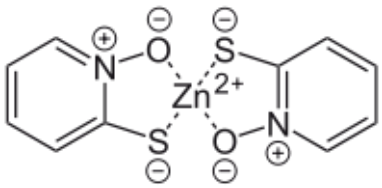
Pyrithione forms complexes with most transition metals *via* the sulfur and the oxygen of its *N*-hydroxythioamide group [4-6]. Metallization of the bidentate ligand **1** often augments its highly biocidal action as in the case of zinc pyrithione.

Zinc Pyrithione (ZPTO, ZPT, $ZnPT_2$, Zinc Omadine®):
$ZnPT_2$, the derivative of PT that is produced prevalently is a well-known chemical. It has many names (Table **1**) and was first reported in the 1930s. Some common information about zinc pyrithione are summarized below:

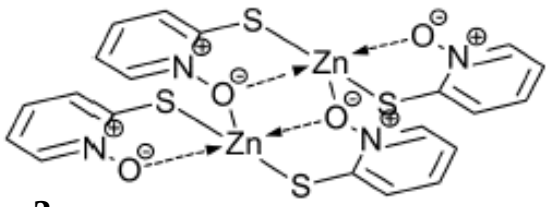
Table 1: Different Names of Zinc Pyrithione

Primary name (INCI Name)	**Synonyms**	**Trade names (Abbreviations)**
Zinc Pyrithione	*bis*[1-hydroxy-2(*1H*)-pyridine-thionato] zinc (IUPAC Name) *bis*(2-pyridylthio)zinc 1,1'-dioxide Pyrithione zinc Zincpolyanemine ZP, ZnPT, ZnPTO, ZPTO Zinc bis(2-pyridylthio)-*N*-oxide Zinc pyridinethione Zinc 2-pyridinethione-l-oxide *bis*(*N*-oxopyridine-2-thionato) zinc (II) 2-pyridinethiol-*N*-oxide zinc salt 2-pyridinethiol-1-oxide zinc salt 2-mercaptopyridine-1-oxide zinc salt	Zinc Omadine Vancide ZP BOTZ Evafine P 50 Finecide ZPT FSB 8332 Hokucide ZPT OM 1563 BC-J Biocut ZP Tomicide Z 50 Tomicide ZPT 50

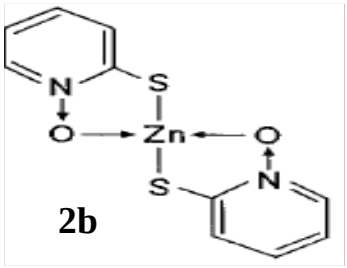
Chemical structure
Zinc pyrithione (**Fig. 2a & b**) is a coordination complex of zinc in which the pyrithione ligands (formally monoanions) are chelated to Zn^{2+} *via* oxygen and sulfur centers. In the crystalline state (solid state), zinc pyrithione exists as a centrosymmetric dimer (**Fig. 3a & b**). Each zinc is bonded to two sulfur and two oxygen centers intramolecularly and third oxygen from the adjacent molecule [7]. In solution, however, the dimers dissociate *via* scission of the intermolecular Zn-O bond.



2a



3a



2b

3b

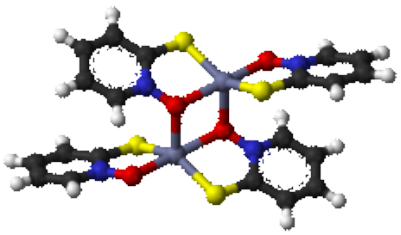


Fig. 2a & b: Molecular structure of ZPTO in solution
Fig. 3a & b: In the solid state ZPTO exists as a dimer with penta-coordinated zinc

Identifiers
CAS No.: 13463-41-7
EINECS No.: 236-671-3
Pub Chem: 26041

Empirical Formula
Emp. Formula: $C_{10}H_8N_2O_2S_2Zn$
Mol. Weight: 317.70 g/mol

Physical Properties
Appearance: white to slightly yellow crystals
Melting Point: 240 °C
Boiling Point: Decompose
Density: 1.782 at 25 °C

Solubility
Very low solubility in most of the solvents:

Solvent	Solubility (in ppm)
Diethyl ether	< 1 ppm
Benzene	3-5 ppm
Castor Oil	10 ppm
Isopropyl-palmitate	10 ppm
Propylene glycol	200 ppm
Ethanol	300 ppm
Isopropanol	300 ppm
Ethylene glycol	500 ppm
Methanol	600 ppm
Acetone	700 ppm
Chloroform	3400 ppm
Dimethyl formamide	8100 ppm
Dimethyl sulfoxide	5.13 %

(Where 100 ppm = 0.01 %)

Note: The addition of amines or amino acids to ZPTO suspensions lead to complete solubility of zinc pyrithione [(***Clear Zinc Pyrithione Preparations:*** Terry Gerstein *et. al., J. Soc. Cosmetic. chem.,* **23**, 90-114, 1972)].

3 Applications

3.1 As an antifungal and antibacterial agent

Medicinally zinc pyrithione is used as antifungal and antibacterial agent [8]. It is employed as a preservative in various commercial products such as cosmetics or industrial fluids. Apart from these, ZPTO is best known for its use as the active ingredient in several anti-dandruff shampoos [9-10] (**Table 2**), lotions and creams in treating dandruff and seborrheic dermatitis. Its effectiveness which is most probably based on its fungistatic as well as light cytostatic qualities is undoubted [11]. It is approved for over-the-counter topical use in the United States as a treatment for dandruff. Clinical tests show that ZPTO is unique among antimicrobials in its ability to reduce surfactant irritation which can cause irritant contact dermatitis [12]. It is also important to mention that ZPTO is partially neutralized by nonionic surfactants and emulsifies in MIC test.

Apart from fungicidal activities, ZPTO also possesses antibacterial properties and is effective against many pathogens from the *Streptococcus* and *Staphylococcus* class. It has been used as an antibacterial treatment for household sponges. Its other medical applications include treatments of psoriasis, eczema, ringworm, fungus, athletes foot, dry skin, atypical dermatitis, tinea, and vitiligo.

Table 2: Various brands of antidandrfuff shampoos and the respective ZPTO % in some of them

Head & Shoulders, Johnson and Johnson ZP-11, Clinic All Clear, Pantene Pro V, Sikakai Powder	
Head & Shoulders	zinc pyrithione 1%
Zincon	zinc pyrithione 1%
Dendrex	zinc pyrithione 1%
Sebulon	zinc pyrithione 2%
DHS Zinc	zinc pyrithione 2%
ZNP Bar	zinc pyrithione 2%
Theraplex Z	zinc pyrithione 2%

3.2 In Paints

ZPTO is an effective algaecide and exhibits a broad action against marine fouling organisms. Due to its low solubility in water (8 ppm at neutral pH) and slow rate of decomposition by ultraviolet light, it is suitable for use in outdoor paints and as a booster biocide in anti-fouling paints that provide protection against mildew and algae [13-15].

Due to the ban of organotin anti-foulants in ship paints established by the International Maritime Organization (IMO), the use of substitute biocides is of increasing significance. For example, Arch Chemicals are the leading producers of ZPTO in the antifouling biocide market [16].

It is important to mention that ZPTO is chemically incompatible with paints relying on metal carboxylate curing agents. Therefore, when used in latex paints and with water containing a high amount of iron, a sequestering agent that will preferentially bind to iron is needed. Similarly, in shampoo formulations, Zn^{+2} can be chelated away from the pyrithione complex if EDTA or any other chelating agents are present therein. In such cases, the transchelation can be prevented by adding zinc ions in the form of a salt.

To prevent the photodegradation of ZPTO, photoprotectants can be incorporated into formulation or into containers.

As a result of bad water solubility of ZPTO, its use is strongly restricted to only 0.0015% of the formulation of products. That's why in practice the usage of zinc pyrithione in the shampoos has been limited to only 0.7 to 2.0% (of late, the concentration limit used in cosmetics in Germany has been set to 0.5%). [*Evaluation*

and Opinion on Zinc Pyrithione, SCCNFP/0671/02, The Scientific Committee on Cosmetic Products and Non-food Products intended for consumers, 22nd plenary meeting, 17 December 2002, Europe].

Requested Use:

- Cosmetic rinse-off hair care products at a maximum concentration of 1.0 %
- Cosmetic leave-on hair care products at a maximum concentration of 0.1 %
- As a preservative in cosmetic rinse-off hair care products at a maximum concentration of 1.0 %.

4 Various Studies on Zinc Pyrithione

4.1 Synthetic Studies

Robinson has reported the synthesis of ZPTO and on the basis of elemental analysis, molecular weight and conductivity measurements proposed the structure of ZPTO to be a monomer comprised of one zinc atom chelated by two pyrithione units by way of sulfur and oxygen atoms. Later Barnett *et. al.* [17] have reported the crystal and molecular structure of ZPTO. In solid state, zinc pyrithione exists as a centrosymmetric dimer (**Fig. 3a & b**), in which each zinc is bonded to two sulfur and two oxygen centers. Further two monomeric units are linked together by two zinc-oxygen bonds. Thus, the zinc atom is pentacoordinate in a distorted trigonal bipyramidal environment.

Table 3: Crystal structure data for ZPTO

	Product I	Product II
Formula	$C_{10}H_8N_2O_2S_2Zn$	$C_{10}H_8N_2O_2S_2Zn \cdot CHCl_3$
Cell, *a*, Å	8.405 (1)	11.664 (3)
b, Å	10.183 (1)	12.120 (3)
c, Å	13.731 (1)	7.892 (1)
Angles, deg	$\beta = 97.24$ (1)	$\alpha = 67.05$ (2), $\beta = 74.82$ (2), $\gamma = 113.18$ (2)
V, Å^3	1165.82	822.99
Crystal dimensions, mm	0.20 × 0.18 × 0.10	0.30 × 0.05 × 0.05
Formula wt	317.685	317.685 + 119.378
ρ(calcd), g/cm^3	1.808	1.764
Z	4	2
Space group	$P2_1/c$	$P\bar{1}$
Total independent reflections	1460	1886
$I > 2\sigma(I)$	1370	1577
$I < 2\sigma(I)$	90	209
R_1, R_2	0.056, 0.079	0.057, 0.045
R_1, R_2 (including $I < 2\sigma(I)$)	0.059, 0.079	0.080, 0.045

The bulk physicochemical properties of molecular materials depend not only on molecular structures but also on the arrangement adopted by molecules in the solid state [18 (a, b)]. Different solid-state arrangements of the same molecule (polymorphs) can display markedly different properties and therefore, control of solid-state structure is very essential to exploit successfully the bulk properties of the molecule. As knowledge of the relationship between solid-state structure and physicochemical properties develops for a particular class of materials there arises the possibility of tailoring structures for applications where specific properties are desirable. Modification of solid-state structure in a controlled manner may allow for production of structural variants of existing molecules with properties enhanced for specific applications. Strategies for structure modification include isolation of polymorphs [18 (c, d)], production of solvates or hydrates [18 (e)] and formation of mixed molecular solids (including both stoichiometric co-crystals and non-stoichiometric solid solutions) [18 (f-h)].

Bond *et. al.* has explored the possibilities of modifying ZPTO's solid-state structure to generate modified bulk physicochemical properties. The strategy of solvate/hydrate formation has led to the synthesis and

characterization of the novel hydrate $[Zn(PT)_2(H_2O)]$ **4** (**Fig. 4** & **6**) [18 (i)]. The hydrate exhibits solubility in water greater than that of zinc pyrithione and dehydrates at elevated temperatures to reform the original material.

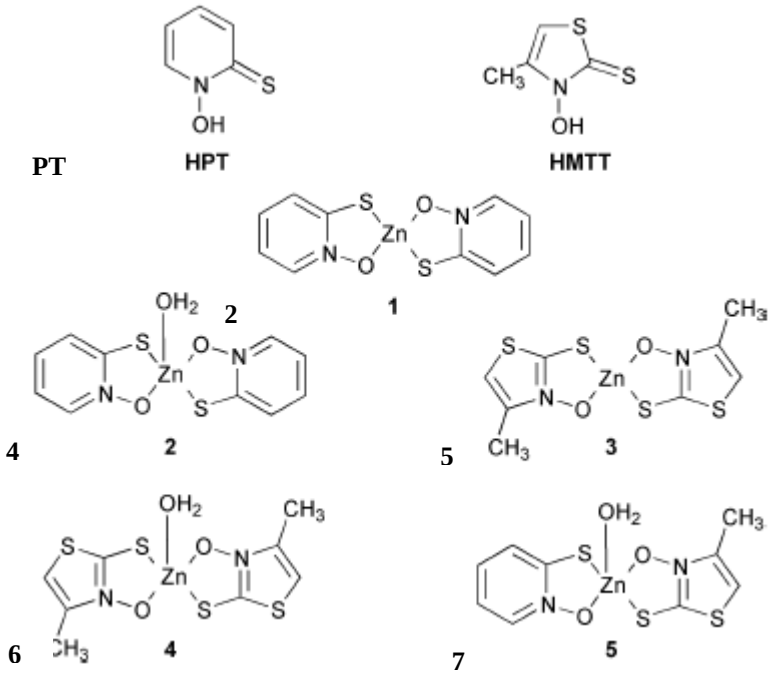


Fig. 4: Various zinc metallo-organic derivatives of PT and HMTT.

Further to this Bond *et. al.* has also focused on the second strategy for structural modification, namely *co-crystal formation*. It may be envisaged that successful co-crystallization of two active molecular species will give rise to a composite material which possesses the activity of both components. If the two components have complementary properties (*e.g.* one biocidal component fills gaps in the activity spectrum of the second), co-crystallization will lead to a combined material with a complete spectrum of desirable properties. If the materials in question were to be applied in solution, it might be expected that application of a co-crystal would ultimately be equivalent to application of a physical mixture. Co-crystallization, however, modifies the solid-state structure of each component such that the combined material may display bulk properties different from those of a physical mixture.

It has been suggested that the fungicidal activity of **1** may be attributed to chelate complex formation [18 (j)] and therefore, Bond *et. al.* has studied other cyclic thiohydroxamic acids with similar chelating groups [18 (k)]. One such molecule is 3-hydroxy-4- methyl-2(*3H*)-thiazolethione (HMTT) [18 (l)] and they have reported the solid-state structures of several divalent complexes of HMTT with d-block elements including that of $Zn(MTT)_2$ **5** (**Fig. 5**) [18 (m-o)].

In continuation of their work on pyrithione type analogous compounds, Bond *et. al.* have reported the synthesis and characterization of a mixed-molecular material [18 (p)] incorporating both the PT and MTT units as part of a hydrated zinc complex over the composition range $[Zn(PT)_x(MTT)_{2-x}(H_2O)]$, $1 \leq x \leq 2$. Extensive crystallographic disorders prohibit unambiguous identification of the molecular components present in the material but additional evidence suggests that the hydrated $[Zn(MTT)_2(H_2O)]$ **6** is not present. Instead, the mixed molecule $[Zn(PT)(MTT)(H_2O)]$ **7** is formed (**Fig. 6**) and this forms a continuous solid solution with $[Zn(PT)_2(H_2O)]$ **4** over the entire composition range, *i.e.*, the material consists of a solid solution of $[Zn(PT)_2(H_2O)]$ in an orientationally-disordered lattice of $[Zn(PT)(MTT)(H_2O)]$ (**Fig. 7**).

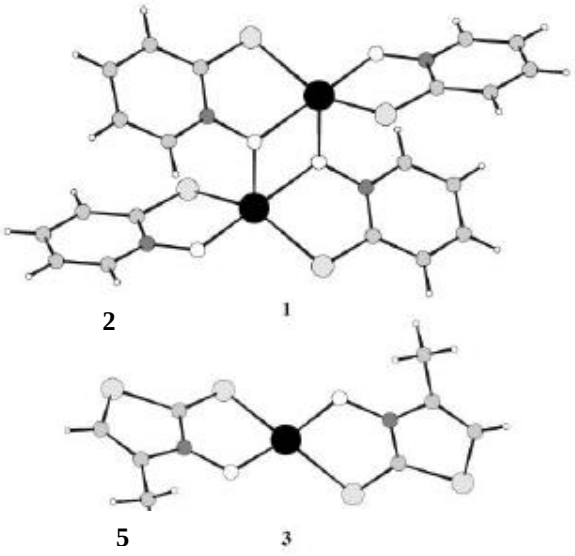


Fig. 5: Molecular structures of **2** and **5** in the solid state.

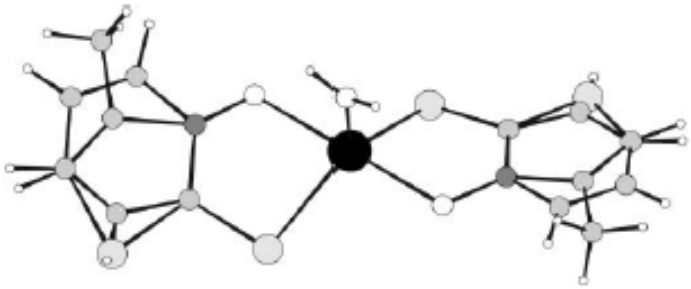


Fig. 6: Molecules of the hydrates **4** and **6** overlaid: ε=0.93.

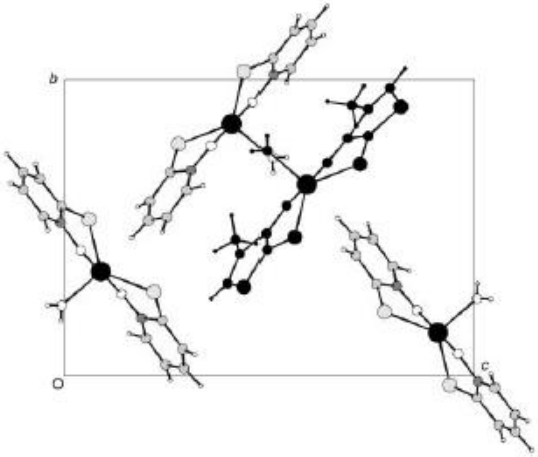


Fig. 7: Model of a unit cell of **4** with one molecule of **4** replaced by **6** (shaded black) corresponding to 25% substitution.

Due to its poor water solubility other metal salts of ZPTO have also been used in shampoo formulations. Herbst and Feistkorn [19] have used aluminum pyrithione in form of a hair lotion for the treatment of dandruff. Daily applications of a lotion with only 0.05% of the active ingredient gave in most cases complete remission or at least a distinct improvement within a few weeks. With 86% of the test persons, good to very good results were achieved (from the results in table **4** it can be seen that the result is achieved relatively fast as there was already a significant improvement seen in the first examination).

Table 4: Number and respective percentage of patients with various degree of dandruff before and after the treatment as well as during the first examination approx. two weeks after the start of the treatment. The numbers in the brackets pertain to the second-treatment.

Degree of Dandruff	Before the treatment		In the first examination		After the treatment	
	Number	%	Number	%	Number	%
Very Strong	9	26	1	3	1	3
Strong	19	54	6	17	-	-
Medium	7(7)	20	15	43	5	14
Little	-	-	11	31	15(5)	43
None	-	-	2	6	14(2)	40

Table 5: Overall assessment of the impact of the anti-dandruff hair lotion with aluminum pyrithione

Overall Assessment	Number of cases	%
Very Good	14	33
Good	22	53
Moderate	5	12
No Effect	1	2

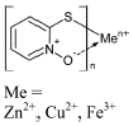
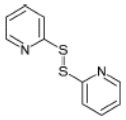
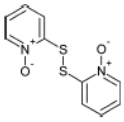
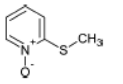
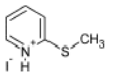
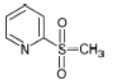
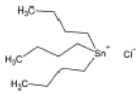
Howes and Black [20] have studied the percutaneous absorption, effect of duration of contact and concentration of ^{3}H and ^{35}S labelled PT and its metal salts (NaPTO, ZPTO & ZrPTO) on the skin of rat, rabbit and guinea pig *in vivo*. The comparative permeability of the animal's skin to these PTs was rabbit > rat > guinea pig. The comparative penetration of the three pyrithione samples was Na > Zr > Zn in all cases. NaPTO penetration was found to be dependent upon duration of contact and concentration in the test solution whereas the penetration of ZnPTO was found to be proportional to concentration but independent of duration of contact of the test solution.

Boekhout *et. al.* [21] have developed some uniquely formulated ZPTO shampoos and lotions which are mild enough to use daily. The epidemiological studies on *Malassezia* yeast by them indicated that frequent shampooing by patients gives better control over *M. globosa* and soothes the inflammatory skin response, itch and tight feeling that is symptomatic of dandruff.

4.2 Studies on Zinc Pyrithione

C.A. Doose *et. al.* [22], on the basis of their studies in rat leukemic cells (IPC-81), presented the toxicological data of zinc pyrithione and several other structural analogs. The *N*-hydroxythioamide functional group proved to play a significant role in the molecular mechanisms related to the biological action. Structural analogs [namely pyridine, pyridine-1-oxide and pyridine-2-thione, *bis*-(2-pyridinyl)disulfide and three methylated metabolites, *viz.*, 2-(methylthio)pyridine-1-oxide (MSPT), 2-(methylthio)pyridine (MSP) and 2-(methylsulfonyl)pyridine (MSO_2P)], which were deprived of one or more molecular interaction or chemical reaction potentials given by this group, exhibit far less toxic potential in IPC-81 cells than pyrithiones (*i.e.*, 2-pyridinethione-1-oxides). In particular the *trans*- metallization products of zinc (ZPTO, iron (FePTO) and copper (CuPTO) pyrithione and the oxidation product *bis*(2-pyridinyl)disulfide-1,1'-dioxide [(pyrithione disulfide, (PT_2)] have been proven to be as toxic as ZPTO and tributyltin chloride in IPC-81 cells. CuPTO, FePTO and (PT_2) need to be considered as environmental transformation products of ZnPTO.

Table 6: Structural analogs selected for IPC-81 toxicity tests

Molecular structure	Chemical name	Abbr.	No.	Molecular structure	Chemical name	Abbr.	No.
 Me = Zn^{2+}, Cu^{2+}, Fe^{3+}	Zinc(II)- Copper(II)- Iron(III)- pyrithione	$ZnPT_2$ $CuPT_2$ $FePT_3$	**2** **3** **4**		Pyridine 1-oxide	PyNO	**9**
					Bis(2-pyridinyl)disulfide	$(PyS)_2$	**10**
	Bis(2-pyridinyl) disulfide 1,1′- dioxide	$(PT)_2$	**5**		2-(Methylthio) pyridine 1-oxide	MSPT	**11**
	Sodium pyrithione	NaPT	**6**		2-(Methylthio) pyridine hydroiodide	MSPHI	**12**
	Pyridine	Py	**7**		2-(Methylsulfonyl) pyridine	MSO_2P	**13**
	Pyridine 2-thione	PyS	**8**		Tributyltin chloride	TBT	**14**

Harding *et. al.* [23] have reported the following observations based on their research on dandruff affected scalp stratum corneum:

- The scalp skin of dandruff sufferers is characterized by decreased intercellular lipids, particularly ceramides
- The intrinsic barrier properties of the stratum corneum of dandruff scalp skin become weak as observed in other scaling skin disorders such as xerosis
- Dandruff sufferers have an increased response to the itch mediator, histamine

Based on these observations, they have proposed that certain individuals are more prone to dandruff due to an intrinsically weakened scalp permeability barrier that renders them more susceptible to skin damage through microbial metabolites.

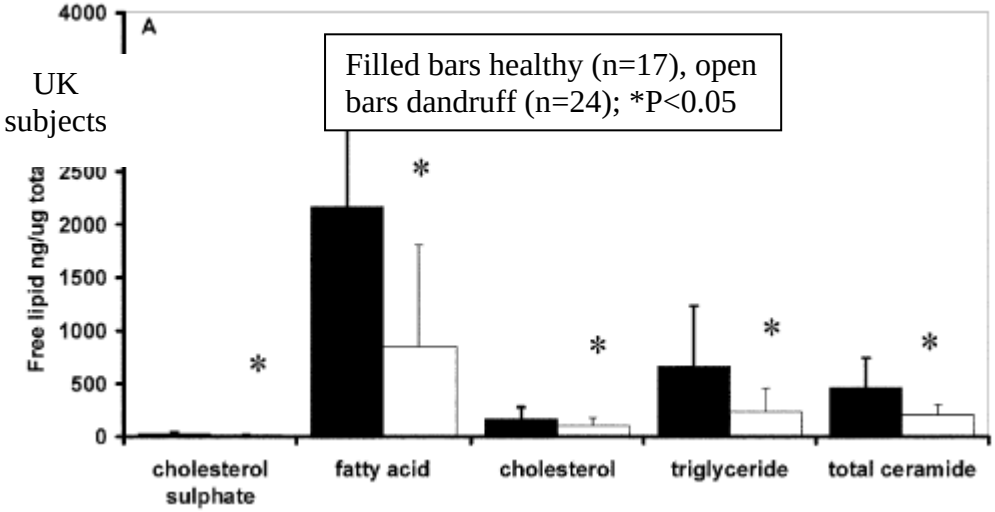


Fig. 8: Dandruff sufferers have lower levels of free lipids. Amounts of cholesterol sulphate, total ceramides, free fatty acids, cholesterol and triglycerides were assayed in tape strips from subjects. The lipids were quantified using a scanning densitometer and were compared with known standards.
A UK subjects; *filled bars* healthy (*n*=17), *open bars* dandruff (*n*=24); **P*<0.05

Park *et. al.* [24] have shown (by MIC test and modified skin disk diffusion assay for *M. furfur*) that the shampoo formulation containing imidazole with zinc pyrithione reduced the dandruff in statistically significant amount after 4 weeks compared to that containing only zinc pyrithione. This study could suggest

that the use of imidazole together with antifungal agent such as zinc pyrithione or piroctone olamine could be more efficient way to reduce dandruff flora and hyperkeratinization.

Data presented at the *Intercontinental Meeting of Hair Research Societies*, 2004 revealed that the bioavailability of antifungal active ingredients is critically important to therapeutic success. For a formulated therapeutic product to be very effective, a potent active is a necessary but insufficient condition to achieve the desired activity because delivery efficiency will be inherently low in such rinse off products. Therefore, the way the active is delivered to the scalp from the product formula, *i.e.*, the product pharmacology is also equivalently importnat. For example, a comparison of clinical flake-reduction efficacy of a variety of 1% ZPTO based anti-dandruff shampoo products demonstrates a wide range of magnitude of therapeutic benefits. Much of this variation is likely due to the varying efficiency of delivery of ZPTO, a particulate active to the scalp surface. Particulate ZPTO delivery is affected by the physical size and shape of the particles. Particles which are flat, cover the scalp surface more efficiently than those that are not. The particle size is also critical as smaller particles provide better surface coverage (**Fig. 9**) but are more difficult to retain on the scalp after rinsing. Therefore, the optimization of the particle size is very critical to maximize the delivery of active. Studies [25 (a)] showed that shampoo product formulations containing optimized ZPTO particles (2.5 μm) for delivery outperform those utilizing only standard forms of the active (**Fig. 10**). The particles that comprise this anti-fungal can be engineered into flat platelet shapes to deliver optimized scalp coverage resulting in improved efficacy [12].

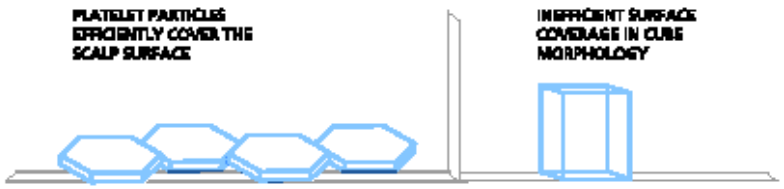


Fig. 9: The smaller size particles provide better surface coverage

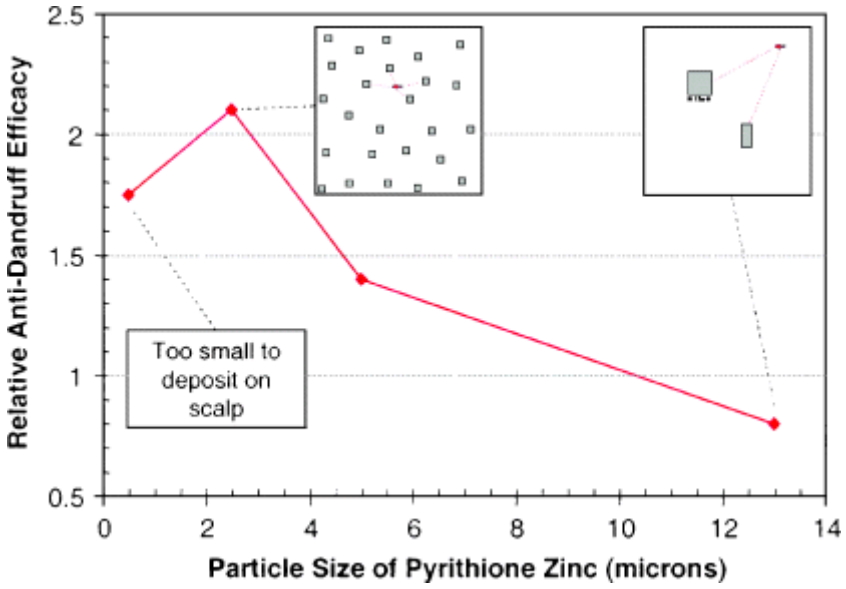


Fig. 10: The impact of ZPTO particle size on clinically observed anti-dandruff efficacy from shampoo matrices. Sub-micron particles are difficult to retain on the scalp after rinsing, whereas the distribution of particles on the surface of the scalp (*inset*) improves as the particles become smaller. These off-setting factors result in an optimum particle size for efficacy of 2.5 μm.

Warner *et. al.* [25 (b)] have evaluated the effect of ZPTO on dandruff treatment and on the ultra structure of *stratum corneum* (SC). Studies showed that dandruff treatment with a commercial small-particle ZPTO shampoo resulted in clinically significant reduction in flaking and improvement in SC structure (**Fig. 11**).

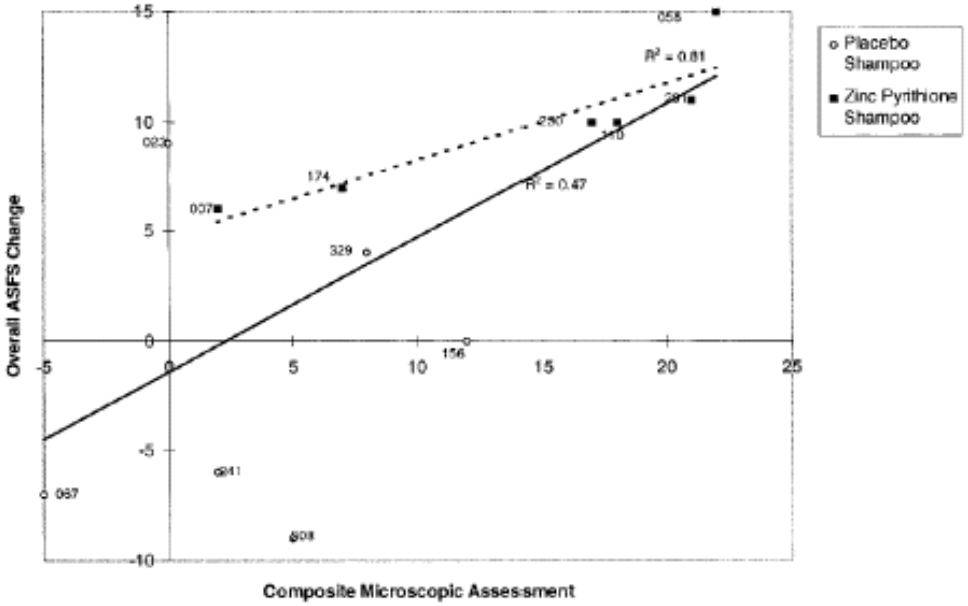


Fig. 11: Correlation of microscopic phenotype with ASFS (Adherent Scalp Flaking Scale) grades. There was good correlation between changes in visual (clinical ASFS) grades and changes in morphological assessment. Correlation of total population (solid line, $R^2 = 0.47$), correlation after ZPTO treatment (dashed line, $R^2 = 0.81$)

Another important pharmacological variable that must be considered is the role of the "non-functional" excipients in the product formulation because they can modulate, either positively or negatively, the activity of the drug active. In this regard, potentiated ZPTO formulas (ZPTO with Zn, zinc salts or other active ingredients) which increase the bioavailability of active ingredients are the latest improvements in dandruff treatment.

The bioavailability of active ingredients can be potentiated by cosmetic ingredients in the shampoo formula. Currently the sophisticated treatments are designed in a way that they will provide greater hairstyle control while inhibiting *M. globosa* and relieving scalp distress. Therefore, ZPTO is being formulated into cosmetic products which enhance hair appearance while actives tame the rapid cell turnover and inhibit the biological mechanism of dandruff. Product versatility will further enhance compliance and efficacy. Potentiated zinc pyrithione is ZPTO with improved bioavailability through the addition of cosmetic excipients such as zinc salts (like zinc carbonate, zinc chloride, zinc sulphate etc.).

A specific example of how product excipients can affect the activity of a scalp care formula is the addition of zinc materials to ZPTO-based therapeutic products [25 (a)]. To understand the mechanism behind this effect, it is important to highlight the importance of the zinc component of the metallorganic complex ZPTO. Evaluation of the *in vitro* anti-fungal activity of the organic component (pyrithione) as well as a number of metal salts of this material (including sodium, zinc, iron, nickel and copper), demonstrates a range of over three orders of magnitude (1000-fold) of potency. The zinc salt (MIC of 8 ppm) is almost 10 times more potent than sodium pyrithione (MIC of 64 ppm) alone and approximately 100 times more effective than the iron salt (MIC of 500 ppm). This clearly highlights the importance of zinc to the activity of ZPTO and establishes the intact ZPTO material as the anti-fungal bio-active species.

Like all metallorganic complexes, ZPTO when in a liquid medium is governed by an equilibrium between the associated zinc-pyrithione complex (*i.e.*, ZPTO) and dissociated zinc and pyrithione components [25 (c)]. Since ZPTO is the bio-active species dissociation has negative consequences on its efficacy. Greater the dissociation the less ZPTO is present in the medium in a form leading to anti-fungal activity. This is represented in the equilibria summarized in **Figure 12** (A, B & C).

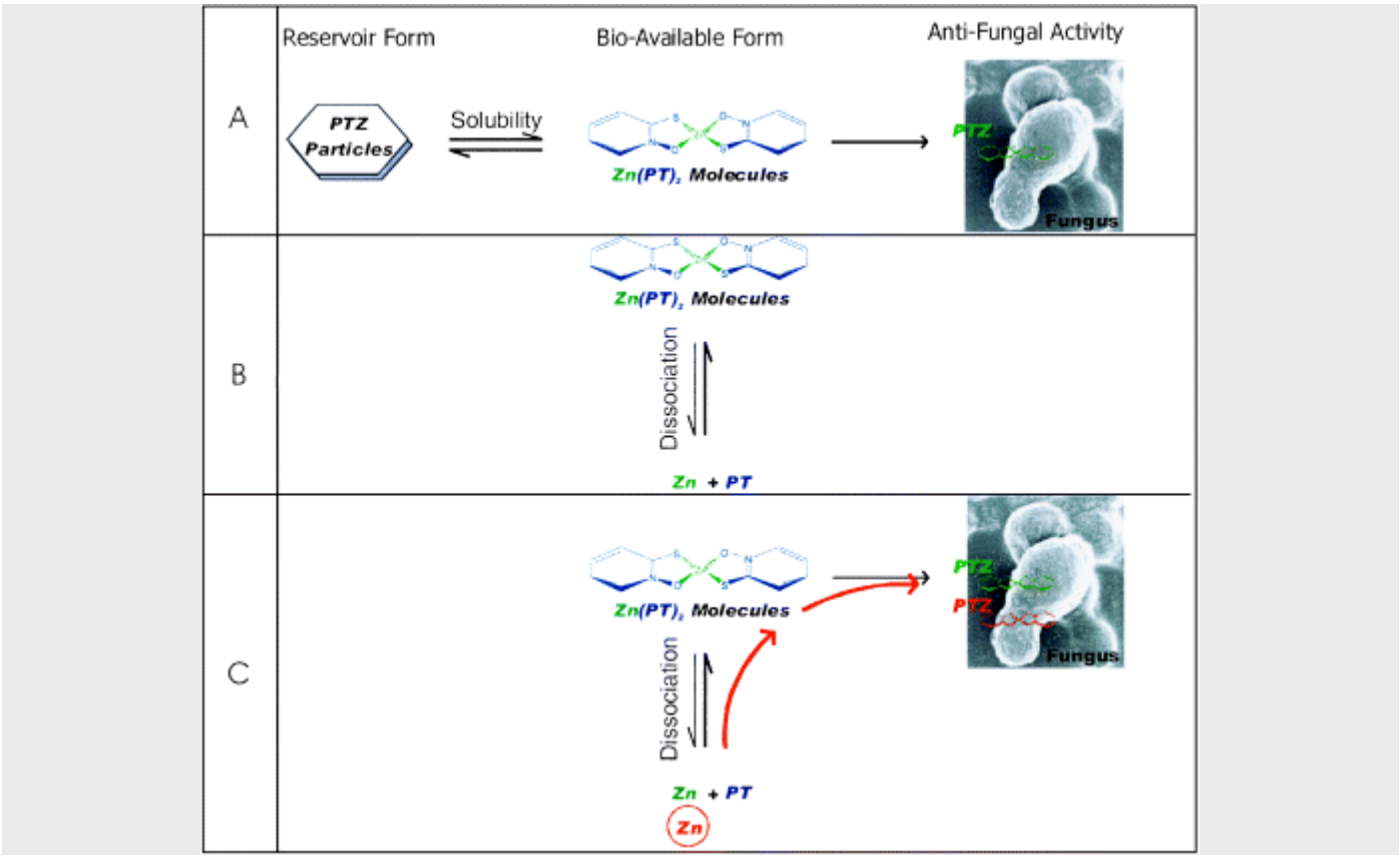


Fig. 12: A summary of relevant zinc pyrithione (ZPTO) equilibria governing realization of anti-fungal activity. **(A)** ZPTO particles yield a soluble portion of ZPTO molecules, which penetrate the fungal cell membrane (*green*) **(B)** ZPTO undergoes dissociative equilibria which reduces the bio-active complexed form (C) The addition of an exogenous source of zinc ions (*red*) shifts the equilibria in the favor of the complexed ZPTO form, thereby increasing delivery to the fungal cells (red) and hence more activity.

Zinc ion doesnot possess any anti-fungal activity of its own but enhances the anti-fungal activity of ZPTO by over an order of magnitude in the *in vitro* evaluations (**Fig. 13**). Apart from this, it also provides a soothing attribute and helps in restoring skin conditions [26], a distinct benefit in shampoos formulated to treat scalps, irritated by *Malassezia*. The mechanism involves shifting the equilibrium between ZPTO and its dissociated components (PT + Zn). The basis for this effect is the well-established principle in chemical equilibria called *LeChâtelier's Principle*. Briefly, it states that a change imposed on a system at equilibrium shifts the equilibrium in a way that reduces the effect of the change. In practical terms, this means that the addition of a material which appears on one side of the equilibrium shifts the equilibrium in the opposite direction. In the case of the ZPTO equilibria, added zinc forces the equilibrium towards the bio-active ZPTO complex side. In effect, additional zinc is acting in the formula to increase the bio-availability of ZPTO to exert its anti-fungal activity and is therefore considered a potentiated ZPTO formula.

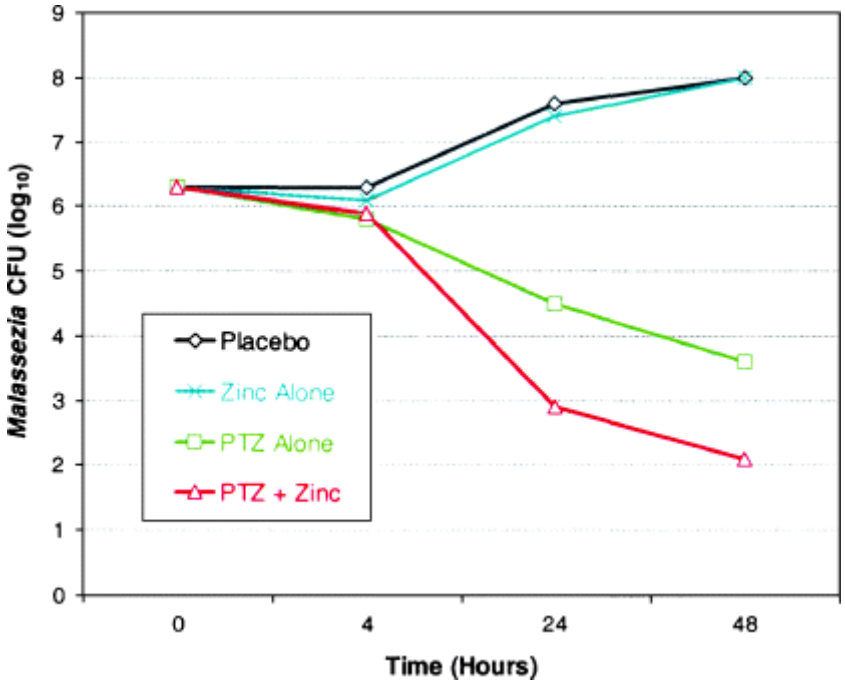


Fig. 13: *In vitro* microbiology assessment of anti-malassezia activity of shampoo prototypes containing no active (placebo), pyrithione zinc (ZPTO) alone, added zinc alone and the combination. Zinc alone has no activity, but it potentiates the activity of the ZPTO formula [25 (a)].

Support for this mechanism comes from quantitation of ZPTO penetration into model mammalian cells or *Malassezia* yeast cells which can be monitered by evaluating zinc levels within cells using fluorescent techniques [28]. Comparison of the zinc levels in cells exposed to either ZPTO or ZPTO plus zinc, shows much higher levels of intracellular ZPTO (as zinc) in the case of added zinc. This is the result of the effect additional zinc has on increasing the bio-availability of ZPTO as the equilibria in **Figure 12 C** demonstrate. The zinc-enhanced ZPTO activity observed *in vitro* and understood in terms of increased bio-availability results in increased performance *in vivo* in complex matrices as well. Quantitation of *in vivo Malassezia* reduction from shampoo matrices demonstrated that ZPTO formulation with zinc is more effective than ZPTO formulations alone. Clinical studies show that potentiating ZPTO in a shampoo formula significantly increases its effectiveness at relieving the five primary symptoms commonly associated with the disorder, *i.e.*, flaking, itch, dryness, irritation, and scalp tightness [12].

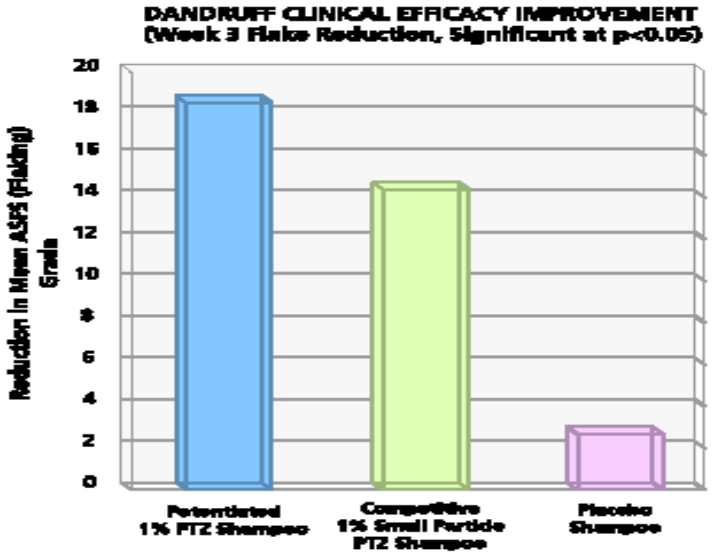


Fig. 14: Clinical efficacy of potentiated ZPTO formulation

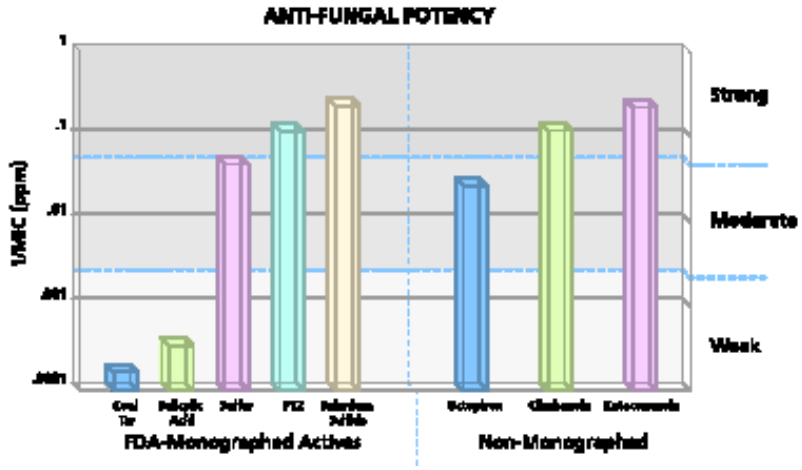


Fig. 15: Antifungal potency of various antidandruff actives

In addition to potentiated ZPTO formula, the prophylactic treatment can also provide long-term relief from dandruff and seborrheic dermatitis. Yearlong studies reveal that long-term prophylaxis prevents recurrence and no evidence has been found which shows that *M. globosa* becomes resistant to ZPTO [27].
To explore the role of zinc Kim *et. al.* [28] have studied the effect of zinc pyrithione on NF-kB activation [29-32] in BCECs (Bovine Cerebral Endothelial Cells). Nuclear factor kappa B (NF-kB) plays a key role in inflammation, cell growth and development [29]. Activated NF-kB enters the nucleus and binds to the kB motif of DNA [30]. Zinc has been noted to have variable effects on NF-kB activity. In vitro studies have shown that zinc is required for NF-kB binding to its DNA motif [31, 32] The results showed that the inhibition of NF-kB activity by pyrithione is time and dose dependent and secondary to its effect on intracellular zinc level in the intact cells. Pyrithione increased the intracellular zinc level within 15 minutes. This effect and the pyrithione induced NF-kB activity is abolished by Ca-EDTA but not by the Zn-EDTA. The potency of pyrithione on NF-kB inhibition and Zn influx was found to be approximately one order of magnitude. These findings establish the regulatory role of intracellular zinc levels on NF-kB activity in BCECs, *i.e.*, ZPTO suppresses NF-kB activity and exhibits cytotoxic effects in BCECs and also these effects were accompanied by an increase in intracellular zinc level.

4.3 pH Stability

Ideally, ZPTO should be used as bactericide/fungicide in the pH range from 4 to 10. Below pH 4.5 zinc complex dissociates into free pyrithione and above pH 9.5, zinc complex hydrolyzes to yield ionized pyrithione and zincate species. Both free and ionized pyrithione are biologically active but being much more water soluble than the zinc complex they are most susceptible to degradation from exposure to light or oxygen.
Some important facts about ZPTO:

- ZPTO is partially neutralized by nonionic surfactants and emulsifies in MIC test
- Zn^{+2} can be chelated away from the pyrithione complex. Thus, it is not compatible with EDTA or other chelating agents. The transchelation can be prevented by adding zinc ions in the form of a salt
- To prevent the photodegradation of ZPTO, photoprotectants can be incorporated into formulation or into containers
- Anidandruff shampoos containing ketoconazole have been shown to be more effective than zinc pyrithione.

4.4 Analytical Studies of Zinc Pyrithione

Several HPLC methods [33-37] have been developed for detecting ZPTO in cosmetic preparations. The direct RP-HPLC analysis [33, 34] of the analyte (ZPTO) is difficult owing to the interaction with reversed-phase packing materials [38] or stainless-steel components of the chromatograph even if Zn^{2+} is added to the mobile phase. In fact iron forms a much stronger complex with pyrithione than does zinc and therefore, zinc

is displaced from the complex. Other procedures [36, 37] involving the conversion of the ZPTO into a copper complex do not take into account the analysis of the commonly found ZPTO- related pyrithiones.
Ferioli *et. al.* [39] have described the reversed-phase HPLC analysis of zinc pyrithione in antidandruff formulations by using the pre-column conversion of ZPT into a copper complex. The proposed method allows the determination of zinc pyrithione and its separation from related pyrithiones and therefore can be adopted for the quality control of commercial antidandruff preparations.
A method for measuring and correlating the rate at which zinc pyrithione dissolves during a shampoo is described by Davies [40]. The rise in zinc concentration of a solution in a stirred vessel following the introduction of a dose of zinc pyrithione suspension was measured as a function of time using atomic absorption spectrophotometry. An analysis of the mass transfer processes occurring during the dissolution of a suspended solid in a stirred vessel was used to correlate the concentration-time measurements. The analysis allows a calculation of the characteristic solution time, the mass transfer coefficient and the diffusion coefficient for the system. In this way the effect of changing shampoo formulation can be systematically studied.
Squiquera *et. al.* [41] have developed an *in vitro* assay to analyze the activity of components of shampoos (Ketoconazole, Zinc Pyrithione and Octopirox Olamine) in eradicating infection by *Malassezia*. The minimal inhibitory effect was attained with 12.5 ng of ketoconazole, 50 μg of octopirox and 4.8 μg of zinc pyrithione. Ketoconazole demonstrated an inhibitory effect on *Malassezia* that was 5000-fold stronger than octopirox and 480-fold stronger than ZPTO.

5 Mode of Action

Pyrithione (2-mercaptopyridine-*N*-oxide) is a fungistatic and antimicrobial agent [8] and its zinc salt enjoys widespread use in antidandruff shampoos [10, 11]. However, the mode of action of pyrithione remains uncertain. Albert *et al.* [42] suggested that pyrithione acts by entering the cell in its chelate form (see **Fig. 2** for the structure) and then dissociates in the cytoplasm. In the cell, pyrithione might act by chelating metal cofactors of enzymes through its sulfhydryl group [8]. Alternatively, pyrithione has been shown to interfere with thymidine uptake [43] and also has been suggested to act as an anti-metabolite for nicotinic acid or vitamin B_6 [44].
In the chelated complex the +ve charge of the metal ion is partially shared with the donor atom and there is π-electron delocalization over the whole chelate ring. This increases the lipophillic character of the metal chelate and favours its permeation through the lipoid layer of the fungal/bacterial membrane. It is also suspected that factors like solubility, dipole moment and cell permeability mechanisms are also influenced by the presence of the metal ion and play a key role in enhancing the activity of metal complex.
Studies by Chandler and Segel [45] have demonstrated that low concentrations of pyrithione (0.1 to 1.0 mM) inhibit plasma membrane transport of a wide variety of unrelated solutes in *Penicillium chrysogenum*. Nevertheless, the primary mode of action of pyrithione on membrane transport remains to be elucidated. Chandler and Segel [45] have hypothesized that pyrithione might act as a general transport inhibitor through weak-acid induced cytosolic acidification and hence partial dissipation of the transmembrane proton motive force (PMF) on which the transport of many organic and inorganic solutes is reliant in fungi [46]. Thus, according to their hypothesis, the uncharged form of pyrithione (pyrithione-SH), which is hydrophobic would diffuse across the membrane, dissociate in the relatively alkaline cytosol to pyrithione-S^- and hence lower the cytosolic pH (pHc). The dissociation reaction has a pKa of 4.7 and would itself tend to sustain the concentration gradient favoring further uptake of the uncharged form.
Direct measurements of cytosolic pH [47] in *Neurospora crassa* with pH sensitive microelectrodes have revealed that weak acids such as butyric acid can indeed acidify the cytosol when applied externally in the millimolar concentration range [48]. However, apriori considerations based on the cytosolic buffer capacity of *N. crassa* militate against a weak acid/acidification mechanism as a mode of action of pyrithione on transport. The buffer capacity is sufficiently high to limit acidification of cytosolic pH to within about 0.1 to 0.2 unit when pyrithione is applied at inhibitory submillimolar concentrations at pH 6.
An alternative manner in which pyrithione might have such far-reaching effects on transport could be through membrane electrical depolarization. Since most H^+-coupled transport systems in fungi carry inward current [46], the membrane potential (*Vm*) forms part of the driving force. Inhibition of transport might then

arise either directly through reduction of this driving force or indirectly as a result of changed cytosolic conditions which result from membrane depolarization.

Ermolayeva and Sanders [49] have used an electrophysiological approach to determine the mode of action of pyrithione on the plasma membrane of the model ascomycete, *Neurospora crassa*. At pH 5.8, pyrithione induced a dramatic dose-dependent electrical depolarization of the membrane which was complete within 4 minutes and exerted a half maximal between 0.6 and 0.8 mM. Zinc pyrithione also induced a similar response but a half-maximal effect was observed at around 0.3 mM. The depolarization is strongly dependent on external pH and almost absent at pH 8.2 at which the concentration of the uncharged form of pyrithione (which is expected to permeate the membrane freely) is markedly lowered. However, quantitative considerations based on cytosolic buffer capacity, the pKa of pyrithione and the submillimolar concentration at which it is active appear to preclude significant cytosolic acidification on dissociation of the thiol proton from the uncharged form of pyrithione. Current-voltage analysis demonstrated that the depolarization is accompanied by a decrease in membrane electrical conductance in a manner consistent with inhibition of the primary proton pump and inconsistent with a mode of action of pyrithione on plasma membrane ion channels. On the basis of the above studies, they concluded that pyrithione inhibits the membrane transport *via* a direct or indirect effect on the primary proton pump which energizes transport and that the site of action of pyrithione is likely to be intracellular rather than extra- cellular.

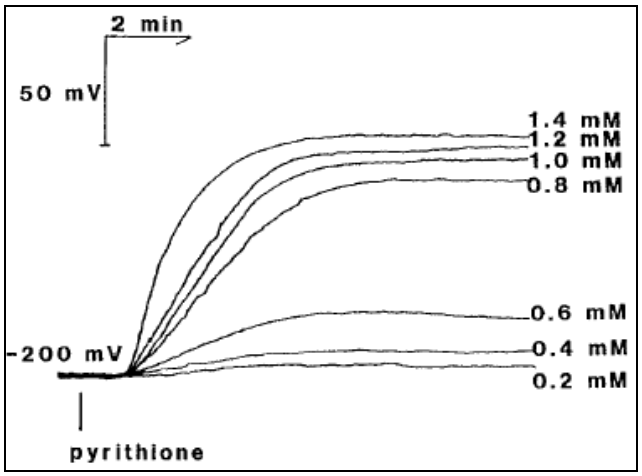


Fig. 16: Time dependence of plasma membrane depolarization in *N. crassa* on application of different concentrations of pyrithione. The lag period between the arrows indicating the time of application is accounted for by dead space between the supply tap and the recording chamber. Each trace is from a different hypha, and resting membrane potentials before the application of pyrithione were in the range of 2198 to 2205 mV.

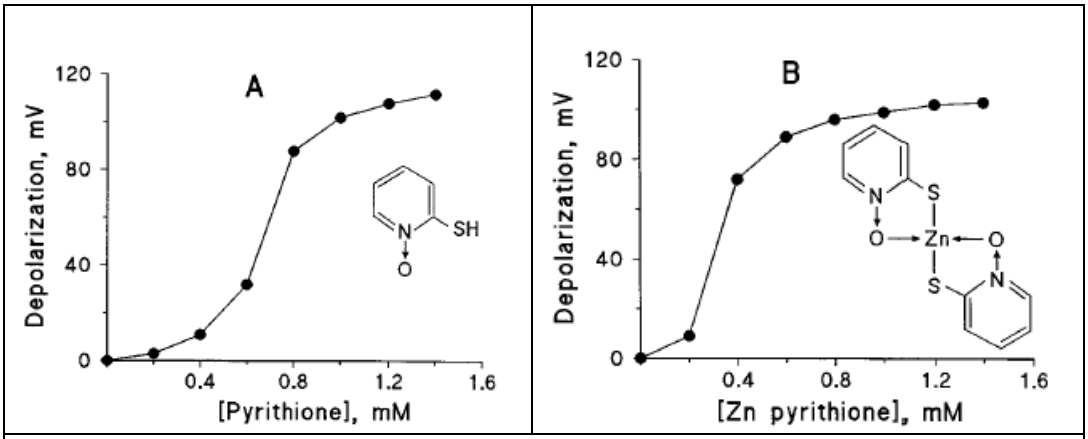


Fig. 17: (A) Dose dependence of the steady-state depolarization induced by pyrithione. Results were obtained from **Fig. 16;** (B) Dose dependence of steady state depolarization by zinc pyrithione.

Investigations by Marks and co-workers [50(a)] with zinc pyrithione shampoo show that this effective treatment for dandruff is unlikely to work by a cytostatic mechanism. During the course of treatment, they measured the labeling index (LI) which reflects the proportion of cells in the epidermis that are in the premitotic DNA-synthesizing stage of the cell cycle and also the mean epidermal thickness (MET). Both the LI and MET would have been anticipated on the half-head receiving the active treatment. Marks *et al* have observed a decrease in yeasts but not in gram-positive bacteria on the treated areas and conclude, with Shuster that the general mechanism is an antifungal one.

Gibson [50(b)] *et al.*have also demonstrated that the inhibition of the cell growth *in vivo* by the antidandruff agent ZPTO is predominantly due to its cytotoxic action. In addition to growth inhibition, ZPTO caused adverse morphological changes and loss of membrane integrity as well as inhibition of chemically induced histamine release from non dividing mast cells. Regarding the topical applications of the ZPTO on epidermal growth, Van Abbe *et. al.* has reported that the ZPTO has no effect on epidermal growth, whereas Imokawa *et. al.* have stated that ZPTO causes a significant decrease in epidermal DNA synthesis and mitosis. Kligman *et. al.* has suggested that ZPTO might exert its antidandruff affect by slowing down epidermal cell division and restoring the normal pattern of cell production in affected skin.

Based on their studies, Gibson *et. al.* has concluded that ZPTO does not work directly by the cytostatic mode of action to clear dandruff. Although, ZPTO treatment will reduce the coenocytes count in individuals with dandruff (Kligman, Marples) indicating the effect on squame cohesion rather than epidermal turnover and squame production. In the absence of demonstrable effects on the epidermal growth, the action of ZPTO must be presumed to be largely restricted to the skin surface and the upper region of hair follicles. Hence, there are the possibilities to investigate the effect of ZPTO on squame cohesion and the process of desquamation. Secondly, as suggested by Van Abbe *et al.* the microbial theory of dandruff needs to be reinvestigated, concentrating on the possible suppression by ZPTO of the lipolytic products generated by micro-organisms causing slight scalp irritation and parakeratosis.

6 Conclusion

In summary, the mechanisms of action of pyrithione and its zinc salt, often found in antidandruff shampoos, have been the subject of extensive research. Several potential modes of action have been proposed, but a definitive understanding remains elusive. Pyrithione may operate through chelation of metal ions, interference with metabolic pathways, and modulation of membrane properties. It can potentially inhibit plasma membrane transport and affect the transmembrane proton motive force in microorganisms. Additionally, it may induce membrane electrical depolarization, which could directly or indirectly impact transport systems.

Research on zinc pyrithione (ZPTO) in dandruff treatment has highlighted its antifungal properties, primarily reducing yeasts while sparing gram-positive bacteria. It may also influence squame cohesion and desquamation, but its effects on epidermal growth are limited.

In conclusion, pyrithione and ZPTO likely combat dandruff through a multifaceted approach, including chelation, membrane interactions, and antifungal activity. While further investigation is needed to clarify the precise mechanisms, these compounds have proven effective in addressing dandruff, especially in their role as antifungal agents. The understanding of their effects on squame cohesion and potential impacts on microbial theories of dandruff offers avenues for future research and development in this field.

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